UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Original

"- Estou a voar! Zorbas! Sei voar! – grasnava ela, eufórica, lá da vastidão do céu cinzento.

O humano acariciou o lombo do gato.

- Bem, gato, conseguimos – disse suspirando.

- Sim, à beira do vazio compreendeu o mais importante – miou Zorbas.

- Ah, sim? E o que é que ela compreendeu? – perguntou o humano.

- Que só voa quem se atreve a fazê-lo – miou Zorbas."

Luis Sepúlveda (1996)*

Dedico este trabalho a todos os que se atrevem, mas acima de tudo, a todos aqueles que acreditam nos primeiros.

Dedico-o a ti, Mãe.

*Sepúlveda, L. (1996). *História de uma gaivota e do gato que a ensinou a voar*. (8ªedição). Lisboa: Porto Editora. Tradução por Pedro Tamen

Acknowledgements.

v

Abstract: PARETIC SYNDROME IN GULLS (LARIDAE) IN THE SOUTH OF PORTUGAL

RIAS, a Portuguese wildlife rehabilitation centre located in Algarve, has been admitting a substantial high number of seagulls, since its opening in October of 2009, with consistent clinical presentations pertaining to a paretic syndrome without cues of a particular disease. This preliminary study describes the clinical signs and microbiological, parasitological, toxicological and pathologic findings of paretic gulls received between 2009 and 2012. It tries to understand if there is an association between the manifestation of this disease and the different species and age classes affected. It seeks to determine possible relations between the geographic distribution of the cases and specific potentially problematic areas or human activities. All in order to additionally determine a probable cause for this disease taking into consideration the species affected, region where the animals were rescued and diseases that could explain the findings observed like: Newcastle disease, Salmonellosis, Aspergillosis, Sarcocystosis, Botulism, Algal toxicosis, Copper/Lead/Mercury intoxication, Organophosphorus/Carbamate poisoning and Thiamine deficiency. Additionally, a treatment trial with three therapeutic protocols (activated charcoal, fluid therapy and thiamine supplementation) was attempted to evaluate their influence in the outcome of the rehabilitation process and their value as a tentative diagnostic tools. Accordingly, digital records of 780 gulls were analyzed, as well as, results of more specific diagnostic ancillary tests used in carcasses and tissue samples in the centre and submitted to the Faculty of Veterinary Medicine of the University of Lisbon. From the 780 admissions, 148 gulls (18,97%) were found to have this paretic syndrome while alive, with *L.fuscus* and sub-adults being probably the classes most affected (p=0,02;p=0,00005). All these gulls, upon admission, were thin and dehydrated and the most frequent clinical signs documented were: depressed mental status without loss of conscious (58,8%); diarrhoea (43,9%), flaccid cloacae (70,3%); generalized muscular weakness (48,6%), moderate muscular weakness (46,6%); posterior paresis (69,6%) and moderate paresis (71,6%). Approximately half of the 148 gulls died while in rehabilitation and gross necropsy findings of paretic gulls were also unspecific and overall inconsistent. However, a high number of these gulls including dead admissions had a thin-walled cloacae distended with diarrhoea and the intestines were also displaying compatible signs of inflammation: oedema, vascular congestion and fluid faeces (32/71). Evidences of opportunistic diseases or development of confounding ailments like probably Aspergillosis were also noted. The differences between the therapeutic protocols were irrelevant (p=0,7422) and could not diagnose this condition. No pathogenic agent (bacterial or parasitic) capable of causing this syndrome was identified in the carcasses submitted (n=9). The necropsy examination and histopathology lesions reported in the faculty were inconclusive as to the cause of the paresis. Lead and Copper levels, analyzed in 2 gulls, were below what is considered in the literature as indicative of toxic. Nevertheless, in one of the gulls submitted a liver sample was positive for the presence of an organophosphorus compound, which could be in accordance with the high association measured between the spatial distribution of the proportion of paretic cases and density of several crops per municipality (Rho>0,5;p<0,05). In this moment, the data here compiled and the results obtained are still insufficient to determine or exclude the diseases in discussion as causes of this syndrome. Inconsistent use of ancillary tests results, paucity in the knowledge of ethologic and ecologic features of these birds in this region, irregularities in the retrieval of sick birds and tourism are some of the factors that may be influencing these results and should be addressed in future investigations.

Key-words: Gulls, Paresis, Infectious diseases, Natural Toxins, Heavy metal intoxication, Pesticide poisoning, Thiamine deficiency, Algarve, Portugal

Resumo: SÍNDROME PARÉSICO EM GAIVOTAS (LARIDAE) NO SUL DE PORTUGAL

RIAS, centro de recuperação de animais selvagens localizado no Algarve, desde a sua abertura em Outubro de 2009 tem recebido um número elevado de gaivotas com um quadro clinico consistente com paresia, sem causa conhecida. Este estudo preliminar descreve os sinais clínicos e achados microbiológicos, parasitológicos, toxicológicos e anatomo/histopatológicos de gaivotas com parésia recebidas entre 2009 e 2012. Tenta igualmente perceber se existe uma associação entre a manifestação desta doença e as diferentes classes de idade e espécies afectadas. Procura determinar relações possíveis entre a distribuição geográfica dos casos e áreas/actividades humanas especificas e potencialmente problemáticas na área em estudo. Tudo com o intuito adicional de descobrir a causa provável desta doença tendo em consideração as espécies afectadas, região onde foram resgatadas e doenças que poderiam explicar os achados reunidos: Doença de Newcastle;Salmonelose;Aspergilose;Sarcocistose;Botulismo;Fitotoxicose;Intoxicação por cobre, chumbo, mercúrio;Intoxicação por organofosforados/carbamatos e Deficiência em tiamina. Três protocolos terapêuticos (carvão activado, fluidoterapia e tiamina) foram igualmente testados para avaliar os respectivos efeitos no processo de reabilitação e o seu valor diagnóstico. Desta forma, foram analisados registos de 780 gaivotas em conjunto com resultados obtidos de métodos de diagnóstico auxiliar mais específicos de carcaças e amostras recolhidas e analisadas no centro ou enviadas para a Faculdade de Medicina Veterinária da Universidade de Lisboa. Das 780 admissões, 148 larídeos (18,97%) exibiam em vida este síndrome, sendo provavelmente as classes mais afectadas: *L.fuscus* e sub-adultos (p=0,02;p=0,00005). Todas as gaivotas afectadas encontravam-se magras e desidratadas, sendo os sinais clínicos mais frequentemente documentados: depressão do estado mental (58,8%); diarreia (43,9%), cloaca flácida (70,3%); fraqueza muscular generalizada (48,6%), fraqueza muscular moderada (46,6%); paresia dos posteriores (69,6%) e paresia moderada (71,6%). Aproximadamente metade destes animais morreu no decurso da reabilitação e as lesões encontradas em necrópsia foram igualmente inespecíficas e inconsistentes. Contudo, um elevado número destes animais, incluindo admissões de animais mortos, apresentavam recurrentemente cloacas com parede finas e distendidas por diarreia e os intestinos apresentavam também sinais compatíveis com inflamação (32/71). Achados de doenças oportunistas ou capazes de provocar sinais/lesões semelhantes foram também reportados (e.g. Aspergilose). As diferenças obtidas entre os diferentes protocolos foram consideradas irrelevantes (p=0,7422) e incapazes de diagnosticar esta doença. Nenhum agente patogénico (bacteriano ou parasita) capaz de causar parésia foi identificado nas carcaças enviadas (n=9) e resultados de análise anatomo-histopatologia das lesões encontradas foram inconclusivos quanto à causa deste síndrome. Níveis de chumbo e cobre, analisados em amostras de fígado de 2 animais, encontravam-se abaixo do que é considerado na literatura como indicativo de tóxico. No entanto, em uma amostra de fígado de uma das gaivotas enviadas foram detectados resíduos de um organofosforado,o que poderá ser concordante com a elevada associação medida entre a distribuição espacial da proporção de casos com parésia e a densidade de diversas culturas por município (Rho>0,5;p<0,05). Neste momento, toda a informação aqui compilada é ainda insuficiente para determinar ou excluir as doenças em discussão enquanto causas. O inconsistente uso de métodos de diagnóstico auxiliar, a escassez de informação relativa à etologia e ecologia destes animais nesta região, irregularidades na recolha de animais doentes e o turismo são alguns dos factores que podem estar a influenciar estes resultados e deverão ser tidos em conta no futuro.

Palavras-chave: Gaivotas, Parésia, Doenças Infecciosas, Biotoxinas, Intoxicação por metais pesados, Envenenamento por pesticidas, Deficiência em tiamina, Algarve, Portugal

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List of Abbreviations and Symbols

= - equal
< - lower than
> - higher than
% - percentage
® - Registed brand
Pb – *Plumbum*
AMAP- Artic Monitoring and Assessment Programme
ANA – Aeroportos de Portugal
Ap. - Appendix
APMV-1 – Avian Paramyxovirus Serotype 1
ASP – Amnesic Shellfish Poisoning
AZP – Azaspiracid Shellfish Poisoning
BID – *bis in die*
CA - Carbamate pesticides
CAOP – Carta Administrativa Oficial de Portugal
cm - centimetre
d – day
dL – decilitre
DGEG- Direcção-Geral de Energia e Geologia
DNA - Deoxyribonucleic Acid
DSP – Diarrhetic Shellfish Poisoning
EDTA – ethylenediaminetetraacetic acid; $NaCa_2$ EDTA – calcium disodium EDTA
e.g. – *exempli gratia*
f – frequency
fig. – figure
FMV-UL – Faculty of Veterinary Medicine of the University of Lisbon
g - grams
GVA – Gross Value Added
GNR- Guarda Ncional Republicana
HAB – Harmful algal bloom
i.e. - *id est*
ICNF – Instituto da Conservação da Natureza e das Florestas
INE – Instituto National de Estatística
IPIMA – Instituto Português do Mar e Atmosfera
kg - kilograms
km^2 – square kilometres
LD_{50} – median lethal dose
LNEG – Laboratório Nacional de Energia e Geologia
m^2 - square metre
mg – milligram
ml – millilitre
n - sample size
NA – absence of logged data on the physical examination
N.O. – not observed
ND – Newcastle disease
NDV – Newcastle disease virus
ng - nanogram
ºC – Celsius degrees
OIE – World Organization for Animal Health
OP - organophosphorus pesticides
PNRF – Parque Nacional da Ria Formosa
ppm – parts per million
PSP – Paralytic Shellfish Poisoning
RIAS – Centro de Recuperação e Investigação de Animais Selvagens
RL – Regulatory limits
S.B.de Alportel – São Brás de Alportel
SEPNA – Serviço de Protecção da Natureza e Ambiente
SID – *semel in die*
SPEA – Sociedade Portuguesa para o Estudo de Aves
t – tons
TID – *ter in die*

V.Bispo - Vila do Bispo
V.R.S.A. – Vila Real de Santo António
Vit. – vitamin
UK – United Kingdom
WAHID – World Animal Health Information Database
WPE – Waterbird Population Estimates

1. Introduction.

It is irrefutable that wildlife in general is subjected to multiple stressors and the anthropogenic ones, for instance predation, habitat destruction/fragmentation/encroachment or overexploitation, chemical pollution, introduction of non-native predator or competitor species, pathogen pollution, climate change; have been pointed out throughout history as one of the primary reasons for the extinction of species, but are also capable of provoking severe and important population declines. Their importance cannot be neglected, as nowadays, these are considered the most significant drivers of the emergence of important infectious diseases (another stressor) by modifying the dynamics of several pathogens, vectors, hosts and consequently infectious and even parasitic diseases. Examples of such declines are numerous in the literature and the consequences that they can pose in the loss of biodiversity and subsequent impacts in ecosystems (disruption of processes and services e.g. migration and pollination), other species and manifestation of diseases including the emergence of zoonotic diseases, are aspects of concern (Daszak, Cunningham & Hyatt 2001;Sekercioglu, Daily & Ehrlich 2004;Wildcove & Wikelski 2008;Keesing et al. 2010). A recent and ongoing population decline was reported by Balk et al. (2009) and has been affecting several avian wild species in the Baltic sea region. Some of the features observed by these authors are similar to what has been found in seagulls admitted in a southern Portuguese wildlife rehabilitation centre, RIAS, since its opening in October 2009. The recurrent admission of gulls exhibiting a paretic presentation, without cues of a particular disease, and given the centre's limited resources for more complex ancillary tests to reach a definitive diagnosis led to the proposition of this theme for a master dissertation thesis. The relevance that such study has is heightened when one considers that the species affected belong to a group of birds with a strong dichotomised nature. Seagulls are considered nuisance species and in this way are object of culling programs in response to the recent increases reported in their number, range of occupied areas and as a preventive measure with the intent to manage their potential threat to other species, human disturbance and public health (Rock 2005;Oro & Martínez-Abraín 2007;Molina 2009). Like any other wild bird, gulls can also carry, disperse and disseminate through their mobility and migratory routes several potential pathogens important for humans and other species (Reed, Meece, Henkel & Shukla 2003;Hubálek 2004). However due to their gregarious nature and a recent tendency to occupy more urban and human made niches the risk for transmission/dissemination of potential problematic pathogens is a prominent concern. In fact, in Portugal they have already been identified as significant reservoirs and carriers of important drug-resistance pathogens like *E.coli* and *Salmonella sp.* (Duarte, Guerra & Bernardo 2002;Simões, Poirel, Da Costa & Nordmann 2010). On the other hand and simultaneously they are a living part of the ecosystem where they dwell, are able to perform taks and influence the processes that are indispensable for the correct functioning of this system, e.g. soil enrichment (Iason, Duck & Clutton-Brock 1986). Like any other living being, they can also be likely threatened by several factors, some of anthropogenic nature, that may untimely lead to deleterious effects with repercussions in important functions like fitness, breeding and survival, as is an example the effects of persistent organic pollutants in northern specimens of gull species cited by several authors, including Sagerup et al. (2009). As a consequence, and given some of their phenotypic, ethologic, ecologic and biologic features, they are considered valuable bio-indicators or environment sentinels, a notion defended by Burger and Gochfeld (2004) that cannot be dismissed.

A great interest in the fields of Wildlife and Conservation medicine and the complexity herein only briefly exposed, were what enticed the author to accept this theme. Thus, in the first part of this thesis a detailed review of selected biologic and ecologic features found in gulls and that may influence the development of diseases is carried out together with a revision of ten of the several diseases that cause paresis or paralytic presentations in birds.This first part is followed by the study **Paretic syndrome in gulls (Laridae) in the south of Portugal** (Algarve) where its main objectives are introduced alongside with the description of the materials and methodologies used, the results obtained and their discussion. Suggestions for future investigations are delivered throughout this paper and in the end conclusions will be drawn.

2. Literature review.
Chapter 1: Seagulls.
2.1.1. Taxonomy and the problematic of classification[1].

Seagulls belong to the order of the Charadriiformes and Laridae family, however the systematics of this group of birds is not well defined yet, and from time to time some modifications do occur regarding the genus, species and also the subspecies as are the following examples, to name a few. Erstwhile all gulls were included in the genus *Larus*, nonetheless this classification was extremely generalist and was not a monophyletic clade. Recent studies including the one by Pons, Hassanin and Crochet (2005) of the philogenetic analysis of mitochondrial DNA sequences, indicated, in conjunction with morphological and behavioural data of other investigations, that larids should be placed in not one or 2 (*Larus* and *Xema*) but in 10 genera*: Rissa, Creagrus, Hydrocoloeus, Pagophila, Xema, Chroicocephalus, Leucophaeus, Ichthyaetus, Larus* and *Saundersilarusi.* The recent integration of the species *Larus ridibundus* in the genus *Chroicocephalus* is another clear evidence of the difficulties that are associated with the taxonomy of these birds. On the other hand and additionally some gulls, previously known to be subspecies, are, recently, considered as independent ones as is the case of *Larus smithsonianus, Larus cachinnans, Larus michahellis* and *Larus fuscus* in relation to *Larus argentatus* (Crochet et al. 2010). All this, in a way, is an additional difficulty when one tries to study a specific gull species. Despite this, there are approximately 50 species of gulls (Schreiber & Burger 2001), about 20 of these were seen and documented in Portugal: *Larus hyperbor*eus, *Larus glaucoides*, *Larus canus*, *Larus marinus*, *L. argentatus*, *Larus atricilla*, *Larus audouinii*, *L. smithsonianus*, *Larus delawarensis*, *Larus genei*, *Rissa tridactyla*, *Chroicocephalus ridibundus*, *Xema sabini*, *Larus minutus, Larus pipixcan, Larus philadelphia*, *L. fuscus, L. michahellis*, *Larus melanocephalus* and *L. cachinnans* (Catry, Costa, Elias & Matias 2010;Aves de Portugal 2012). And so the following text focuses only on those.

2.1.2. Phenotipic features and their importance in studies.

In general terms, these features differ with species, age and moult as is clear in the figures of appendix 1.2 (Olsen & Larsson 2004;Aves de Portugal 2012).

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[1] In appendix 1.1 a table with a list of the scientific names used and corresponding common names in English and Portuguese is available for consultation.

Seagulls are medium to large sized birds, whose length can range from the 25-30 centimetres in *L.minutus* to the 68-79 cm in *L.marinus*. Their wing-span varies accordingly, from the 70-78 cm in the former to the 152-167 cm in the latter, with weights ranging from 88 to 162 grams in the first to 1,4-2,3 kilograms in the second (del Hoyo, Elliott & Sargatal 1996 quoted by ARKive 2012). In spite of males being tendentiously bigger than the females, in general, sexual dimorphism isn't recognizable in these species as are examples figures 15, 21 and 54 in Ap.1.2 (Schreiber & Burger 2001;Olsen & Larsson 2004). These birds own strong bills with the tip arranged in a slightly hook format displaying variations in its size and proportion and some species have pronounced gonys (Aves de Portugal 2012). Regarding its colour, it varies with the species and season from black-brownish to yellow, including red, green, with or without other colour notes, Ap.1.2. fig.6,18,28 and 51 (Dwight-Jr. 1901). The head and body, including the two wings, vary in colour from a combination of a solid dark brownish colour interspersed with white spots usually in younger birds, to numerous gradations of solid grey to black, but remaining, frequently, the ventral surface of the body and alar appendages white (Ap.1.2 fig.1,3,17,32,34,39,40,41,66,68).The head may display several spots of different extensions and colorations, varying also with the moult, species and age, in the same manner as the eyes (iris and ocular contour). The hindlimbs are characterized by the presence of interdigital membranes and can be yellow (Ap.1.2 fig.68), orange, rosy (Ap.1.2 fig.32), red, greenish or even almost black (Ap.1.2 fig.50), but then again always with four digits, except some specimens like *R.tridactyla.* (Schreiber & Burger 2001;Olsen & Larsson 2004;Aves de Portugal 2012).

Given the wide variability of these features, one could consider that the determination of the species or age of an individual would be an easy task to accomplish, however this isn´t the case. Considering the differences between species: juveniles are extremely similar among different species - immatures of *L.argentatus*, *L.michahellis* and *L.fuscus* are indistinguishable (Ap.1.2 fig. 46,66); and some adults also are, like *L. fuscus* and *L. marinus* in Ap.1.2 fig.44 and 68 (Catry et al. 2010;Aves de Portugal 2012). Hybrids, along with certain subspecies, are a complete puzzling challenge (Olsen & Larsson 2004). With regard to the age, sometimes, it is genuinely difficult to determine this parameter because, for instance, wintering plumages are identical between sub-adults and adults and, in addition, some characteristics of immaturity persist in advanced ages, like what is seen in a few species – *L.argentatus* as an example (Olsen & Larsson 2004;Aves de Portugal 2012).

2.1.3. Migration/Dispersion/Distribution, Global tendencies and Portuguese numbers.

Of what is seen and inferred from ringing birds, these 20 species use differently the Portuguese territory (Ap.1.2 map figures). Some are accidental in Portugal like *L.atricilla*, *L.smithsoniannus, L.hyperboreus*, *L.glaucoides*, *L.pipixcan*, *L.cachinnans* and *L.philadelphia*. Others are uncommon passing by migrants such are the cases of *L.audouinii*, *X.sabini* and more rarely *L.genei*. Some winter, like *L.fuscus*, *C.ridibundus (*both fairly common and passing by migrants), *L.melanocephalus*, *R.tridactyla* (both uncommon to common and the former is also a passing by migrant uncommon to common) *L.marinus, L.minutus* (both are rare to uncommon, *L.minutus* is also a passing by migrant uncommon/rare)*, L.canus* (rare), *L.argentatus* and *L.delawarensis* (both rare to accidental). In turn some birds of the species *L.audouinii*, *C.ridibundus* and *L.fuscus* reproduce (rarely) in Portugal, whereas *L.michahellis* is resident in the Portuguese continental territory (Catry et al. 2010). These

differences can be explained by the so called "bird movements" described for the generality of birds by Newton (2008). The majority of such dislocations are seen in these birds. However, normally some discrepancies, attributable to differences in species, are perceived and are responsible for their distinct global distribution and may define seasonal patterns of concentration of individuals in specific sites and time intervals. These movements are usually established between the nesting sites, feeding areas, wintering grounds and moulting regions, but in certain occasions are seen within the same area set for each one (Newton 2008;BirdLife International 2012). Taking as an example the definition of the six kinds of bird movements displayed, referred by Newton (2008), seagulls, in general, are capable of performing daily routine dislocations in short distances from their resting/roosting places or nesting sites in several directions foraging for food. One way dispersal movements (either natal, reproductive or wintering) are also equally common to the totality of birds and seen in this group.This behaviour, described when birds do not establish in the same location but fairly close by from one year to the next, is likely found in juveniles and some adults normally due to reproductive failure in the previous year (Schreiber & Burger 2001;Newton 2008). Migration, *per se*, regular return movement on specific times for specific destinations, over distances superior to the other movements (hundreds to thousands to millions kilometres e.g.Ap.1.2 fig.20) and with a more restricted and fixed direction between the wintering regions and the breeding grounds and vice-versa (Newton 2008); is also seen in gulls. However this phenomenon doesn't happen in certain species called resident or sedentary like *L.michahellis*, Ap.1.2 fig.70 and in others like *L.canus*, *L.genei*, *C.ridibundus*, *L.cachinnans*, *L.genei*, *L.atricilla*, *L.glaucoides* and *L.melanocephalus* is replaced by dispersive movements (Newton 2008;Catry et al. 2010;BirdLife International 2012). Invasion migration is another movement described, especially regarding yearly variations that occur in the occupation of wintering grounds. It appears to be present to some extent in these birds, thus ensuring wintering areas of grand extensions (Ap.1.2 fig.25), and possibly justifies the recent existence of new breeding sites of *L.audouinii* outside the Mediterranean region, Ap.1.2 fig.30 (Newton 2008;Catry et al. 2010). Nomadism, the sixth movement, defined as the exploitation of vast extensions of land for feeding, roosting and reproduction, non-restricted in terms of time intervals and space, does not appear to be part of the behaviour of these avian species (Olsen & Larsson 2004;BirdLife International 2012), despite Schreiber and Burger (2001) admitting a tendency while roosting. These dislocations, namely the case of migration, have differences when concerning the species in question, as to the point of origin, routes used, number of kilometres covered, wintering site, time of the year and stop-overs (BirdLife International 2012). Differences are also seen between different populations of the same species and this probably explains the distinct behaviour reported when it is considered different latitudes, as is the case of *C.ridibundus*: northern breeding populations are migratory, whereas southern are sedentary or only dispersive (BirdLife International 2012). These differences are not only attributable to these factors, certain natural phenomena can also exert its effects on these events. Animals whose life cycles take place in America (Ap.1.2 fig.5,10,11,12,53) and are seen in Europe when strong adverse weather conditions manifest, is are examples of such (Catry et al. 2010). Futhermore, other types of vagrancy, in species like *L.atricilla* and *L.fuscus,* are also valid reasons for the anomalous global distributions of these birds (BirdLife International 2013). Newton (2008) also indicates that the differences found in these movements are found also within the same population and can be explained as owing to dissimilarities in sex and age. These disparities are evident in the distances travelled, chosen routes,

times of departure and the behaviour exhibited during the movement and the same ensues in gulls. Commonly the younger individuals (including the already apt for reproduction) initiate migration later, disperse more and to a larger extent (Schreiber & Burger 2001;Jorge, Sowter & Marques 2011). Another possible reason for this is what was probably seen on the Portuguese coast with *L.fuscus,* where pressure from older birds or their tendency to winter closer to the breeding grounds, probably influenced the migratory behaviour of the immature (Marques et al. 2009). In this way younger specimens begin with short movements to gain experience, unlike adults, and tend to stay further south than the latter during their annual cycle (Marques et al. 2009;Jorge et al. 2011).Nevertheless in other species the youngest or non reproductive can remain instead in the wintering grounds during the reproductive season, while they don´t attain full maturity, like gulls of *L.audouinii*, *C.ridibundus* and *L.genei* species (Equipa Atlas 2008;BirdLife International 2012). From the above one can conclude that migration, and the diverse movements defined, are responsible for alterations in the number of birds in the world in a relatively seasonal manner (Newton 2008) and may condition their exposure to different ecosystems and stressors.

As already stated, larid populations have increased globally in the last decades, however this increase might not be real. In accordance with the data available, this trend depends on populations trends of several species and many of such tendencies result of data obtained only of a few populations for each species, so a careful analysis must be made before admitting such conclusions (Delany & Scott 2006;BirdLife International 2013). In the available data, some species are presented as having an unknown status (*L.atricilla, L.minutus* and *L.michahellis*), in others the global tendency indicates an increase or stability with the existence of a few populations that are stable or decreasing in the number of individuals (*L.marinus*, *L.genei* and *L.fuscus*). In some, like *C.ridibundus,* apparently there is a tendency for a decrease, in spite of the fact that there are populations lacking information, whereas in the case of *R.tridactyla,* considered stable, some populations are in decline. In other species, *L.argentatus* or the *L.canus,* it is impossible to discern a global tendency since several studies indicate an increase or decrease or even unawareness in the totality of the populations studied, probably owing to the inability to study such gulls because of the vast extension that is explored by them (Delany & Scott 2006;BirdLife International 2013). Considering the stable species, there are the ones who really are stable and the ones that appear to be (*L.audouinii* and *L.glaucoides*). *L.cachinnans* and *L.melanocephalus,* for instance are stable only because there isn't any evident threat or decline. Available global estimations for each species vary greatly between them (Delany & Scott 2006;BirdLife International 2013). The far most abundant species is perhaps *L.argentatus* with 2,7-5,7 million counted gulls, but these values included several of its subspecies. On the other end is *L.audouinii* with, apparently, only 63.900 to 66.900 individuals. Then again, for species like *L. cachinnans* and *L.michahellis* information regarding their number is lacking, owing to the recent taxonomic split of *L.argentatus*.

Considering the Portuguese territory and the established counts regarding the number of individuals of each species, there are several surveys: some were taken during wintering season, some encompass all the year, some are restricted to certain sites and others try to comprise the whole country. One of such survey (Bermejo, Carrera, De Juana A & Teixeira 1986) was organized in January of 1984 and was conducted mainly on the coastline of the Iberian Peninsula, possibly covering up 70 to 80% of the real panorama of wintering species of gulls and terns. During this effort, in Portugal were counted 103

individuals of *L.melanocephalus*; 21 *L.minutus*; 146 *R.tridactyla* (one in Algarve); 43.023 *C.ridibundus*, 2.400 in Algarve (the most abundant in the peninsula, but since inland habitats were not included this number is a estimation by defect); 48.880 *L.fuscus* (the third most abundant in the peninsula) of which 6.450 in Algarve; 3.700 *L.argentatus*, ten in Algarve (second most abundant, count included *L.argentatus* and *L.cachinnans*) and nine *L.marinus.* Afterwards and considering the remaining organized surveys and census (including other gull species) there has been a noted increase in the numbers of birds seen. This increase is due to several factors like: adverse weather conditions (in cases of species like *R.tridactyla*), more interest and accuracy of the observer in the identification of these birds, and population growths of several species expressed in the number of animals migrating and some breeding in the country, like the cases of the Mediterranean *L.audouinii* (began breeding in 2001 in Algarve) and *L.michahellis* (nuisance species object of culling programs) (Equipa Atlas 2008;Catry et al. 2010).

2.1.4. Habitat, possible source for disease.

Because these birds are extremely versatile, equally capable of flying, walking, swimming and migrating, they can inhabit a broad range of ecosystems from Arctic to Antarctica, occupying every continent and travelling through every ocean (BirdLife International 2012). They are frequently found in habitats or places with predominance of water bodies and it is possible to point out 5 potential settings in their life cycle: reproductive sites, feeding areas, moulting regions (e.g. *R.tridactyla* among others) stop-over sites (typical of species like *L.fuscus*) and resting/roosting grounds in the winter (Catry et al. 2010;BirdLife International 2012). They can display specific preferences, are philopatric and may show foraging site fidelity (Schreiber & Burger 2001;BirdLife International 2013). To some extent, these may condition all their life cycle and certainly influence the possibility of exposure to a pathogenic agent or toxic that causes disease.

Seing as they are often termed as marine birds, the kind of habitat where, preferentially, they are found or where they dwell and develop their life cycle is the marine or one with its strong influence (Schreiber & Burger 2001). In accordance, they are seen in the coastline of several beaches of several subtracts (Ap.1.2. fig.3), estuaries, bays, costal lagoons, saltpans and river deltas. The sea, itself, can also be their habitat as is the case of *R.tridactyla* and *X.sabini* in upwelling regions (Catry et al. 2010;BirdLife International 2012). Considering the definition of a Marine Bird, it is exceptionally difficult to include all seagulls in this definition, since they are not restricted to the typical marine ecosystems (Schreiber and Burger 2001). For that reason it is possible to find specimens along rivers and in more interior locations like: lakes, marshes (*L.philadelphia*), ploughed/flooded fields (*C.ridibundus, L.cachinnans* and *L.pipixcan*), great river weirs and plains, e.g.*L.fuscus* was found on the central plains of Alentejo, Portugal (Catry et al. 2010;BirdLife International 2012). More urbanized regions or places where human activity is certain, in spite of either its close proximity to sea or the interior, are also preferred sites, particularly fishing harbours (Ap.1.2. fig.17), resorts, areas of sewage outfall, wastewater treatment areas, landfills (*L.hyperboreous, L.glaucoides, L.argentatus)*, refuse dumps (*L.fuscus*), aquaculture ponds (*L.michahellis* and *L.genei*), dams, golf courses (*L.melanocephalus*) and in the centre of some cities as is the case of *L.fuscus* (Catry et al. 2010;BirdLife International 2012). Nevertheless, it's becoming frequent for certain species like *L.michahellis*, that are coastal marine and that do not venture inland, to start invading the interior

accompanying the course of rivers and taking advantage of cultivated fields and dams (Catry et al. 2010;BirdLife International 2012).

2.1.5. Reproduction, potential for disease and population declines.

The need to breed is one of the reasons that motivate migration, one of the events that dictate population numbers, growth tendencies and can be affected by disease or be its possible cause.

Upon arrival to the breeding grounds, on the word of ARKive (2012) taking as an example *L.atricilla*, the courtship starts with the male offering food to the female, Ap.1.2 fig. 21 and 54. The couple arrives at the place before the ideal moment for laying, normally one month before breeding, with the exception of *R.tridactyla* that returns in January and only reproduces in May-June (ARKive 2012;BirdLife International 2012). In some species the date of posture depends highly on the latitude of the nidification zone or climate conditions like are the cases of *C.ridibundus* and *L.delawarensis*, the latter tends to lay eggs in late April and in May, but these dates are likely to differ due to snow cover on the northern range of the territory (BirdLife International 2012). The next step is the construction or reconstruction of the nest by both parents (Ap.1.2. fig.49). It can be erected in isolated nestings sites or in colonial arrangements ranging from a few pairs in *X.sabini* to 30.000 in *R.tridactyla* (Schreiber & Burger 2001;BirdLife International 2012). These agglomerations can be monospecific, can encompass several gull species (e.g.*C.ridibundus* and *L.melanocephalus* or *L.fuscus* and *L.argentatus*) or other avian species, e.g. *L.melanocephalus* and *Thalasseus sandvicensis* (BirdLife International 2012).

The nests have distinct characteristics when one considers certain gull species, habitats where they dwell, ecosystems where they are embedded and the material used for their construction. Accordingly, the nests can be simple mild depressions in the soil made by simply scratching/digging the ground that can be: dry, with vegetation, sandy, rocky, muddy or swampy. The nest can be placed in saltmarshes among scrub, on rocky cliff-edges (Ap.1.2 fig.50), islands or islet, on buoyant structures in rivers, on ice or snow or even on glaciers (BirdLife International 2012).The material used can be grass, mud, seaweed, moss, sticks (Ap.1.2. fig.27,62), twigs, cork, lichens, feathers, pebbles, human waste and even old carcasses (frequent in *L.michahellis*) (BirdLife International 2012). Despite its format or material used, the nest is typically placed in close proximity to water (Ap.1.2. fig.44) and in sites where nature, itself, can be used as a protection against predators and adverse weather, Ap.1.2 fig. 28 (BirdLife International 2012). They can even be erected on top of trees and bushes (typical of *L.philadelphia*), in regions of strong industrial and agricultural influences or even on buildings, e.g. *L.fuscus and L.michahellis* (Catry et al. 2010;ARKive 2012;BirdLife International 2012). Inland regions far from the seashore can be used as nesting grounds as is archetypal of *L.pipixcan* (inland prairies) and *L.cachinans* (steppes and semi-deserts). These regions can additionally be used by *C.ridibundus* (sewage lakes, pits, agricultural irrigation channels, salt exploitation sites and aquacultures ponds), *L.michahellis* (dams) and *L.canus* (nest boxes) (Catry et al. 2010;BirdLife International 2012).

Females lay, on average, 2 to 3 eggs per year. Their incubation is done by both parents alternately and last 23 to 25 days in *C.ridibundus* to 24 to 27 days, in for instance *Larus fuscus,* and 28 to 30 days in species like *L.canus* (Catry et al. 2010;BirdLife International 2012).The hatchlings, precocious (Schreiber & Burger 2001), after hatching are kept under the care of both progenitors that alternately bring food every 3 to 4 hours,which is fed by regurgitation of what was captured/found (Ap.1.2 fig. 55). The young are capable of flying on the 21-24th day, *L.minutus,* (del Hoyo, Elliott and Sargatal 1996

cited by ARKive 2012), 30-45th day (for most species) or on the 45-50th in *L.hyperboreus* (Avibirds 2012).

2.1.6. Food, another source to consider.

Again the great versatility and adaptation, abilities manifested by these beings, reflects positively but also, and unfortunately in certain cases, negatively in their survival when the binominal animal-food is considered. Since they can inhabit a vast selection of ecosystems, some entirely distinct, the scope of food items provided by each is also very vast and diverse and encompasses several trophic levels. There are variations in these items that can be attributable to climatic changes and their availability may also be affected by the competition established between these wild birds and human activities like fishing (Schreiber & Burger 2001). The way by which these birds can obtain such items is also something to consider, since it can be responsible for the exposure of these avian species to numerous potentially pathogenic agents or toxics.

Seagulls tend to aggregate in locals of high abundance of nutritional resources in close proximity to where whales, dolphins and predatory fish forage and following fishing vessels (Camphuysen, Van Dik, Witte & Spaans 2008). But, they can also gather in more humanized places, sometimes accompanying for example some agricultural practices like *L.atricilla* or *C.ridibundus* (Ap.1.2 fig.60). In these settings they aggregate in flocks more or less dimensioned, where other avian species may be seen (Schreiber & Burger 2001).

When in the presence of prey (or other type of food) seagulls can: simply catch it by walking along the shoreline; pick it up in flight or fly over the water surface and collect it; swim and submerging their neck and head capture unsuspecting prey; and they can also dive from the surface or plunge-dive to get the less superficial food items. In certain occasions gulls drop some of the most problematic items on stones to break/stun or kill them, which is typically seen in *L.argentatus* and *L.marinus* (Catry et al. 2010;ARKive 2012). Cleptoparasitism (Ap.1.2 fig.33), is another option and is common in every single one of the species here discussed. Nonetheless, it can also be the preponderant manner by which food is acquired in some, as is possibly the case of *L.michahellis* and *L.atricilla* (BirdLife International 2012). Another technique used, primarily described in *L.audouinii*, is night foraging in order to reduce cleptoparasitic and agonistic behaviours of other gull species, particularly in regions of strong fishing activities (Arcos, Oro & Sol 2001;Catry et al. 2010). Similar nocturnal behaviour may be present in wintering *L.melanocephalus* in Portugal (Poot 2003).

Thus, larids can feed on several species of: fish (e.g.*Salmo* spp., *Sprattus* spp. and *Clupea* spp. are the choices of *L.glaucoides*), terrestrial or marine/aquatic invertebrates (krill, crustaceans, molluscs, worms and insects), vegetal matter (tubers, seeds and berries), small mammals (rodents, lagomorphs – Ap.1.2. fig. 67 and even ill cubs of small ungulates as is seen in *L.marinus*), reptiles, eggs (e.g.*X. sabini* eats *Sterna paradisae* eggs) and birds both young and adults (Ap.1.2 fig.45) (ARKive 2012;BirdLife International 2012). Urban waste in cities, garbage dumps, landfills, outfall sewage zones and fishing wharves are also subject to attention by these birds, like *L.delawarensis*, *L.fuscus* and chiefly *L.michahellis* (Catry et al. 2010). Furthermore, the ability to consume decaying organic matter like fish, frequently in individuals of *L.argentatus*, *L.marinus* and *L.fuscus* is another alternative for survival (Catry et al. 2010;BirdLife International 2012) and could be another cause for disease.

Diet does not remain so diversified all the time, there are alterations in its composition associated with, evidently, the availability of these resources, but in addition habitat changes during and after migration and, as BirdLife International (2012) indicates for some species, preferences and the reproductive season. Moreover, different habitats are not explored in the same way by distinct species as are examples the species *L.fuscus* and *L.argentatus* in Holland in the study of Camphuysen et al. (2008). In this study they found that in Kelderhuispolder, in Texel, the more pelagic species *L.fuscus* tended to extent its foraging distant from the coast for more than 5 kilometres than the latter, in spite of both being dependent on fishing boats in the limits recognized.

Human activities like fishing or waste management (Ap.1.2. fig.63) can influence the feeding and foods of these animals and also affect, possibly, the reproductive output of a colony. When present, they are a regular and abundant sources of food and when they disappear it affects the stability of a population in the breeding season (Kilpi & Ost 1998).

Knowing that gulls are animals that take advantage of everything that they may find (are opportunistic feeders and some are scavengers), what is seen in certain colonies, and perhaps flocks in the winter is that it appears to exist some kind of specialization in terms of type of food searched for and technique used to obtain it, at least in what concerns *L.argentatus* (Avibirds 2012). In this manner, in the same colony there are: those who utilize regularly landfills or refuse tips (when these are in close proximity); the ones who hunt and among these, the more efficient (that do not disperse greatly form the original site) and the ones who cover more meters or kilometres; and lastly the specimens that steal food from others, normally and in accordance with Schreiber and Burger (2001) these birds are the youngest and usually are less competent in foraging by other means.

2.1.7. Interaction, a step towards disease.

Larids, like most marine birds are tendentiously gregarious and colonial, as was stated previously, because evolutionarily the advantages granted in these formations, particularly the colonial (protection against predation, socialization, and possibly synchronized reproduction) greatly outweigh the cons of death due to overpopulation, competition for sites/partners/food, scarcity of food or even reproductive failure (Schreiber & Burger 2001). In this way the closest proximities between individuals are found: in the colonial nesting sites, with densities of about one nest per square metre in *L.audouinii* in monospecif colonies ranging from 10 up to 10.000 pairs; in roosting moments in wintering areas (Ap.1.2 fig.52); in foraging flocks or in certain cases during migration as is reported in *C.ridibundus, L.canus* and *L.argentatus* (Schreiber & Burger 2001;Catry et al. 2010;BirdLife International 2012).

The number of such examples is very high, but in spite of the all the pros, according to Schreiber and Burger (2001) this great proximity facilitates the transfer of microorganisms and parasites between specimens. The exploitation of the same sites by birds, year after year, also allows the accumulation of such agents and subsequent infection or intoxication of the new generations or individuals in the upcoming years. The high densities seen in certain sites is another potential factor for disease since a high number of individuals are exposed to a specific potential pathogenic infectious or toxic agent, when they gather in large numbers in certain regions like outfall sewages zones, as is seen in *C.ridibundus* and *L.fuscus*, whose flocks are frequently found in these locations (Catry et al. 2010).

Chapter 2: Paresis and Paralysis in Avian species.

Paresis and Paralysis are two concepts related with the neuromuscular system and, although distinct, in certain cases they are misinterpreted as equals or may overlap and create serious difficulties on the correct identification, subsequent description and accurate diagnosis of disease with the appropriate ancillary tests. This last one is certainly of great importance because it dictates the therapy instituted and the prognosis. When considering the rehabilitation of wild birds its significance far exceeds what it is common in other birds, because it is a key point that restrains the efforts to rehabilitate the bird or not and its future release into the wild (when considering, for instance, complete paralysis without recuperation).

Paralysis according to Boden (2005) is a symptom and

> (…) in its widest sense, may mean loss of nerve control over any of the bodily functions, loss of sensation, and loss of the special senses, but the term is usually restricted to mean loss of muscular action due to interference with the nervous system (pages 511-512);

whereas **Paresis** is a

> (…) slight or incomplete paralysis, and includes animals that can make purposeful attempts to rise without being able to do so, those that are able to rise with assistance, those that are able to rise and walk with major difficulty including frequent falling, and those able to stand and walk without assistance, but with slight errors (Blood & Studdert 1999, pp.843).

These two definitions, although old and not specific for birds, are still useful and cited in numerous text books like the *Avian Medicine* of Samour (2000), chapter of Bailey. They are clear in establishing the differences between these two terms, indicating that paresis is like a "fleeting paralysis", with irregular muscular function, while in paralysis the limbs are completely flaccid, there isn't any kinds of resistance when passive movements are attempted though the pain sensation can be elicit (Boden 2005). A variety associated with the wings can also be present (White 2011). But even so, it appears to be easy to differentiate, certainly when observing wild animals in captivity, the new environment, the contact with humans (in spite of its briefness) may possibly mask paresis for paralysis or vice-versa. So the two terms in this dissertation are used together not in a sense of equality of meaning, but more like a continuum observed in the progression of several illnesses.

There is a multitude of conditions that can affect birds (in its most general term) and are documented as being the causative agent of these particular unspecific signs. Some of them are included in table 1, for all accounts this is not the full list of diseases regarding these signs, such task would be impossible to accomplish given the multitude of species studied, the discovery of new diseases like the recent vacuolar myelinopathy and probably the lack of studies or reports of cases concerning certain afflictions in selected species and of course the difficulties associated with the distinction between paresis/paralysis.

These diseases are divided by type of pathological agent/genesis and so there are nine categories: trauma, infectious (for easy organization parasites were included in this one), vascular, toxic, metabolic/nutritional, reproductive, neoplasic, congenital and unclassified (all that could not be place in the others, because they are specific situations like misplacing rings, or they are diseases that are new scientific discoveries and/or they are not fully known yet, or concerning the way these signs develop cannot be placed in the others).

Table 1 – Differentials for Paresis and Paralysis in Avian Species (Wild, Ornamental and Domestic). Adapted from: Ritchie, Harrison & Harrison (1994);Jones & Orosz (1996);Friend & Franson (1999);Bailey (2000);Tully-Jr, Dorrestein & Jones (2000);Samuel, Pybus & Kocan (2001);Harcourt-Brown (2002);Stocker (2005);Harrison & Lightfoot (2006);Pollock (2006);Schmidt (2006);Coles (2007);Thomas, Hunter & Atkinson (2007);Fowler (2008);Fowler & Miller (2008);Mitchell &. Tully-Jr. (2009);White (2011) and Miller & Fowler (2012).

Categories	Possible cause
Trauma	Cranial concussion, Vertebral fracture/luxation, Multiple fractures, Pelvic or sinsacrum fracture, Joint sub/luxation, Ligament luxation, Nerve avulsion in old fractures, Peripheral traumatic event with direct or indirect lesion of the Nervous system, Predation and Electric shock.
Infectious	**Viral:** Alphavirus (Eastern equine encephalitis virus and Western equine encephalitis virus), Birnavirus (Transmissable viral proventriculitis), Bornavirus (Borna disease paralytic syndrome and Proventricular dilation disease), Flavivirus (Tick-borne encephalitis group virus, West Nile virus, Turkey meningoencephalitis virus, Tembusu virus, and Bagaza virus), Herpesvirus (Inclusion body disease, Pigeon herpes encephalomeningitis, Duck enteritis, Duck hepatitis, Pacheco's disease and Marek's disease), Paramyxovirus (Newcastle disease), Parvovirus (Muscovy duck), Picornavirus (Avian encephalomyelitis), Polyomavirus (Hemorrhagic nephritis and enteritis of geese and Budgerigar Fledgling disease),Poxvirus, Reovirus (Pancreatitis and Peking duck reovirus) and Retrovirus (Reticuloendotheliosis); **Bacterial:** *Listeria* sp.; *Mycoplasma* sp., Spirochetosis, *Enterococcus* sp.; *Staphylococcus* sp.; *Mycobacterium avium*; *Yersinia pseudotuberculosis*; Clamydophilosis; *Erysipelothrix rhusiopathiae*; *Pseudomonas* s*p.; Salmonella* sp.; *Echirichia. coli; Streptococcus* sp.; *Pasteurella* sp.and *Klebsiella* sp.; **Parasitic:** Ascaridiosis, Giardiosis, Coccidiosis (*Caryospora* sp., *Eimeria* sp. and *Isospora* sp.), Sarcosporidiosis, Baylisascariosis, Atoxoplasmosis, Toxoplasmosis, Leucocytozoonosis, Encephalitozoonosis, Nematodiasis cerebrospinal/verminous encephalitis/central nervous system larval migration, Visceral larval migration and Crysptosporidiosis; **Fungal:** *Aspergillus* sp. and *Criptococcus neoformans;* **Granulomatosis disease** (chronic infection by fungi or bacteria).
Vascular	Atherosclerosis, Immune-mediated haemolytic anaemia, Intravascular cartilaginous emboli in the spinal cord and Isquemic myelopathy
Toxic	**Natural:**Botulism, Fumonisin, Aflatoxicosis, Algal toxicosis, Tick-paralysing toxin, Salt toxicosis and Snake bite (toxin); **Drugs:** Dihydrostreptomycin, Levamisole, Procain penicillin, Cholinesterase inhibitors, Furazolidone, Ionophores; **Heavy metals**: Lead, Zinc, Mercury, Arsenic; **Pesticides:** Organophosphorus or Carbamates pesticides; **Others:**Carbon monoxide toxicity.
Metabolic/ Nutritional	**Deficiency**: Vit. A, B_1, B_2, B_6, D_3, E, Selenium, Chlorine, Zinc, Calcium, Phosphorus or combinations; **Excess:** Protein and Calcium; Fatty liver and Kidney syndrome and Hepatic lipidosis in turkey (FLKS); Neuronal ceroid lipofuscinosis; Osteoporosis, Articular/visceral gout; Metabolic Bone disease and pathological fractures; Other Neuronal storage diseases.
Reproductive	Egg-biding/dystocia/retained egg, Calcium tetany, Ectopic egg.
Neoplasic	**Nervous system neoplasias**: cranial and intracranial; **Gonadal, adrenal and kidney neoplasias**: Renal and Ovary adenocarcinoma; Granulosa tumors, Fibrossarcoma, Teratoma, Embryonic nephroma; **Space-occupying lesions**: Hematoma, Multiforme Glioblastoma, Coroid plexus neoplasia, Schwannoma, Astrocytoma, Pineal neoplasia, Sarcoma and Hemaphioma; **Paraneoplasic syndrome**: Exostosis and Polyostotic hyperostosis;
Congenital/ malformation	Cerebellar hypoplasia, Hydrocephalus, Spondylopathy, Kyphosis, Lordosis, Scoliosis and other malformations/deformations.
Unclassified	Incorrect ring placement; Abcess/celulitis/granuloma post vaccine in commercial birds; Capture miopathy; Intestinal disorders (foreign bodies, tumors, strictures, intussusceptions, volvulus, incarceration of intestinal loops, pseudoligaments, adhesions, severe enteritis and local peritonitis, embolism/thrombosis of splanchnic vessel, intoxication, cloacaliths); Impactation/trauma/perfuration by proventricular and ventricular foreign body; Uroliths; Amyloidosis; Progressive Tetraparesic Syndrome of Lorikeets; Vacuolar Myelinopathy.

In accordance with the authors cited when adapting the present table, some diseases result in alterations directly and mainly in the nervous system (ex: Brain neoplasia or Tick paralysis), others affect this system and others, like for an example septicaemia by *Listeria* sp. Localized lesions,

infections, neoformations or even abnormal physiological responses can also contribute to paresis/paralysis by direct/indirect local effects in parts of the neuromuscular system such as: Joint luxation (wing/leg); *Criptosporidium* spp./*Eimeria* sp./*Isospora* sp. (leg), Bumblefoot (leg) and Aspergillosis (wing/leg); Adrenal carcinoma (leg), and Egg-biding (leg), respectively. In certain cases the affection is particularly described in older birds (Mycobacteriosis) and in some circumstances what was due to a disease it's complicated by another pathogen like an exposed Fracture and Osteomyelitis, or according to Harcourt-Brown (2002) the obvious lesion may not be the only problem. These are just a handful number of examples to substantiate the fact that the number of differentials is vast and furthermore the way they commence these signs is limitless. Thus, for the purpose of this dissertation, study of paresis in wild birds in particular larids in the south of Portugal, one can see that some of the diseases presented can be excluded just by taking into consideration the species in question, free-living status, number of animals afflicted (including almost all ages and the two genders) and their life cycle, particularly in the southern region of Portugal, Algarve. In this way of thinking differentials like, Progressive Tetraparetic Syndrome of Lorikeets (together with viral and bacterial ones described only in a precise limited number of species - frequently domestic and ornamental), intoxication with pharmaceutical drugs like Penicilline, and granuloma post – vaccination are the least probable to cause paresis in these larids. Causes like neoplasia, reproductive anomalies, traumatic events cannot fully justify the high number of animals that enter a rehabilitation centre with paresis and are in default of signs that are consistent with them. And so given the singularity of the current study, the author decided to explore every category and only what was thought to be the most reasonable explanation, taking into account the species, the data recorded in RIAS and the geographic, socio-economic, climatically distinctiveness that is Algarve.

2.2.1. Newcastle Disease.

Newcastle disease (ND) is an infectious disease caused by a virus (NDV) known as Avian Paramyxovirus Serotype 1 (APMV-1), belonging to the family *Paramyxoviridae* and the genus *Avulavirus* (World Organization of Animal Health [OIE] 2009). The ND virus is highly contagious, it possesses a variable pathogenic potential and is extremely resilient in the environment, especially when protected by organic matter such as faeces, water, soil, carcass, eggs and feathers (Leighton & Heckert 2007;OIE, 2009). This recent disease is of utmost significance in poultry industry and its deleterious effects in this group of birds are a crucial cause of drastic production and economic losses, and so it is one of the most critical afflictions in the whole world (Docherty & Friend 1999;Leighton & Heckert 2007). And given that is found in the whole world, with a greater expression in some regions of Africa, Asia, Central and South America (principal reservoirs of virulent strains in commercial poultry, Leighton & Heckert 2007) and despite its control in Canada, USA and in several countries of the western part of Europe, outbreaks of Newcastle disease can develop in any country of any part of the world because wild birds are possible carriers of this virus (OIE, 2009). Additionally, wild birds can also suffer from this disease and some of its presentations encompass neurological signs such as paresis and paralysis, and consequently taking into consideration the above, this viral disease is one of the probable causes for this paretic syndrome and of paramount importance in view of the relationship between wild birds and domestic ones, and the consequent effects in both groups.

2.2.1.1. Epidemiology and Pathogenesis.

ND affects numerous avian species, in accordance with Docherty and Friend (1999) all the variety of poultry species are included as well as over 230 species of more than half of the 50 orders of birds (Leighton & Heckert 2007). Both males and females of any age are susceptible to the infection by the NDV, but the clinical presentation appears to be more severe in younger individuals in both commercial and wild birds - probably owing to the under developed immune system (Leighton & Heckert 2007).

The principal routes for infection implicate the direct contact with virulent material in respiratory secretions, faeces or even carcasses (viscera and meat) normally by: ingestion, inhalation or fomites. In a few non-virulent strains vertical transmission may occur (Docherty & Friend 1999;Leighton & Heckert 2007;OIE, 2009).

The incubation period varies greatly between different avian species. The periods of viral elimination, persistence of the virus in the organism and antibody persistence in serum are also extremely variable (Falcon 2004;Leighton & Heckert 2007;OIE, 2009). Moreover it is unknown, in several species, if they are susceptible to re-infection or how long the viral excretion will last in both infected and re-infected animals (Leighton & Heckert 2007).

Several clinical presentation of ND are documented, usually classified by their pathogenesis and post-mortem findings in gallinaceous, notwithstanding one has to understand that the signs reported don't always have a linear association with each determined category. In spite of this, the following variants are reported: viscerotropic velogenic (highly virulent, causes acute and lethal infection with mortalities reaching the 100%, in which the most evident findings are hemorrhagic/difteric lesions on the digestive tract); neurotropic velogenic (also highly virulent, causing acute and lethal infections and is responsible for neurologic and respiratory signs, the morbility can reach the 100%, but the mortality depends on the age of the bird, being 90% in the youngest and 50% in adults, these last ones display productive losses in the course of the diseases); mesogenic (provokes neurologic signs, but only the young usually die, exhibiting the adults persistent and rapid decreases in the productivity) and lentogenic that originates, in the majority of cases, mild respiratory signs with a higher prevalence in younger birds, but it can also initiate asymptomatic or enteric presentations (Docherty & Friend 1999;OIE, 2009). These presentations, especially the most severe, according to Munir, Sharma and Kapur (2005) with microarray technology, are due to the suppression of genes of the interferon response and among others the down-regulation of genes potentially mediators of apoptosis. Thus, when the virus is inside the organism, it suffers two replication phases, one in the epithelium mainly in the respiratory tract, and the second after viremia in other organs (this last one in the most virulent cases), resulting in several observable signs and lesions. Death normally is due to intravasal coagulopathy and organ dysfunction (Leighton & Heckert 2007;Kalefa 2012). Nevertheless, when considering wild birds it is necessary to bear in mind that the previous categories in the pathology classification of the various strains and on the word of Docherty & Friend (1999) are not transposable to wildlife, since presentations of high pathogenicity may not have a correspondence in poultry and vice-versa.

Considering the distribution of the viruses that inflict these presentations, it is acknowledged that the lentogenic and mesogenic are disperse in the world, while the ones of high virulence apparently experienced a differential dispersion in the globe from 3 panzooties: in 1940 in the south-eastern Asia

and in 1960 and 1970 in the Middle East (linked with poultry and recreational/ornamental species and their products), and so are endemic in America, Asia, Middle East and Africa (Docherty & Friend 1999).

With regards to wildlife, it has already been mentioned its potential involvement in the maintenance of the virus in circulation, generally lentogenic variants of several genotypes in individuals of the same species and in the same locations with an apparent annual cyclic expression in circulation (Kim et al. 2007;OIE 2009). In accordance with Leighton and Heckert (2007), it is also valid their involvement in the manifestation of ND, with the possible occurrence of intraspecific and interspecific pathogenic strain reservoirs in cormorants and rock pigeons species. These groups of birds can also be the source of die-offs and outbreaks of disease owing to the conversion of low virulence strains to high virulence strains that prompt disease in commercial birds. In this aspect and despite the recent believe that the pathogenicty variation of the NDV depends on several genes (Wakamatsu, King, Seal, Kamal & Brown 2006), according to Miller et al. (2009) a mutation of 2 nucleotids in the cleavage region on the end of the fusion protein is sufficient to cause an emergence of a virulent form of NDV. Although such events are exceptionally rare (the site, in question, is highly conserved, and the negative selection mechanisms as well as the low mutation rate preclude this from happening), this phenomena already took place as are examples what Miller et al. (2009) and Kim et al. (2007) state citing Alexander et al. (1992) and Gould, Kattenbelt, Selleck, Hansson, la-Porta and Westbury (2001): in the Republic of Ireland in 1990 from a wild coastal reservoir and between 1998-2000 in Australia by the circulation of a low virulence strain in poultry commercial practices.

As previously noted, ND has already been identified in wild populations, probably owing to 3 processes in accordance with Leighton and Heckert (2007): "overspill" from poultry industry; the variant of NDV of pigeons and migration. However studies and reports of mortality and disease in these birds are rare (particularly in Europe, where only identification of the virus in carcasses was frequently accomplished, Leighton & Heckert 2007) and old, as is the case of an epizootie in *Phalacrocorax auritus*, *Pelecanus erythrorhynchos* and *Larus spp.* in Canada (Alberta, Saskatchewan and Manitoba) in 1990 (Wobeser et al. 1993) and the epidemic in the same species of cormorant also in Saskatchewan in the year of 1995 (Kuiken et al. 1998) (another epizooty of great relevance and in spite of initially affecting racing pigeons in the 80's in Middle East, Africa and Europe, disseminated to the North America, Japan and all over the world and clearly feral and wild species of birds were affected as is stated by Leighton & Heckert 2007). These descriptions are normally based upon estimations and strongly depend on local claims, or are unexpected findings encountered deriving from other studies in progress in specific locations – e.g. studies of breeding colonies during which they were the focal point of disease (Kuiken et al. 1998). Thus, wildlife ND events, although infrequently documented, are possibly the 4th panzooty for the dispersion of highly virulent strains (Docherty & Friend 1999). The outbreaks mentioned arose in the wild populations and apparently didn't extend to poultry, but certainly there was exposure in the latter, this possibility was suggested by Kim et al. (2007) via the detection of low virulence virus in cloacal samples, phylogenetically close between market birds and coastal/aquatic avian species in the USA from 1986 to 2005. It is thought that the origin of the virus isolated in cormorants and a gull in 1990 was in the panzooty of 1970-74 in poultry birds (viscerotropic velogenic – they had similar monoclonal antibody profiles) and it could have reached the North of the country by means of the natural migration patterns of these species that

winter in the South of America or perchance it can be enzootic in these birds, hypothesis that weren't ruled out in 1998 (Wobeser et al. 1993;Kuiken et al. 1998).

Considering only the deleterious effects of APMV-1 in wild birds, the first report of ND in wild birds dates from 1897 in *Phalacrocorax carbo* and *P. aristotelis*, in Scotland (Leighton & Heckert 2007), from 1975 to the present days several outbreaks with severe consequences in wild birds took place mainly affecting *P. auritus*, *Columba livia*, psittacines and other tropical birds involved in commerce (Leighton & Heckert 2007). The virus could also have been the cause of reproductive problems in 1997 in cormorants in Californian colonies where 2000 died. In the epizootic of 1992 in North Dakota in the summer, a domestic turkey flock in close proximity to a colony of wild cormorants was infected and had mortality associated to ND. But the inverse might also have happened in *P.auritis*, *P. aristotelis* and *Morus bassanus* in respect to the die-offs of 1949-1951 in poultry (Docherty & Friend 1999).

2.2.1.2. Signs and lesions.

The described outbreaks indicate that they took place in breeding colonies with mortalities between March and September, but with a higher expression in the summer (Wobeser et al. 1993;Kuiken et al. 1998;Docherty & Friend 1999). The signs were more exuberant in cormorants, mainly juveniles, and included: sudden death, weakness/prostration (sometimes the only sign), dirtied and damaged plumage, vent feathers dirtied with green-grey excreta, dyspnoea, ophthalmic signs (Kuiken et al. 1999 described aqueous exudates, friable yellow whitish plaques in the conjunctival sacs, reddened and opaque cornea/sclera/third eye-lid, bilateral pupil dilation and blindness) and neurologic findings. This last group of signals can be considered as the most representative of the ND set of signs and lesions, and in these reports the birds displayed: abnormal behaviour; disorientation; loss of fear or notion of predator or human presences; ataxia (associated with circling and goose stepping); variable degrees of difficulty or inability to fly, swim or dive; several grades of paresis/paralysis of one limb or combinations of the four with signs of compensation and its consequences (extension/flexion of the wing, lameness, wing slapping, leaning of a number of body parts against the floor for support, sternal recumbency, abduction of the affected limb or its leaning in the floor by its most proximal articulations and clenching feet), inability to maintain the neck erect (torticollis or twisting), tail deviation from the medial line (Glaser et al. 1999) and head tremors associated with an inability to beak and thus in general compromising the apprehension of food (Wobeser et al. 1993; Kuiken et al. 1998;Glaser et al. 1999). In the study of Glaser et al. (1999) of several cases of mortality by NDV in several states of the USA and provinces of Canada, in which the clinical descriptions are by far more exhaustive for the various affected species (*P. auritus*, *P. erythrorhynchos*, *Larus* sp. and *Nycticorax nycticorax*) contrary to the works above cited and available that could only focus their attention on cormorants, afflicted gulls only displayed paresis/paralysis (unilateral to bilateral of the wings and/or legs) and probably because they were in the proximity of a cormorant colony, 3.100 birds died.

In the case described by Kuiken et al. (1998) the peak of the observations regarding the clinical signs exhibited by the juvenile cormorants happened at the 2nd week and the peak of deaths 10 days later, but the first manifestations of the clinical signs described could have developed within the 2 days of infection as is indicated in experimental inoculation of mallard ducks. But counterpoising the variability

of the infectious parameters in poultry and ornamental birds to wild ones the information available is still limited regarding, for instance, the minimal infectious dose of ND for the generality of wild birds.

There aren't macroscopic pathognomonic lesions of ND (OIE, 2009).The infection by the virus can cause lesions in one or more than four organs or systems: central nervous system, kidneys, gastro-intestinal tract and respiratory apparatus, in spite of the fact that in wildlife the common cases are the ones in which the central nervous system is affected (additionally the kidneys) or situations whereupon the infection generalizes and causes abrupt death together with very few signs or lesions (Leighton & Heckert 2007). Results of the necropsy evaluation, in the described outbreaks alluded to: low body score, almost or complete lack of adipose tissue, absence of ingesta in the gastro-intestinal tract (thick black material possibly digested blood in the stomach and intestine of many was a frequent finding), high parasitic burdens (*Contracaecum* spp*.* in the ventriculus and various species of lice). Less frequent findings included hydropericardium and good body condition. Regarding the alterations seen with light microscopy, there was a high frequency of findings in the central nervous system: non-supurative encephalitis and/or myelitis, neuronal necrosis, spongiform alterations, lymphoplasmocitic vasculitis, mild and focal perivascular lesions and gliosis of the grey matter particularly in the cerebellar folia with loss of Purkinje cells - in this same area vacuolation and white matter separation was found) (Wobeser et al. 1993;Kuiken et al. 1998). Axonal degeneration, endothelial hypertrophy and mononuclear perivascular infiltrations were also noted in the brachial and lumbosacral plexus by Kuiken et al. (1999). Nevertheless, mononuclear perivascular infiltrates and endothelial hypertrophy could be the only findings in this organic system (Kuiken et al. 1999). Additionally in some cases lymphocitic necrosis with depletion of the cellular populations of the thymus, bursa of Fabricius and spleen were also reported by these authors. Small foci of lymphocitic inflammation and even mononuclear were found in one or more organs (pancreas, liver, kidneys…) of the described cases, usually accompanied by non specific alterations, like hepatomegaly, swollen mottled spleen that could belong to the clinical picture of ND, or could be a result of concurrent diseases, or even due to the presence of other pathogens like was said by Kuiken et al. (1998/1999) in relation to *Salmonella* sp., *Plesiomonas shigelloides* and *Pasteurela multocida*.

2.2.2. Salmonellosis.

Taking into consideration the same category of infectious diseases, but this time the bacterial ones that are capable of causing neuromuscular signs, particularly paresis and paralysis, one of the most important and was previously pointed out is Salmonellosis. The inclusion of this affliction in the present differential list is related to the fact that, despite being practically fully characterized in scientific terms and being frequently associated with disease and colossal economic losses in animal production and human health, is, in this day and age, an emergent disease in which seagull species perform a significant role and likewise constitutes a valid possible cause to paresis.

The pathogen responsible for this disease is a gram-negative non spore forming bacteria of the genus *Salmonella*, capable of affecting both vertebrates of warm and cold blood and originating severe clinical presentations of disease (Kapperud & Rosef 1983). Despite all the changes in the classification of this kind of pathogenic agent, the disease is caused by *Salmonella* bacteria, and the identification of at least 2300 serotypes is possible (of two species *S. enterica* and *S. bingori*). Each subtype can, in turn, comprise several biotypes (Tizard 2004) turning the dynamics of this pathogen

life cycle one of the most complex, given also the subsequent consequences of infection that will be discussed next.

The presence and importance of this pathogen in the wild populations, with emphasis in birds, is the prime reason for its new status of Emergent Disease (Tizard 2004). According to the same author this emergence is justified by the increase in its prevalence in the wild bird populations owing to: the pathogen ability to be part of the intestinal flora of the host; persistence in the same by environmental contamination, without causing disease – the bird functions as a transmissible vector and maintenance host to other vertebrate species including farm animals and humans (from among various cases reported, the case of Coulson, Butterfield and Thomas (1983) is an example in which by possible circumstantial evidences larids of the species *L. argentatus* were implicated in the transmission of *S. montevideo* to bovine and ovine stocks in Scotland and the outbreaks of salmonellosis in the same regions between the years 1975 to 1982); or existing in the microbiota, and in particular cases, like for instance immune system deficiency, is responsible for illness, causing die-offs of high mortality (Tizard 2004). In addition and due to the ability of certain wild species for phenomena like dispersion and migration (mostly in birds), the probability for disease dissemination to several different regions is increased and overall this fact is another reason for the reemergence of this affliction (Kapperud & Rosef 1983).

2.2.2.1. Epidemiology and Pathogenesis.

In wild birds, the isolation and identification of numerous subtypes of *Salmonella* is frequently reported in studies conducted on outbreaks of other diseases (e.g. studies cited in the other sub-chapters), and so repeatedly this group of birds are only considered as asymptomatic vectors in the transmission of the disease, which is frequently the case of gulls. However and although rarely documented, outbreaks of diseases do occur in this group of birds including seagulls, such occurrence is the case of Quebec described in 1992-1997 affecting larids, possibly 38 *L.delwarensis* and one *R. tridactyla* that entered the Quebec branch of the Canadian Cooperative Wildlife Health Center (Mikaelian, Daaignault, Duval & Martineau 1997). But then again, the scarcity of reports and the few number of birds afflicted were insufficient in the past to draw attention to this problem (Friend 1999c;Daoust & Prescott 2007).

Whether or not the disease is present, the preferential transmission pathway in birds is the feacal-oral route although in selected individuals predation is also important (Gaffuri & Holmes 2012). Literák, Cizek and Honza (1992) indicated that gull eggs collected during their study were negative to the presence of *Salmonella* sp., which could indicate that vertical transmission does not occur, but still remains the doubt concerning this route of transmission, since vertical transmission happens in other birds, and in this study it was not proved that the parents of those eggs were negative as well. From these assumptions one can tell that the most affected and more documented species are carnivorous/piscivorous (Kirkpatrick 1986) or omnivorous, that feed in the soil or on easily contaminated pavements with faecal matter, or that live or pass most of their life cycle in contaminated aquatic environments or forage for food on them as is described by Literák et al. (1992). These authors indicate that in a colony of *C.ridibundus,* juveniles and chicks, were positive for the presence of *Salmonella* bacteria (36,84% n=171 juveniles and in 20,83%, n=96 young gulls, respectively) with origin in the contamination by sewage effluents of the aquatic reservoir of the Nové Mlýny Water

System in the Czech Republic in 1991. Furthermore, ecosystems in the proximity of animal farms, slaughterhouses, manufacturing industrial units and regions where sub-products of animal origin and sewage residues are applied as fertilizers are also areas of risk (Friend 1999c).

In view of the above all these aspects are common to the majority of gull species and so the acquisition of these pathogens, essentially, takes place in sites of abundance of organic residues like landfills, dumps, sewage treatment plants and regions of strong sewage outfall and influence. Fact that is confirmed by several papers published that show higher frequencies of isolation of more than a few serotypes in these birds in these locations (Fenlon 1981;Fenlon 1983;Quessy & Messier 1992), although Ramos, Cerdá-Cuèllar, Ramírez, Jover and Ruiz (2010) defend that a study that combines and tests the association of dietary analysis and carriage determination to establish a correct association is still missing.

Some of the scientific studies conducted to investigate the presence of this pathogen in gulls are European and the only available regarding Portugal is the one of Duarte et al. (2002). Duarte et al. (2002) reached the same conclusion previously presented, there was a higher frequency of positive isolation and less diversity of serotypes in beaches with strong human influence (34,8% n=69 Santo Amaro and only 6,3%, n= 95, in Fonte da Telha). In this Portuguese study from the 285 faecal samples collected, 37 were positive for this pathogen; in 37,8%, *S. tiphymurium* was identified, in 18,9% *S. derby* and among others with variable frequencies *S.enteriditis* was found in 10,8%, which correlates with what serotype of *Salmonella* is frequently identified in other studies, i.e. *S. typhimurium* (Gridwood, Fricker, Munro, Shedden & Monaghan 1985;Tizard 2004;Palmgren et al. 2006;Cívek, Dolejská, Karpískova, Dedicová & Literák 2007;Hall & Saito 2008). Another conclusion reached in these isolations and identifications in larids is that in the same sample it is possible to differentiate two or more serotypes (Quessy & Messier 1992;Duarte et al. 2002), as is the example of Brand, Windingstad, Siegfried, Duncan and Cook (1988) who found *S. tiphymurium* and *S. Heidelberg* and *S. thiphymurium* with *S.manhattan* in a study in the Jamaica Bay in New York in gulls on the summers of 1981 and 1982. Nonetheless the serovar *S. typhimurium* is still the most identified and is additionally pointed out as the most important cause of salmonellosis and outbreaks of deaths in the greater part of the whole group pf wild birds species (Daoust & Prescott 2007;Gaffuri & Holmes 2012).

The majority of these published research, which focus on the association of salmonella and seagulls, are always projects with the objective of determining environmental contamination markers, with special prominence in the public health component and so, in most of them, the clinical evaluation of the individual is always a subjective observation of the general condition, whereupon it is indicated, continuously, an absence of disease cues, as are the cases of the study of Palmgren al (2006). These findings apparently reinforce the notion that these birds are passive vectors and carry quantities that probably do not produce disease or they are subclinically infected without harm for their health (Kapperud & Rosef 1983;Butterfield et al. 1983;Quessy & Messier 1992;Palmgren et al. 2006). Another problem found when considering this relationship, results of the identification of the same subtypes isolated in larids and their counterparts in commercial animals (Coulson et al. 1983;Gridwood et al. 1985;Palmgren et al. 2006;Cívek et al. 2007), or human beings for the same period of time and region (Butterfield et al. 1983;Fenlon 1983;Gridwood et al. 1985;Ferns & Mudge 2000;Palmgren et al. 2006;Cívek et al. 2007); and cases where it is proved that the transport of serotypes to other sites occurs, and it is due to the former migration patterns (Coulson et al.

1983;Palmgren et al. 2006). These are co-substantiated by the presence of strains in wild populations with signs of antimicrobial resistance to several active principles, giving an extra significance to the possibility that gulls reflect the contamination of the ecosystem where they dwell, but also is a proof that there is an interaction between commercial/ornamental animals, humans and seagulls and the origin of the resistance is in the formers (Hilbert, Smulders, Chopra-Dwasthaly & Paulsen 2012), which in turn, gives rise to important health issues and public debates and certainly conditions the direction of the scientific inquiries and the public perception of the importance of these birds.

From the information gathered the pathogen apparently is not endemic in gulls (Kapperud & Rosef 1983), since a study conducted by Gridwood et al. (1985) revealed that the faecal elimination of the bacteria by isolation-positive *L. argentatus*, captured and kept in controlled conditions in an experimental design, is of 2 to 4 days and the maximum value obtained by isolation in positive samples was of 170 organism/grams with a mean of 22 (similar observation was found by Fenlon 1981 on the field).This is supported by the findings of Palmgren et al. (2006) in the case of the recapture of previous positive birds that were in the second analysis negative, and this corroborates with the opinion that these bacteria are not part of the normal enteric flora of gulls: the carriage is of short duration and the infection level is low. However, and in spite of the meaning of this value, the gregarious nature of these birds, typically seen when feeding and roosting in pastures, is appropriate for the accumulation and concentration of *Salmonella* in the soil and water (Coulson et al. 1983;Gridwood et al. 1985) and even in areas of recreational activities (Lévesque et al. 1993) due to the new colonization of more humanized habitats. Subtypes can even persist in the nesting sites for the next season or sometimes over years (study of Cívek et al. 2007 citing Literák, Čížek, and Smola 1996), besides the possibility for bioaccumulation in more simpler trophic levels that further aids in this aspect (Monfort, Piclet & Plusquellec 2000).

The carriage rates determined in gulls are extremely variable (Kapperud & Rosef 1983), 13,0% was the value determined in the Portuguese study by Duarte et al. (2002), but percentages of 55% were found in cases in which gulls used, frequently, areas where sewage treatment plants were located differing the value obtained by the degree of regional contamination, human density and salmonella incidence in the local human population (Fenlon 1983). On the other hand the human population is also responsible for differences in seasonality and geographic distribution of the findings. The species studied and its eating habits are 2 other reasons for the discrepancies found in the carriage rate: Gridwood et al. (1985) accounted for *L. argentatus* 38% and *L. fuscus* 26,1% in their study. From several studies the carriage values appear to be higher in the autumn (between August and November) and in the reproductive season and lower in winter (Gridwood et al. 1985;Ferns & Mudge 2000). The younger apparently have superior values in comparison with the adults (Butterfield et al. 1983;Kapperud & Rosef 1983;Lévesque et al. 1993;Tizard 2004;Palmgren et al. 2006) perchance owing to their feeding habits, or insufficient immune response and/or insufficient intestinal microbial competition (Palmgren et al. 2006;Gaffuri & Holmes 2012). Gender differences in the carriage rate are possible, and in gulls, particularly *L. argentatus* it is due to differences in behaviour, namely dominance over foraging sites by males and the consequent use of refuse dumps by females (Monaghan, Shedden, Ensor, Fricker, Girdwood 1985 cited by Gaffuri & Holmes 2012).

Taking into consideration another aspect of the pathogenesis, it is unknown the infectious dose in wild birds in general, but it is acknowledged that it varies with the susceptibility and serotype and the latter

pathological mechanism (Gaffuri & Holmes 2012). The pathogenic agent, after its ingestion by the host, colonizes and invades the intestinal wall, which is followed by the dissemination and replication in various cells of several organs and tissues leading in some cases to septic shock (Gaffuri & Holmes 2012).

2.2.2.2. Pathology.

As a disease all the species are susceptible to infection by *Salmonella* spp. The severity contrast with the species, virulence, infectious dose, phagotype, stress, age (severe clinical presentations are often observed in younger birds particularly chicks and juveniles) and concomitant presence of other diseases (Friend 1999c;Tizard 2004). Once again, and normally, the bacteria in question are rarely the cause of mortality, since it acts like an adjunct condition in the terminal stage of other illnesses (Brand et al. 1988;Tizard 2004;Daoust & Prescott 2007). The project of Hall and Saito (2008) to analyze the database of the US Geological Survey National Wildlife Health Center, particularly passive reports in America between 1985-2004, is clear in this aspect. It showed that in 4,5 million causalities in 3.472 events, 186 were documented as being related to *Salmonella* sp. (as a cause or with an important role in the mortality) with lethality of 1 to 11.888 specimens by event (single or multi-species), in a total of 68.000 estimated causalities in 98 species of 12 orders of hosts, being the Passeriformes and colonial waterfowl the most affected. The mortality was found to be superior in the Passeriformes (21,5% of all the mortality events) followed by the Charadriiformes (inferior to 5%), which is in accordance with other studies that indicate that these two groups are the more importantly afflicted – Passeriformes and aquatic/marine birds (Tizard 2004).

The disease can develop and has been detected during the whole year, yet the mortalities tend to happen with a higher significance and expression in periods of stress: winter (for Passeriformes due to birdfeeders) and summer (in colonial birds and also Passeriformes), the latter season probably accompanying the high carriage rates (Friend 1999c;Hall & Saito 2008).

After ingestion of this pathogen, 3 forms of disease can arise: asymptomatic/subclinical infection, acute septicaemia with or without enteritis, or localized chronic infection (Daoust & Prescott 2007;Gaffuri & Holmes 2012).

The clinical signs exhibited by infected birds are unspecific and their descriptions result of very few reports, the latest are efforts to describe the recent mortality of Passeriformes with regard to birdfeeders. Considering the generality of the available descriptions it can be found: sudden death; fluffed-out or ruffled feathers (in small and big species, respectively); rapid and profound breathing; shivering; chemosis; hypopyon; eyelids swollen and "fused" together due to the presence of exudates; blindness; wing-drop; tremors; weakness; lethargy; indifference to external stimuli; incoordination, seizures; coma and death can befall in 24 hours. In small birds, thirst and dysphagia can also be frequently seen. If present, enteritis can be responsible for the presence of diarrhoea and the cloaca and tail's feathers are dirtied by urates and fluid faeces. In animals with a chronic presentation, the signs can vary accordingly with the location of the lesion, like for an example arthrosynovitis is usually identified in the umero-radio-ulnar and tibio-metatarsic joints and causes lameness, inability to fly, local swelling, and the animal is emaciated. Focal or multifocal aerosaculitis is another lesion described that can be asymptomatic or is accompanied by signs that relate to loss of flight

performance or loss of body condition (Mikaelian et al. 1997;Friend 1999c;Daoust & Prescott 2007;Hall and Saito 2008;Gaffuri & Holmes 2012).

Taking into consideration the findings detected in the necropsy examination, again and equally as to what was said regarding the clinical presentation, the lesions presented depend on the course of the disease, virulence and the resistance of the host (Friend 1999c). There are situations in which it is impossible to observe alterations (acute cases) and subacute or chronic ones where the ensemble of lesions is variable and suggestive of bacterial involvement. In this way, birds can have a good body score in the first example, or inferior and along with it: pulmonary and renal congestion, hepatomegaly and splenomegaly with tiny focal points of haemorrhage and necrosis. With the progression of the disease an increase on the friability and congestion of the tissues is reported alongside with the development of spots/nodules/granulomas in several organs (initially reddish then copper-whitish composed of inflammation and caseous necrosis). These formations can extend throughout the coelomic cavity and be seen in the microscope, or even at naked eye, in the cases when there are plaques or granular abscesses in the pectorals and other organs/tissues associated with fibrin or fibrin-purulent inflammation of the pericardium, coeloma and air sacs among other findings. Only in Passeriformes can be visualized the presence of yellow caseonecrotic nodules/plaques in the surface of the esophagus and crop, ingluvitis and esophagitis, deemed responsible for the dysphagia mentioned earlier (Tizard 2004;Daoust & Prescott 2007). Animals that exhibit enteritis and tyflitis (frequently described in adult birds), in turn, initially develop intestinal congestion and ulceration particularly in the distal portions of the small intestine and in the large intestine (including the ceca), but with the course of the disease it can be possible to distinguish in the mucosa small adherent yellow plaques of fibrinous origin, abscesses or necrosis cores. In these enteric cases, it can also be detected exudates giving an opaque dark brown rough appearance to the intestines. Considering the chronic manifestation of salmonellosis, arthritis is the key finding, but mainly in the form of a bursitis and tenosynovitis characterized by the presence of fibrous tissue with yellow viscous fluid and fibrin; in the same animal focal or multifocal osteomyelitis and aerosaculitis can also be described (Duncan, Stroud & Locke 1983;Mikaelian et al. 1997;Friend 1999c;Daoust & Prescott 2007;Gaffuri & Holmes 2012).

Birds with neurologic behaviour alterations and symptoms have deviations from the normal aspect, texture, colour and cellular organization of some of the nervous system organs such as caseous necrosis and mononuclear inflammatory infiltrates (Tizard 2004). In these cases, in the histopathology analysis it is identifiable necrotic lesions and inflammation associated with bacilli gram-negative: in the beginning it is characterized by multifocal aggregates of heterophiles and fibrin surrounded by necrotic cells then progresses to a mixture of histiocytes, multinucleated giant cells and lymphocytes (Daoust & Prescott 2007).

With respect to seagulls, the only document available (Gaffuri & Holmes 2012) indicates purulent arthritis, pneumonia, perihepatitis and nephritis as the common findings seen in sick birds.

2.2.3. Aspergillosis.

Aspergillosis is a fungal infection, primarily affecting the respiratory tract, but can be a plausible cause for mortality outbreaks, can initiate acute or chronic cases of disease, or may be an opportunist disease jeopardizing the prognosis of a debilitated animal. This fungal disease is the most frequently

documented in birds and given that it can be responsible for paresis/paralysis, is, in this way, another affliction to consider in the differential diagnosis. Additionally, its significance in wild birds is greater than it was assumed in the early stages of avian medicine, since it is regularly described as the primary cause for disease and mortality in wild birds captured and maintained in captivity, and so constitutes an important risk that can threaten efforts of conservation/translocation/recuperation and rehabilitation of several species , particularly the ones imperil that depend on conservation projects, or even the ones already endangered, as are examples the works of Sanchez and Murray (2005) and the one of Perrot and Armstrong (2011).

2.2.3.1. Pathogen and Epidemiology.

This mycosis is due to a fungus of the genus *Aspergillus*, and in the case of wild fauna the most preponderant is *A. fumigatus* (Friend 1999a) occasionally *A. flavus* is found and the least frequent are *A.terrus*, *A. glaucus*, *A. nidulans*, *A.niger*, *A. amstelodami* and *A. nigrescens,* but cases of mix infections are possible like what is stated by Redig, Fuller and Evans (1980); Xavier et al. (2011) and Tarello (2011). The reason for the recurrent isolation of *A.fumigatus* in wild birds perhaps is due to the small sized spores in comparison with the other *Aspergillus* species (Richard & Thurston 1983 cited by Beernaert, Pasmans, Waeyenberghe, Haesebrouck & Martel 2010).

The fungi, thermophilic, are saprophytic and hence can establish in sites with a strong human influence (e.g. farms), sites where humid terrains, decomposing vegetation, organic residues and animal feed prevail (Friend 1999a). They are capable of producing aflatoxins, whose role in wildlife pathology is subject to recent scientific research (Lawson, MacDonald, Howard, Macgregor & Cunningham 2006), and can infect all living beings, in spite of commensality (Friend 1999a).Nonetheless, they are especially deleterious in birds, possibly because of: the inexistence of a epiglottis and diaphragm in these animals, limitations in the pseudo stratified ciliated columnar cells' distribution and the presence of air sacs that are poorly vascularised and where there are high concentrations of oxygen (Tell 2005 quoted by Garcia, Lanzarot, Rodas, Costas & Blanco 2007;Bauck 1994 cited by Xavier et al. 2011), although Jansson (2012) states that such assumptions may require more evidence, given that studies regarding the association of immunity to avian aspergillosis are scarce in the moment.

 Aspergillosis is a non contagious disease characterized by acute or chronic presentations, both probable in the wild, and is responsible for mortalities of a high number of adults and young, mainly in acute cases (Friend 1999a). Nonetheless, and in spite of being recognized as a respiratory disease in birds since 1800, on the word of Friend (1999a) the association of mortality events to the pathogenesis of this agent is practically only achieved in isolated post-mortem evaluations than in outbreaks in large scale, where there are still present and surviving few animals. However, exceptions do occur and correspond to what is said by Adrian, Spraker and Davies (1978) that indicate the two die-offs in North Colorado on October of 1975 and 1976 in mallard ducks and the case described by Zinkl and Hyland (1977), in which more or less 10% out of 10.000 to 15.000 *Corvus brachyrhynchos* died in Nebraska in the Fall of 1974. In certain cases, aspergillosis it is only identified when anthropogenic causes are present and responsible for its development, like capture or captivity of wild individuals, as is what Friend and Trainer (1969) stated: from the 146 juveniles *L. argentatus* captured

for pesticides experimentations, 140 survived the transportation and from these 32 died of aspergillosis.

The exposure usually takes place by inhalation of fungal spores, but for the disease to arise, factors like the integrity and correct function of the immune system are extremely important, thus conditions that lead to immunosupression or immune depression are the most important (Friend 1999a). Other routes described for exposure include infection of eggs by means of penetration of the pathogen through the egg shell when present in large concentrations in the environment (causing the "brooder pneumonia" in outbreaks on Spring) or inoculation of the pathogenic agent by puncture wounds (Friend 1999a;Jansson 2012). The inhalation of *Aspergillus* sp. spores is the most frequent route of infection, but rarely is pathogenic in healthy individuals (Young, Cornish & Little 1998;Shin et al. 2004 cited by Garcia et al. 2007) and since *Aspergillus sp.* is ubiquitous, some birds can carry the agent without developing disease (Garcia et al. 2007) and possibly constitute a source of infection for other animals.

In respect of the clinical presentations seen, the acute is reported in nature due to consumption of discarded grains and in contaminated ensilage pits during adverse climatic conditions, where good conditions for the growth of the fungus are reunited, while the chronic ones are also seen in the same places as the former but are also more likely documented in captivity situations, rehabilitation centres, zoologic parks and bird feeding stations (Friend & Trainer 1969;Burr 1981; Friend 1999a; Sanchez & Murray 2005;Nardoni, Ceccherelli, Rossi & Mancianti 2006;Jansson 2012). Nevertheless both presentations can happen anywhere in the world, perhaps the exception being Antarctica (Friend 1999a;Converse 2007a).

The outbreaks, when described, tend to occur in the Autumn and early Winter, but singular cases can take place during all the year (Cork, Alley, Johnstone & Stockdale 1999). Normally, the temporal distribution coincides with periods of great challenges to the immune system (the principal predisposing factors), whether because the pathogen is already present in the animal or by the simultaneous occurrence of spore inhalation and these factors (Friend & Trainer 1969). These factors can act alone, but is recurrent their association, with for instance: lesions/traumatic events (Nardoni et al. 2006); oil spillage (Balseiro et al. 2005); disease/intoxication/toxicosis or concomitant presence and effect of certain pathogenic agents like in botulism (Brand et al. 1988), in plumbism (Souza & Degernes 2005), intoxication by organophosphates (Jung, Kim, Lee & Kim 2009), parasitism (Beaudette 1945;Young et al. 1998), among other diseases (Tarello 2011); capture/transportation/captivity (Friend & Trainer 1969;Mihaylov, Petrov, Marutsov, Simeonov & Simeonova 2008); prolonged treatment with antimicrobials and corticosteroids in wildlife rehabilitation units; release of animals in inadequate ecosystems (Cacciuttolo, Rossi, Nardoni, Legrottaglie & Mani 2009); constant presence in landfills and dumps (Davis & McClung 1940); innutrition (Friend & Trainer 1969); migration (Redig et al. 1980); thermal stress; hierarchy and social interactions (Redig et al. 1980); reproduction – nest material (Burr 1981) and stress (Cork et al. 1999); high population densities in a specific site (Redig et al. 1980); or climacteric conditions favourable and justifying for infection like snow (Adrian et al. 1978), or environmental increase in the number of viable spores, such as warm temperatures, low humidity and dust (Zinkl & Hyland 1977).

Several avian mortalities have been reported to be due to aspergillosis and it is believed that all species are susceptible (Friend 1999a). The more repeatedly documented as being more affected

comprise: waterfowl like *Anas platyrhynchus*, *Aix sponsa* and *Branta canadensis*; marine birds like gulls; crows and ravens, and raptors in captivity (Redig et al. 1980;Friend 1999a;Converse 2007a). Despite the abundance of such reports in these cases, which can possibly imply that these species can be more susceptible, according to Zinkl & Hyland (1977) this illation might be due to the regularity with which it is investigated this fungus in these groups of birds when compared with others, including specific geographic areas or data (Converse 2007a). Avian groups like singing birds, upland game species, diurnal and nocturnal raptors, egrets and rarely wading birds have also been subject of investigation in cases of aspergillosis, which corroborates this previous position (Redig et al. 1980;Friend 1999a). In respect to age, younger birds appear to be more sensitive to the pathogenesis of this agent than adults (Friend 1999a), as is clear in one of the die-offs already mentioned of anatids studied by Adrian et al. (1978). Identical observation had Souza and Degernes (2005) in swans and, probably this situation is due to immune system immaturity (Olias et al. 2011). Additionally regarding a gender differential predisposition, in accordance with the findings of Adrian et al. (1978) apparently this is non-existent, yet the study conducted by Souza & Degernes (2005) states the contrary given that it was determined that males were two times more prone to develop this mycosis. However these same authors admit that this affirmation has some weaknesses, particularly because there wasn't a laboratorial confirmation for the presence of *Aspergillus* sp. in the identified lesions and it was impossible to explore the possibility that differences in immune competence are the reason for this result, and so this standpoint needs further validation.

Regarding the infectious dose for the pathogenesis, the data are still limited, contradictory and strongly dependent on factors like: species, age, immunity, virulence and in what experimental conditions was carried out the study (Jansson 2012).

2.2.3.2. Disease: pathogenesis, signs and necropsy findings.

As was previously stated, there are two clinical presentations of aspergillosis, one acute and the other chronic. The clinical progression of the acute form is of a few days and is associated with the exposure to a high quantity of spores from one source (Redig et al. 1980) and/or is related with a strong state of immunosuppression (Xavier et al. 2011 quoting both Abundis-Santamaria 2003 and Kearns & Loudis 2003). It can initiate suddenly and last for only 7 days, with the vast majority of deaths occurring on the first days (Adrian et al. 1978), or the causalities happen in 24-48 hours (Xavier et al. 2011) without apparent signs of disease (Converse 2007a). This kind of clinical presentation and high mortalities, generally, tends to occur in gregarious species, perchance because larger groups of birds facilitate a higher exposure to the source of spores, or a larger number of birds are exposed to it, or given the high number of individuals sick or dead gathered in the same place is much easier their detection. In the case of the chronic variant the animals die over weeks or months and this form is essentially due to immunosupression, which allows a small quantity of spores to provoke disease (Redig et al. 1980), and so, adjoining this presentation and fatalities are clues/signs of the predisposing factors (Converse 2007a).

Regarding the pathogenesis of this disease, dismissing the two varieties, the spores when inhaled reach the lungs. From their development, obliteration of the air ways can be seen due to the growth of the hyphae and accumulation of inflammatory cells and in this case death may result of asphyxia (Friend 1999a;Jansson 2012). In other cases, the inhalation may result in a clinical presentation owing

to, not only the injuries caused by the fungal presence/growth and inflammatory response pertained, but the effects exerted by metabolites produced by *Aspergillus* sp. (Sutton, Newcombe, Waring, and Mullbacher 1994 cited by Abou-Rawash et al. 2008;Joseph 2000). When the spores reach/colonize the lungs and air sacs in quantities that compromise the immune response and/or the latter is already deficient, the lesions formed can generate gradual respiratory deficits and the fungus can spread to other organs and tissues (e.g. liver, intestinal wall and other viscera) by direct extension from the respiratory system or haematogenous route either/both directly or by interposition of constituents of the immune system (Richard & Thurston 1983 and Dahlhausen, Abbott & VanOverloop 2004 both quoted by Beernaert et al. 2010) turning the respiratory presentation into a systemic and lethal one (Friend 1999a). In cases where there are not only mortalities or hyper acute/acute forms of aspergillosis, the clinical signs described are unspecific and depend on the infectious dose, spore distribution, previous existence of other diseases in progress and the immune response (Dahlhausen et al. 2004 cited by Beernaert et al. 2010). For the most part, the signs of aspergillosis are identical in acute and chronic disease, but there is predominance of some related to certain organs and systems, particularly in chronic aspergillosis. In most cases described, when they are not only reports of mortality, it is documented dyspnoea which tends to become, progressively, severe with: gaping, panting, open-beak respiration, tachypnoea, nasal discharge (exudative rhinitis), cyanosis and vocalization alterations (Threfall 1967;Friend & Trainer 1969;Ward, Fairchild & Vuicich 1970;Redig et al. 1980;Zinkl & Hyland 1977;Burr 1981;Cork et al. 1999;Nardoni et al. 2006;Mihaylov et al. 2008;Tarello 2011;Jansson 2012). The birds can equally have various degrees of weakness or signs associated with the localization of the lesions, and in this way, signs such as unthrifty/ruffled feathers, wing-drop, lameness, debilitation, lethargy, inability to flee when approached combined with incapability to walk or fly may be seen (Zinkl & Hyland 1977; Davis & McClung 1980;Redig et al. 1980). Additionally and conjoined with the previous signs can be noted emaciation, weight loss, dehydration, thirst, loss of appetite, diarrhoea, anorexia and regurgitation/vomit (Friend & Trainer 1969;Ward et al. 1970;Zinkl & Hyland 1977;Davis & McClung 1980;Redig et al. 1980;Nardoni et al. 2006;Mihaylov et al. 2008).

Other signs described and likely to be evaluated depend on the degree of dispersion of the agent in the organism. When the nervous system is compromised, these may include: ataxia, head and neck twisting, torticollis, head tilt and disorientation (Friend 1999a;Cork et al. 1999;Nardoni et al. 2006;Jansson 2012); but ataxia and paresis/paralysis are also likely described in birds but instead owing to kidney or spinal cord invasion (Joseph 2000).

The presence of spores in the air can originate ocular lesions and signs, such as: blepharospasm, photophobia, periorbital oedema, epiphora, swollen eyelids with adhesions, corneal opacity, mycotic keratitis and yellow caseous exudates in the conjuntival sacs (Beckman et al. 1994 and Hoppes, Gurfield, Flammer, Colitz & Fisher 2000 cited by Beernaert et al. 2010;Jansson 2012).

Taking into consideration the lesions observable in the necropsy examination, they tend to vary in accordance with the quantity of the inoculum and the course of disease - acute or chronic (Adrian et al. 1978), although, and on the word of Converse (2007a), in spite of acute and chronic aspergillosis exhibiting gross differences, these lesions are not exclusive of each kind, being frequent situations in which they coexist (Xavier et al. 2011). In the study by Cacciuttolo et al. (2009) of several avian species subjected to different stressors but with aspergillosis diagnosed, the differences observed in

the necropsy, namely the gross ones, are also attributable to the host and the efficiency of its immune system. Therefore, normally in the acute cases the animals are in a good body condition and have good fat deposits, being rare examples of low body scores or emaciated individuals – frequent in chronic cases (Threfall 1967;Pearson 1969; Adrian et al. 1978;Jansson 2012). Food can be seen in the crop and/or ventriculus, but seldomly (Zinkl & Hyland 1977;Adrian et al. 1978). Frequently in both presentations the lesions are found in the respiratory apparatus (lungs, air sacs, syrinx and bronchi) which correspond to the primary sites for the development of this pathogen, in particular the lungs and air sacs, because they maintain spores that create dormant infections or chronic ones, without apparent clinical signs or lesions (Zinkl & Hyland 1977;Davis & McClung 1980;Nardoni et al. 2006;Converse 2007a).

In the acute manifestation of aspergilosis and according to Adrian et al. (1978), Friend (1999a), Xavier et al. (2011) and Jansson (2012) gross findings such as: thickened air sacs with luminal white nodules or plaques; pulmonary congestion, oedema and haemorrhage; and parenchyma and pleural white-yellowish nodules (coalescing, round to irregular in shape, homogeneous of caseous appearance and elastic/cartilagene consistency but dry at the cut) are commonly documented and result of the contact of high doses of spores and the subsequent reaction without cues of other diseases (Redig et al. 1980;Friend 1999a;Atasever & Gumussoy 2004;Nardoni et al. 2006;Cacciuttolo et al. 2009). Conversely in sub-acute to chronic cases these lesions are in a lower number, more dimensioned and more confined to the respiratory apparatus (Zinkl & Hyland 1977;Adrian et al. 1978): the air sacs' membranes are thickened, opaque and heavily vascularised sometimes associated with fibrinopururlent exudates and displaying adherent flat caseo-necrotic plaques with frutivorous bodies (that can extend to other air sacs and tissues as nodular focal formations similar to tubercles with hyphae in the center and reactive inflammation in the periphery almost resembling granulation tissue, documented in the serosa of the celomic cavity, spleen, pericardium sac, liver, kidneys or invanding several layers of the gastro-intestinal tract or bones); the alveoli, bronchiole, bronchi contain in their interior mucus, fibrin, fragments and cellular debris, sometimes with inflammatory cells and even mycelia masses (in the case of Nardoni et al. 2006 diffuse fibrin or catarrhal pneumonia in a *L. cachinans michahellis* was additionally seen); the lungs are congested; and blood can be seen in the

parabronchials along with the nodular lesions previously described (Davis & McClung 1940; Pearson 1969; Friend & Trainer 1969; Ward et al. 1970; Adrian et al. 1978; Cork et al. 1999; Abou-Rawash et al. 2008; Xavier et al. 2011). In this type of aspergillosis it is possible to distinguish, particularly in the air sacs, velvety plaques of grey, green to brown or blackish colour of fungal reproduction particularly massive in the case of gulls with fruiting organs in the air sacs and pulmonary parenchyma, fig.71 (Coon & Locke 1967; Friend & Trainer 1969; Redig et al. 1980; Friend 1999a; Nardoni et al. 2006; Cacciuttolo et al. 2009;Jansson 2012).

Figure 71 – Lesions in lungs and air sacs of a *L. argentatus* (whitish caseous material with a grey-greenish mould). Adapted from**:** Cacciuttolo et al. (2009).

Without fungal involvement of other organs than the respiratory ones, grossly, the recurrent observation in two works, Adrian et al. (1978) and Xavier et al. (2011), was slightly to moderately enlargement of the spleen and in the latter petechial or ecchymotic haemorrhages were found on the epicardium.

In the histopathological analysis in acute cases, pleuritis, interstitial pneumonia with the presence of infiltrates of heterophils, macrophages, fibrin, lymphocytes and pyogranulomatous to necrotic coalescing lesions with adjoining fibrous tissue are seen. Sometimes severe supurative and/or pyogranulamatous pneumonia can be found (Jansson 2012). In subacute cases the lesions are identical, but with multinuclear giant cells (Adrian et al. 1978), the air sacs have similar inflammatory responses with additional perivaculitis and oedema and with a lesser involvement of lymphocytes and plasma cells (Jansson 2012). In turn, in the chronic phases, conidae, germinating conidae and hyphae are seen in the centre of the lesions among pyogranulomas, necrotic sites and abudant giant cells (Adrian et al. 1978;Jansson 2012). Other microscopic finding in this last presentation are signs of angioinvasion (infiltration of the subendothelial and mural compartiments by polymorphonuclear cells, hyphae, thrombi and coagulation necrosis) and sporulated conidiophores in aerated tissues, particularly in exudates in these locations (Atasever & Gumussoy 2004; Abou-Rawash et al. 2008;Jansson 2012). The brain, in both acute or chronic disease, rarely is affected to the extend to see lesions, although when neurologic signs are present, findings like solitary abscesses with necrotic centres and infiltration by heterophils and macrophage from the periphery, or meningoencephalitis with encephalomalacia are seen (Cork et al. 1999; Friend 1999a; Converse 2007a;Jansson 2012). Other findings, less frequent or more inconsistent, are necrotic cutaneous granulomas or similar "growths" (in chickens and pigeons) or even "cheesy" plaques on the surface of the eye or beneath the third eye lid (Friend 1999a).

2.2.4. Sarcocystosis.

Sarcocystosis is a parasite disease, generally asymptomatic and non-lethal caused by coccidian protozoas of the subphylum Apicomplexa, clade Sporozoa. These pathogens, of the genus *Sarcocystis,* can infect several species of mammals, reptiles, fish and birds and can cause occasionally paresis/paralysis with different clinical and histopathological presentations (Moorman, Baldassarre & Richard 1991; Tuggle & Friend 1999;Prakas & Butkauskas 2012).

2.2.4.1. Pathogenesis.

The life cycles of parasites of the genus *Sarcocystis* are not fully known and only the ones concerning some species of animals, particularly the commercial, are complete as is stated by Dubey and Morales (2006) citing Dubey, Speer and Fayer (1989), and Prakas and Butkauskas (2012). These parasites have indirect life cycles that require an intermediate host for its completion before transfer to a definitive host, usually a carnivorous, in what is commonly termed as an obligatory heteroxenous predator-prey two-host life cycle (Tuggle & Friend 1999; Dubey, Lindsay, Rezende & Costa 2000; Greiner 2007). There are numerous possible "combinations" between predatory and prey, however recent studies indicate that a co-evolution in many of the associations between certain species of *Sarcocystis* with their definitive hosts may exist (Dolezel et al. 1999), and in accordance with Dubey et al. (2000a) quoting Dubey et al. (1989) there is a certain specificity regarding the intermediate host. This apparently is not restricted only to the genus of the host, like is evidenced in the studies of Box and Duszynski (1978) on the subject of transmission of *Sarcocystis* from members of the family

Icteridae (cowbirds and grackles) to others distinct like the Ploceidae and Fringillidae, by means of sporocysts from the *Didelphis virginiana*, the definitive host.

Birds in these cycles, as a rule, have an intermediary role by the ingestion of eggs or oocysts in food or water contaminated by carnivore faeces (Tuggle & Friend 1999;Greiner 2007). It is known that approximately 22 species of *Sarcocystis* have birds as intermediate hosts, although 12 have been denoted as parasitizing avian definitive hosts (like the *Tyto novaehollandiae* and *Tyto alba* in the study of Munday 1977), and additional two resort to these animals for both parts (Greiner 2007). Once within the gastro-intestinal tract the oocysts (enclosing 2 sporocysts with 4 sporozoites each) reach maturity, and release the sporozoites. These are able to penetrate the intestinal wall and in the arterial endothelium of the mesenteric lymphonodes, takes place the merogony, initial asexual multiplication phase of the cycle (Greiner 2007), which can also occur subsequently in other endothelial cells and organs, including the brain as are the cases of *S. falcatula* and *S. neurona* (Spalding, Yowell, Lindsay, Greiner and Dame 2002 citing Teglas, Little, Latimer and Dubey 1998, Dubey et al. 1991 and Aguilar, Shaw, Dubey & Redig 1991;Dubey, Quist & Fritz 2000b;Wunschmann et al. 2010). Is at this stage, when meronts are in the early stages of infection in the locations previously mentioned, multiplying/invading these tissues, that all the pathology ensues (Dubey et al. 2000b quoting Dubey et al. 1989;Greiner 2007). Accordingly, it is possible to find several clinical presentations with signs in relation with the tissue or organ affected, for instance: obstruction of the lungs, liver and heart capillaries by schizonts causing pulmonary oedema and haemorrhage (in the first); or affecting the liver, heart and spleen can cause death, as was what was seen in parrots and pigeons, without apparent signs of disease (Dubey et al. 2000b and Latif et al. 2010 quoting Ecco et al. 2008 and Dubey et al. 1989).

In these organs and tissues, takes place a new set of multiplications that originate merozoites, that upon arrival at the voluntary muscles develop into septate cysts with villae protrusions on the walls or intramuscular macrocysts, a distinct feature from other cyst forming coccidian species (Dubey, Cawthorn, Speer & Wobeser 2003). In this last phase, they can suffer a new asexual reproduction, endopolygeny, forming bradizoites (Tuggle & Friend 1999;Greiner 2007). The transmission to the suitable definitive host, is the next step of the cycle and is via ingestion of a bird carcass with these cysts, giving continuity to the cycle in the carnivorous host, namely in the epithelium with the formation of new oocysts from the bradizoites through sexual reproduction, without involvement of other systems or organs (Tuggle & Friend 1999). It is also in this compartment that the last stage of asexual reproduction comes to pass, sporogony, and the sporolated/infective oocysts are excreted in the environment (Greiner 2007).

The full range of species affected by this parasite is quite diverse comprising several dabbling ducks (like the *A. platyrhynchus*) with evident gross lesions, whereas in other species of ducks, geese and swans, or even gulls and terns this presentation is less frequently documented or the investigation of this parasitic disease is uncommon (Hoppe 1976; Tuggle & Friend 1999; Prakas, Kutkiené, Sruoga & Butkauskas 2011). The majority of the reported cases and the gathered knowledge focuses on the exposure of waterfowl to this pathogen, but the possibility for these parasites to cause lesions in other species cannot be neglected (Tuggle & Friend 1999) and new studies are necessary in this regard, as is an example the one carried out in Australia to determine this parasite prevalence in several families, including: 43 of birds, with representatives of 129 species; 4 families and 14 species of reptiles; 2

families and 9 amphibian species and 5 families of fish with 5 species (Munday et al. 1979). In this project, gulls, like *L.pacificus* and *L.novaehollandiae* were taken into consideration and from the 18 samples collected, 3 were positive. Given that information concerning the particularities of these life cycles, seasonality pattern, age of the affected individuals, species afflicted and so forth is limited, further studies are crucial (Hoppe 1976;Tuggle & Friend 1999). Studies in wild life also appear to be restricted to cases where gross lesions were easily detected underestimating the true value that this parasitic disease can have in these animals (Crawley, Ernst & Milton 1962), in view of the fact that it is possible the formation of only microcystis as was documented by Crawley et al. (1962) citing Drouin and Mahrt (1979); and Latif et al. (2010). Additionally, several studies focusing only on specific cycles exist and are the ones by Spalding, Atkinson & Carleton (1994) in Ciconiiformes and Munday (1983) focusing on the *Tyto novaehollandiae*/mice and rat cycle in Australia.

The factors that enable exposure to *Sarcocystis* sp*.* and the occurrence of this parasitic disease are identical to other diseases herein discussed and include: diet, season of the year, habitat where foraging takes place, number of sporozoites ingested, sarcocystis and host species, immune status (Duszynski & Box 1978;Cawthorn & Rainnie 1981;Spalding et al. 1994) and the likely transport by arthropods, such as cockroaches in the life cycle of *S. muris,* for instance (Munday et al. 1979 quoting Smith and Frenkel 1978 and Latif et al. 2010 citing Clubb and Frenkel 1992).

This disease apparently has a global distribution (Tuggle & Friend 1999), nevertheless in certain regions there is a tendency for an unequal distribution of the cases, according with Hoppe (1976). In terms of a temporal distribution, disease events are found throughout the year, though the observations tend to concentrate in specific time intervals, namely in the hunting seasons (Constanzo 1990) which can overrate certain locations, species and time periods (Wickware 1944;Tuggle & Friend 1999). These distributions can also be influenced by the reporting of only the most severe cases of sarcocystosis (Wickware 1944). Adults seem to be more prone to infection/lesions in several species as Hoppe (1976) and Costanzo (1990) declare, the last one in his study and citing Chabreck (1965); but also Spalding et al. (1994) and Tuggle and Friend (1999). The high prevalence of parasitism in adults could be due to, possibly, an absence of exposure in the young while still protected in the colonies and also the need for a longer time interval for its development (Hoppe 1976 citing Fayer and Johnson 1974; Constanzo 1990; Moorman et al. 1991;Tuggle & Friend 1999): 85 days were necessary in *Anas clypeata,* for the observation of microscopic immature cysts in muscles and 154 days for macroscopic small, mature and infective cysts, values obtained in a study where the *Mephitis mephitis* was the definitive host by Cawthorn and Rainnie (1961) supported by the cited Drouin and Mahrt (1979). Significant gender differences appear to be non-existent as is suggested by Spalding et al. (1994), however Munday et al. (1979) quoting Fayer and Kocan (1971) found a higher prevalence in *Cassidix mexicanus* and *Quiscalus quiscalus* females, perchance because they are responsible for the construction of the nest and probably are more exposed to this pathogen; the same finding was reported by Box and Duszynski (1977), but in *Molothrus ater*.

2.2.4.2. Disease: signs and findings.

Visible signs of this parasitic disease are uncommon and rarely the presence of lesions is an indicative of the cause of death (Moorman et al. 1991;Tuggle & Friend 1999). Several wild avian species may have an elevated number of *Sarcocysts* sp. in their tissues and do not display clinical signs of disease

(Greiner 2007). Only in severe cases, loss of muscle tissue can result in a clinical presentation of this illness with lameness, weakness or even paralysis, as the most frequent observations. Thus, this pathogen is usually considered as a non-lethal factor, but since it can cause weakened conditions leading to reduced survival, it is an adjunct to predation and other legitimate causes of death (Moorman et al. 1991 citing Tuggle 1987; and Tuggle & Friend 1999).

When other organs or tissues are involved, sarcocystosis can be the cause of death and clinical signs and both gross and microscopic lesions are found, as is an example the mortalities caused by *S. falcatula* in wild *Haliaeetus leucocephalus* and *Aquila chrysaetos* in rehabilitation in North America documented by Wunschmann et al. (2010). These birds were incapable of flying, they had several neurologic deficits varying in degree of severity (e.g. consensual reflexes, proprioception, toe pinch and grasp) displayed tremors, hock sitting, wing-drop, were in ventral recumbence and the neck ventroflexed. The severe case in 48-96 hours exhibited complete loss of response to stimuli, ventral recumbence in extension, and severe dyspnoea. Identical signs were also found in other species like *Bubo virginianus* (including slight head tilt) by Wunschmann et al. (2009); *H.leucocephalus*, together with loss of equilibrium, muscular weakness of the legs, repetitive movements of the head and impaired vision, in the study of Oslon, Wunschmann and Dubey (2007); or even in more marine species like *Morus bassanus*, with recumbence, opisthotonus and convulsions, in spite of the identification of other pathogens by Spalding et al. (2002). Depression, polyuria, torticollis, opisthotonus, paralysis and trembling followed by death were signs reported by Olias et al. (2010) in pigeons experimentally infected to reproduce the emerging fatal neurologic disease in this species, *Columba livia* in Berlin, whose signs were previously reported in 2009 by Olias et al. (2009) and suspected to be due to *Sarcocystis* species (apathy, weakness, depression, mild diarrhoea, torticollis, opisthotonus, muscle tremor, paralysis an trembling were the signs described). Overall, all these cases are examples of the nervous involvement of this pathogenic agent in the clinical presentation of paresis and paralysis.

In the necropsy evaluation visible forms of the cysts are evident upon removal of the skin, given that they resemble rice grains, because they are wihitish opaque cylindrical structures that typically run in parallel streaks in the muscles both in superficial and in more profound layers, at least in aquatic species as Wickware (1944); Spalding et al. (1994); Tuggle and Friend (1999) say. Nevertheless, studies regarding other species do not substantiate this predilection for the development of cysts in the breast muscles but indeed, indicate other tissues and organs as possibly affected, like other skeletal muscles and even the myocardium (Box & Duszynski 1977;Tuggle & Friend 1999;Crawley et al. 1962;Spalding et al. 2002;Wunschmann et al. 2010) with different intensities in their presence like what Latif et al. (2010). The shape and size of the cysts differ from one species to another, in relation with the type of host cell, *Sarcocysts* species, degree of maturation of the cyst and calcification (Tuggle & Friend 1999;Prakas & Butkauskas 2012). Normally these muscular lesions are not accompanied by myositis or myodegeneration like is stated by Spalding et al. (1994), but when it is present can be a lymphohistiocytic, granulomatous and eosinophilic myositis with marked Zenker's degeneration and loss of affected fibres as was found by Olias et al. (2009), despite the development of a plasmocytic, hystiocytic and polymorphonuclear myocarditis with cardiomyocite degeneration in association with the presence of schizonts and merozoites that are occasionally detected when this

muscle is influenced (Spalding et al. 1994;Dubey et al. 2000b;Olias et al. 2009;Wunschmann et al. 2010).

When other organs or tissues are affected with a consequent clinical manifestation of signs, other findings are identifiable like: encephalitis or meningoencephalitis (perivascular inflammatory infiltrates composed of lymphocytes, macrophages and plasmatic cells) with encephalomalacia, gliosis, in addition to the presence of schizonts and merozoites in various stages of development (Spalding et al. 2002;Oslon, Wunschmann & Dubey 2007;Wunschmann et al. 2009;Olias et al. 2009;Wunschmann et al. 2010). Disseminated endothelial pulmonary infection and according to Olias et al. (2009) protozoa in the lumen of the capillaries coexisting with mild multifocal lymphoplasmacytic, histiocytic and polymorphonuclear infiltration of the interstitial space is another finding, as well as embolism in large pulmonary veins (particularly of fragmented striated muscles), could be seen in one specimen of Olias et al. (2009). Lymphohistiocytic interstitial nephritis and eosinophilic/lymphocytic glomerulonephritis; chronic follicular hyperplasia in the spleen; multiple foci of inflammation, necrosis and oedema in liver, spleen and lungs are other documented lesions (Dubey et al. 2000b;Olias et al. 2009;Olias et al. 2010;Wunschmann et al. 2010). Sometimes similar lesions, as the ones herein described can be identified in the nervous system, but without a direct involvement of the presence of these protozoa like was reported by Dubey et al. (2000b) and Olias et al. (2010), and in accordance with the latter, occurrence of brain lesions and in the absence of protozoa could indicate a possible indirect mechanism for encephalitis, yet unknown.

2.2.5. Botulism.

According to Rocke and Bollinger (2007), botulism is the most important disease to consider when regarding wild avian species, particularly aquatic/marine, including the migratory. Its importance in this differential description is highlighted because it can produce various clinical presentations of paralysis and so it is a valid differential for paresis/paralysis. Additionally, its significance is emphasized because it can affect countless species in an outbreak, presents a potentiality to induce serious and significant population declines in quite a few and become another threat to the survival of others that are already endangered, like the case described by Rocke (2006).It is also a supplementary difficulty that hinders the success of several conservation projects such as the one referred by Work, Klavitter, Reynolds and Blehert (2010) of A*nas laysanensis*.

2.2.5.1. The Pathogen and Epidemiology.

The pathogenic agents in this disease are the neurotoxins produced mainly by bacterium *Clostridium botulinum* (Coffield & Whelchel 2012). Of the seven that can be produced by these bacteria, the C and E types are the more frequently pointed out as globally responsible for outbreaks of botulism in wild birds (Rocke & Friend 1999), despite Lindberg, Skarin, Knutsson, Blomqvist and Baverud (2010) and Skarin, Lindberg, Blomqvist, Aspán and Baverud (2010) suggesting the possibility of a mosaic typeC/D toxin be a reasonable cause for intoxication and source of die-offs in both domestic and wild avian species.

Botulism type C has been reported since 1900 and been identified in all the continents with the exception of Antarctica (Rocke & Bollinger 2007). Only taking into consideration the European

continent this disease has been documented affecting seagulls in Sweden in various outbreaks, between 2000 and 2004, in the Baltic Sea region. The estimation of causalities reached at least 10. 000 deaths, comprising primarily *L. argentatus* (Neimanis et al. 2007). Other countries with a history of reported botulism events are as an example Denmark, United Kingdom and The Netherlands. On the other hand, type C die-offs with more than 50.000 estimated deaths are more frequent in the USA, Canada and Russia (Rocke 2006). Type E Botulism, in turn, being less recurrent (Rocke & Friend 1999), has only been identified and documented past the 60's, initially in Lake Michigan and later in the Great Lakes. Between 2000-2008, only on lake Ontario fairly 67.793 deaths were estimated by extrapolation, including gulls of the species: *L. delawarensis*, *L. argentatus*, *L. marinus*, *L. fuscus* and *L. philadelphia* (Adams, Roblee & Stone 2009;Hannett, Stone, Davis & Wroblewski 2011). In Europe the disease has been detected especially in the North, but other countries like France have identified this kind of intoxication in events affecting gulls (Rocke & Bollinger 2007). By the examples given the outbreaks of these kind of disease are objectively distinct from the majority of the rest caused by other illnesses and among the various problems associated with these kind of die-offs is the fact that one event can affect several different species, reach high mortalities, can also potentially and differentially affect from year to year different species, numbers and regions, or can persist numerous years in the same area, like what was seen in Lake Ontario in the time span of eight years, 2000-2008 (Hannett et al. 2011). Every single particularity here presented and the ones that will be described latter depend on characteristics of the pathogen and all the complex pathogenic and ecological mechanism that it is intrinsic to these diseases and thus will be discussed ahead.

Clostridium botulinum is a gram positive, strict anaerobic and ubiquitous bacterium that forms dormant heat and drought resistant spores in order to forearm its survival in adverse environmental conditions. It can persist for an unlimited time in a certain ecosystem, but dispersion to others new is possible (Rocke & Friend 1999). Therefore, it is conceivable the occurrence of numerous outbreaks in the same area, year after year, due to its permanence in: terrestrial and aquatic soils, tissues of several invertebrates (insects, mollusks, crustaceans), fish (e.g. *Oreochromis mossambicus* introduced into the USA, as indicated by Nol, Rocke, Gross and Yuill 2004) and birds (Rocke & Friend 1999;Rocke 2006). Or disease outbreaks can happen in new locations, apparently without known suspicion, by bird transfer through the elimination of spores in the excreta or by their presence in the feathers (Matveev & Konstatinova 1979 quoted by Rocke 2006).

The toxin is only produced after the germination of the spores when the bacteria grow, multiply and the vegetative forms develop. The toxin exerts its effect only after the ingestion of the pre-formed toxin in food, by environmental contamination of the food item or even by means of consumption of intermediaries in which it is concentrated (intoxication), or, although less significant, when the bacteria colonizes the gastrointestinal tract and of its growth the toxin is manufactured (toxi-infection). Secondary infection of wounds can be another possibility, though quite rare or significant in wild birds (Duncan & Jensen 1976;Getchell & Bowser 2006;Rocke 2006;Rocke and Bollinger 2007;Neimanis & Speck 2012).

For the production of toxin to take place and for the intoxication to initiate (especially in the case of botulism type C) it is necessary for certain environmental factors to be present essentially in the aquatic medium and in the most suitable conditions namely (extrapolating from experimental data), there would be a higher chance for an outbreak to occur, if: the pH is between 7,5-9,0; the redox

potential is negative; the average temperature is superior to 20º C (ideally 23-30ºC for type E, production of this toxin is not compromised at temperatures as low as 9ºC, but temperatures superior to 30ºC for several days hinder toxicity of carcasses, contrary to type C, Smith, Turner & Till 1987); and the water levels have undergone major shifts or are at their lowest. The invertebrate, organic matter and bird densities, other parameters related to water (ex: osmolality and depth) and the existence and concentration of specific sediments are other determinant factors that predispose an outbreak (Locke & Friend 1989;Rocke 2006).

The role of the maggot cycle in botulism is another important factor, as it can be the trigger and can maintain an outbreak active, and even extend it to the next seasons as was probably the case presented by Wobeser, Raininnie, Smith-Windsor & Bogdan (1983) that extended till Winter and even Spring. The decomposition of animal carcasses (in spite of the cause of death being or not botulism and provided that the bacteria is present) with the coexistence of invertebrates enables the best conditions for spore formation or dissemination of toxins. The carcass is an optimal nutritional and energetic medium for bacterial growth with high temperatures and anaerobiosis (conditions required by this bacteria), and vectors like various invertebrates can act like a source of toxin and a form of dispersion to regions not so near. The consequent ingestion of invertebrates of the carcass with toxin, by other birds, their demise and decomposition associated with spore environmental contamination allow this cycle to self-perpetuate (Duncan & Jensen 1976;Rocke & Friend 1999;Rocke & Bollinger 2007;Coffield & Whelchel 2012;Neimanis & Speck 2012). In these cases, additional contributing factors must be equated and correspond to the density of insects; temperature and wind speed; carcass density; facility for egg shedding; larval development and insect dispersion. (Rocke 2006 quoting Reed & Rocke 1992 and Wobeser, Baptiste, Clark & Deyo 1997) The presence of animals that prey upon sick individuals or ingest toxic carcasses can prevent the cycle or mask it, however if there is a sudden overload in respect to the carcass-scavenger ratio owing to the death of a high number of birds (due to: sudden alterations on the quality or water levels, hailstorms, chemical spillage, death of fishes, harmful algal blooms, limited access of scavengers, anomalous management of the carcasses and diseases) this cycle can be unstoppable (Wobeser 1997;Rocke 2006;Rocke & Bollinger 2007;Evelsizer, Clark & Bollinger 2010). The maggot cycle, typical of botulism type C, can be equally found in respect to type E, because it is believed that there is a variant in which the presence of "scavenger" fish and the ones that eat the toxic invertebrate are the cornerstones for the constitution of a similar cycle, whereupon their death followed by the ingestion of their remains by wild birds and eventually the latter deaths can initiate and perpetuate it (Yule, Barker, Austin & Moccia 2006a).

In this way bearing in mind the previous considerations it is justifiable and common the documentation of this disease in the hot months of Summer and Autumn, even though reports in Winter and Spring months are possible, albeit with less frequency. If in the former cases the favourable environmental conditions are responsible for the mortalities observed, in the latter two seasons the explanation for the deaths is due to cases that occurred in the previous season, and the possible preservation of maggots in carcasses during the winter and/or deposition of them in the bottom of aquatic ecosystems where they will be harnessed by diving birds (in type C). The natural turn-over of the same ecosystems can be another reason for these last two seasons, particularly in the case of type E botulism (Wobeser et al. 1983;Locke & Friend 1989).

Other factors that may influence the occurrence of a die-off are still not fully acknowledged, such is the case of type C botulism, since the toxin's gene has its origin in bacteriophages. The disease may depend on constraints that alter the regulation of infection and viral replication (Rocke & Friend 1999) and that would probably justify why no-toxinogenic specimens are isolated together with the normal ones in several studies including the one by Hannett et al. (2011).

In this point, it is extremely complicated to identify when there will be a die-off, where it will occur and what was the original source, excluding those regions were the disease is endemic. So it is indispensable to consider all the above factors in every single case to diagnose and assess what made possible its expression. This conduct becomes ever more pressing when associated with these mortality events is a possible human interference in the environment, broadening the spectrum of the type of environment in which botulism can develop and increasing the availability of the toxin (Rocke & Bollinger 2007), like are examples the studies of: Forrester et al. (1980);Hubalek, Skorpikova and Horal (2005);Neimanis et al. (2007);Woo et al. (2010) and Shin et al. (2010) that imply a combination of artificial factors like pesticide use (organophosphates), precariously treated sewage effluents, sediment lagoons of mines and transforming industries, and climate changes (pollution, increased temperature and drought).

However, the effects of the presence and human activities aren't always direct. Some bird species, while trying to adapt to human artificial structures and more urbanized environments, can be victims and/or triggers of an outbreak, like what can be inferred from the cases regarding landfill sites, dumps and seagulls. The landfill sites have been numerous times associated with die-offs of type C in gulls of Britain, Scotland, Ireland and the Virgin Isles, nevertheless it is believed that the source of the pathogen is not the landfill, *per se*, because, according to Ortiz & Smith (1994) in a survey conducted in 19 UK landfills in 1994, about 63,2% were contaminated with types B, C and D and 5,2 % with type E, conversely none of the more recently collected wastes were contaminated. In the same study was verified that the prevalence of these spores in these sites was higher than in the more natural counterparts (3% C e E, e 1% D n=554 in mud samples, Ortiz & Smith 1994) This discrepancy is more likely due to gull transfer, birds that recently opted to forage for food in this kind of places. The high concentration of organic matter and the presence of optimal environmental factors in the landfill sites seem to substantiate the maintenance of the cycle allied to the participation of the seagulls as transfer (Rocke 2006).

2.2.5.2. Pathology: pathogenesis, signs and necropsy findings.

Every bird is susceptible to this affliction (Rocke & Friend 1999). Type C botulism is more frequently found in animals that search for food in subtracts and feed by filter feeding, probing, dabbling and diving (Anatidae and individuals of the orders Scolopacidae and Recurvirostridae). Species that by habit take advantage of decomposing carcasses (except bird like vultures and other scavengers that appear to possess a certain innate/acquired resistance) are alo the most affected. In the case of type E, it is usually described in species that feed on molluscs and fish, by and large in decomposition, like gulls, loons and even bald eagles (Rocke & Friend 1999;Rocke 2006;Caudill & Zimmerman 2010). Botulism type E, like type C, is also associated with the presence of exotic and invasive species, as what was stated in a series of die-offs that took place in beaches of the North of Lake Michigan, affecting principally *Gavia immer*, *Podiceps grisegena* and *Clangula hyemalis,* perhaps by the

ingestion of *Neogobius melanostomus* (for that particular location an abundant invasive), that in turn, exhibited paralytic signs (attracting birds) owing to the consumption of toxic and exotic invertebrates (Michigan Sea Grant College Program, 2007). These cases are evidence to the possible vector role that live fish can have in the transfer of the toxin to birds (Hannett et al. 2011) and being the preferred ones because they develop signs of disease that aid in the capture of ill fish by birds, such as the ones suggested and proven experimentally in the projects of Yule et al. (2006a) and Yule, LePage, Austin, Barker & Moccia (2006b): alterations in equilibrium/swimming, loss of voluntary motor function, frequent breaching, tachipnoea and pigmentation discoloration.

The distribution of cases by age and gender possibly reflects more the local presence, abundance and type of foraging technique used by the bird (or even physiological differences) than a higher or lower intrinsic susceptibility (Rocke & Brand 1994;Rocke & Bollinger 2007).

Concerning the dose capable of provoking disease or mortality, it is well established that these neurotoxins are the most toxic substances known to man (Rocke & Bollinger 2007) and the necessary quantity for disease or death to occur depends on the affected species and the strain in question. Taking as an example the gulls as a whole group, the oral LD50 for neurotoxin C is 2.500.000 mouse lethal doses and about 20.000 for type E (for a certain strain, 026-080X), values higher than the determined for other species (Monheimer 1968 and Haagsma 1987 quoted by Rocke & Bollinger 2007).

Upon absorption the botulinic toxins C and E cause progressive flaccid paralysis of the skeleton muscles in birds. The severity, duration, progression and expression of the clinical signals, death or recovery depends on the initial dose (quantity and type), affected species, exposure time, quantity of toxic material and possibly synergism, when simultaneously present (Rocke 2006;Rocke & Bollinger 2007). In a very simplistic way, the pathogenic mechanism of botulism is the result of the effects of these toxins on the organism. Because these toxins are closely related zinc - metalloprotease enzymes and their main targets are the nervous terminals rich in acetylcholine. After the intestinal absorption, they connect preferably to the pre-sinaptic region of the neuromuscular junctions preventing the release of acetylcholine, by cleavage of proteins responsible for the ducking, fusion of synaptic vesicles and liberation of the neurotransmitter (Rocke & Bollinger 2007; Schiavo, Matteoli & Montecucco 2000 cited by Coffield & Whelchel 2012), and so, they interfere with nerve impulse transmission. Consequently, there is loss of motor control, initially, with paresis of the legs and then ascendant flaccid paralysis that becomes complete and is associated with muscle atrophy (Rocke & Brand 1994; Neimanis et al. 2007;Woo et al. 2010). Yet and on the word of Simpson (1981) cited by Rocke and Bollinger (2007) autonomic ganglia, parasympathetic post-ganglionar terminals, symphathetic post-ganglionar nerves and adrenal glands are other locals where these toxins can act and of which little information is available or documented regarding specific signs related to these interferences.

In the study of Neimanis et al. (2007), previously mentioned in which the most affected specimens were *L. argentatus*, the clinical presentation disclosed indicated a variety of neurological signs with temporal progression in terms of severity. The first sign found, indicative of abnormality, was the presence of birds that engaged in flight at a later time than the rest or, the flight was uncoordinated in its various stages, but they were still capable of escaping when approached by humans. The mildly affected gulls couldn't fly, but were standing with a slight ataxia, seen when they took a few steps.

With the progression of the disease the moderately afflicted ones were frequently in sternal recumbency with drooped wings at the shoulder articulation, held far from the body (fig. 73).These birds made efforts to stand and walk for a short distance, however in the majority of these attempts they could not reach a complete standing positioning and they tended to remain on their tarsometatarsi (fig. 72) using this portion of the body as a base point to walk. In the severe cases there was a clear loss of the ability to walk and they resorted to the wings and beak to propel themselves as if "crawling". In the terminal stages, the eyes were partially closed and observations like nictitating membrane prolapse (paralysis), intermittent nystagmus and rapid constriction and dilation of the pupil, although seen, were rare. Also in this last group, dyspnoea with slow open beak breathing was documented, as well as occasional "bobing" or ventroflexion of the neck. During the course of the disease the tonus of the wings and the strength to beak decreased with the severity of the signs and since there wasn't a loss of conscience, in spite of the immobilization of the body, they could still respond to external stimuli and sometimes be extremely aggressive (in dying birds there was a decrease in this responsiveness). In other outbreaks, described in other species (other than gulls), the signs reported are extremely similar and in addition include: dysphagia, anorexia, acute dehydration, diarrhoea (Neimanis et al. 2007 only stated that the feathers next to the vent were stained with urates and bright green faeces), cloacal impaction, "epiphora" (accumulation of tears due to closed eyelids), "limberneck posture" (paralysis of the neck, fig. 74), complete absence of movement in the final phases, and coma (Martinez & Wobeser 1999;Mereb et al. 1999;Rocke & Friend 1999;Rocke 2006;Rocke & Bollinger 2007;Chou, Shieh & Yu 2008;Work et al. 2010;Shin et al. 2010;Neimanis & Speck 2012). As it is said by these authors, almost no bird can survive these final stages, only the ones with paresis (sub-lethal cases) are the ones with the best prognosis with complete recovery or persistence of intermittent neurologic signs. Rocke and Bollinger (2007) also state, and similar to what has been observed in humans, that there can be cases in which residuals effects may persist and affect the survival of a bird aforementioned regarded as completely rehabilitated.

Figure 72 – *L. argentatus* unable to stand completely, walked with its tarsometatarsi in Sweden. Adapted from**:** Neimanis et al. 2007.
Figure 73 – *L. argentatus*, from Blekinge, Sweden, alert with wing-drop. Adapted from: Neimanis et al. 2007.
Figure 74 – *L. argentatus* affected by type C (prostrated and with limberneck). Adapted from: Neimanis & Speck (2012).

In a die-off, the greatest part does not survive and death usually arises due to paralysis and can be attributed to respiratory insufficiency, drowning or as result of the combination or not of dehydration, famine and predation. Regardless of the cause, the animal is found in recumbence with the legs and head extended (Martinez & Wobeser 1999;Mereb et al. 1999;Rocke 2006;Neimanis & Speck 2012). Considering the progression of the disease and here conveyed, the image most illustrative of these sort of die-offs corresponds to the presence in the same place and simultaneously of healthy birds of

several species, sick birds with several degrees of paresis and paralysis and those that are dead and in different stages of decomposition. These last two sets are preferentially found on the receding water lines or in the immediate vegetation, or even in peninsulas/islands (Locke & Friend 1989;Rocke & Friend 1999).

Necropsy findings are unspecific as are the signs displayed in life (Rocke & Friend 1999). There isn´t a macroscopic or microscopic lesion in organs and tissues that is evident or pathognomonic of botulism (Rocke & Friend 1999;Shin et al. 2010). The majority of the corpses are dehydrated and the gastrointestinal tract is usually empty (case stated by Neimanis et al. 2007 indicated that it was empty and bile stained, the gall bladder was full, the cloaca was distended with urates and faeces and so could be parts of the large intestines), despite exhibiting a good to moderate body condition (Brand, Schmitt, Duncan & Cooley 1988a;Neimanis et al. 2007; Rocke and Bollinger 2007;Shin et al. 2010). When the death was due to drowning, concordant lesion can be identified (Brand et al. 1988b;Work et al. 2010 and Hannett et al. 2011 citing Smith 1977). It can also be detected and pointed out signs of traumatic lesions owing to predation or necrophagy, or even other afflictions, like parasitism (Neimanis et al. 2007;Work et al. 2010) or incidental findings, such as lead shots (Neimanis et al. 2007). Fly maggots can also be present on the carcass and accompany its decomposition (Wobeser et al. 1983;Brand et al. 1988;Work et al. 2010 b12,24,16). But still, the findings when present are unspecific and little consistent, taking as an example the work of Mereb et al. (1999) on waterbirds in Argentina: vascular congestion of the intestinal wall, cloacal distension, focal vascular congestion of the central nervous system and subpericardium region were reported in illed specimens. In the histopathological analysis of these carcasses it was detected centrohilar necrosis and lymphocytic infiltration of the pericardium, liver and muscles, sometimes the result of debilitation, stress and manipulation of the birds in life, which is the also the case of the study of Neimanis et al. (2007). These authors found degeneration of skeleton muscle cells from moderate to acute in the legs and pectorals (15% n=20) and 10% of the cases had sub-acute, mild to moderate, necrosis in the legs. This reason may also explain similar results in the works of Chou et al. (2008).

2.2.6. Algal toxins.

Algal toxicity is a condition that usually occurs in natural phenomena commonly denominated Harmful Algal Blooms, HAB (Creekmore 1999). These events are the outcome of a sudden and rapid proliferation of several microscopic or multicellular organisms in aquatic environments capable of causing lesions or producing toxins that, directly or indirectly, harm or compromise the survival of several aquatic or terrestrial living beings in freshwater/marine ecosystem (Landsberg, Vargo, Flewelling & Wiley 2007). The effects of an HAB are diverse and include those that are directly felt in lower trophic levels like: predation (of fish for instance by *Pfiesteria piscicida* on the word of Bushaw-Newton & Sellner 1999), oxygen depletion (overgrowth, solar occlusion, death and nocturnal respiration stated by Anderson 2009), hunger (in molluscs by, as an example, *P.minimum* that modifies enzyme production of these beings, indicated by Bushaw-Newton & Sellner 1999), lesions or irritation (Bushaw-Newton & Sellner 1999 report that it can be caused by the presence of theses organisms or the action of their specific structures in fish), ultimately leading to massive killings of invertebrates, plants and fish; and those that depend on the effects of toxins produced by these organisms that apart from causing disease and/or death in molluscs, crustaceans and fish can turn

them inedible food items to other animals (Creekmore 1999;Sellner, Doucette & Kirkpatrick 2003). And thus, these biotoxins can be the cause of disease and mortality outbreaks in reptile, amphibian, fish, mammal and bird species, with clinical presentations that encompass: the digestive tract, respiratory apparatus, cardiovascular and nervous systems (Shearn-Bochsler 2008). Even the integument of such species can be affected, as was reported in a mass stranding of marine birds in the winter of 2007 in Monterey bay, California, caused by a surfactant producing red tide on the word of Jessup et al. (2009). Indirect effects such as immune depression/immunosupression, cancer, or reproductive failures are also extremely important and may have serious repercussions in a number of conservational efforts to protect vulnerable or even endangered species as is suggested by Shumway, Allen and Dee Boersma (2003) and Goldstein et al. (2009).

Nowadays the reporting and documentation of HABs is increasing and since these biotoxins were identified as possible causes of numerous mortalities in wild birds, including seagulls (Creekmore 1999), as well as eliciting paresis or paralytic presentations, the addition of this differential in this review could not be disregarded.

2.2.6.1. Pathogenic agents and epidemiology.

The entities in these algal blooms are microalgae, like phytoplankton, algae such as dinoflagellates, (e.g. *Gymnodinium* sp*., Karenia* sp., *Alexandrium* sp…), diatoms (*Pseudonitzschia* sp.), but cyanobacteria (ex: *Microcystis* sp., *Anabaena* sp., *Aphanizomenon* sp., *Nodularia* sp., *Oscillatoria* sp…) are likewise capable of causing these phenomena (Creekmore 1999;Landsberg et al. 2007). Several species of such groups were identified as causative agents of HABs, however new species, previously considered innocuous, may belong to the toxic groups, given that new techniques/procedures to detect and identify these organisms are being developed and an increase in the awareness to this kind of pathogen and environmental factors that influence their life cycle has been seen (Glibert, Anderson, Gentien, Granéli and Sellner 2005 citing Burkholder 1998). Regardless of this fact, in general, these organisms are found all over the world in aquatic/marine environments (Shearn-Bochsler 2008).

The life cycle of these species has several phases. Initially there is a rapid reproduction stage, triggered by species-dependent optimal conditions (high temperatures, nutrients, among others), that is followed by an exponential growth, regarded as the toxic period, during which a progressive depletion and degradation of the ecosystem continues. The stationary phase is the next point in the cycle and, in certain cases, is the one responsible for the discoloration of surface waters, seen and named as red tides or blue/green blooms due to the association of this finding with high densities of algae or toxic protists' pigmented cells (Creekmore 1999;Shearn-Bochsler 2008). During these periods cellular death is a constant and can culminate in a "population crash" leading to the so called "dead zones" owing to the massive death of the toxin producing organisms, consequent release of such toxins, and bacterial growth that originates and maintains a toxic, and hypoxic zone incompatible with life (Shearn-Bochsler 2008).

The types of phycotoxins that can be produced in the exponential growth phase are numerous and exhibit different characteristics: chemical structures, intrinsic potentials, pathogenic mechanisms and thus, different clinical presentations and necropsy findings can be found. Additionally, different global distributions of such events defined by the occurrence of the producing organism, sometimes in

specific regions and depending on the optimal conditions required for its development, influences the production of these toxins and the geographic expression of disease or mortality outbreaks (Creekmore 1999;Sellner et al. 2003;Shearn-Bochsler 2008;Anderson et al. 2011;Brand, Campbell & Bresnan 2012;Trainer et al. 2012). Accordingly, toxins that exert their effects in more marine habitats like Domoic acid and its isomers are produced by *Pseudonitzchia sp.* and have been found almost with a worldwide geographical distribution; Saxitoxins and derivatives are produced by *Alexandrium sp.*, *Gymnodinium sp.*, *Pyrodinium sp.* (among others) and were detected all over the globe; brevetoxins of *Karenia sp.* (*Gymnodinium sp.*) exert their effects more on the American coast (but are found worldwide); Okadaic acid and Dinophysistoxins of organisms like *Dinophysis sp.*, *Procentrum lima* and *Procentrum concavum* are identified globally; and Ciguatera toxins, normally noted in coral reefs and warm waters globally, are produced by *Gambierdiscus toxicus* among others (Creekmore 1999;Sellner et al. 2003;Shearn-Bochsler 2008). Cyanobacterial toxins of the species *Anabaena sp.*, *Planktothrix sp.* and *Nodularia spumigena* are normally found in freshwater environments, such are the cases of anatoxins (globally in rivers and lakes), microcystins (globally in similar ecosystems as the previous), cylindrospermopsin (tropical and subtropical regions of Africa, Australia, Central/South America and Asia, but also in temperate regions of Europe, Central Asia and North America) and nodularins (in waters of New Zealand, Australia and in the Baltic sea); however a few species of cyanobacteria like *Anabena circinalis* and *Cylindrospermopsis raciborskii* can also produce some algae or protist toxins, for instance saxitoxins (Creekmore 1999;Shearn-Bochsler 2008;Litaker et al. 2010;Brand et al. 2012).

The association of HABs with disease cases and die-offs in wild birds is rare because, in accordance with Creekmore (1999), Shumway, et al. (2003) and Landsberg et al. (2007), information of the effect of these toxins in wild species is limited as are the diagnostic techniques available, and deaths may occur in inaccessible areas or well after the bloom was noticed. Nevertheless, the co-ocorrence of HABs and avian mortalities is frequent and these toxins are often suspected of influencing these causalities, hence numerous species were identified as having possibly suffered and died from the toxic effects of such molecules. Various species of ducks, geese, grebes, songbirds, pelicans, cormorants, fulmars, shags, murres, loons, shearwaters and scaups are examples of invertebrate or fish–eating birds that were identified as being victims in these events as is stated by Creekmore (1999) and several studies cited by Landsberg et al. (2007). Some of which allude to cases where seagulls like *L. argentatus*, *L. marinus, L. fuscus, L. canus, C. ridibundus, R. tridactyla, L. pipixcan* and *L. atricilla* were affected by saxitoxins, brevetoxins, anatoxins-a, microcystins or domoic acid, in continents like Africa, America and Europe (several countries: England, Russia, Norway…). Nonetheless, factors like: toxin and its characteristics, producing organism, susceptibility of the species and location where the blooming event took place dictate what species could be affected (Creekmore 1999). Nevertheless, cues of behavioural displays of detection, aversion and rejection of toxic food items (or the most toxic tissues), as well as regurgitation by some birds to prevent intoxication exist (Kvitek & Bretz 2005;Landsberg et al. 2007) and for instance were documented in wild adults, juveniles and chicks of *L. glaucescens*, in a controlled feeding experiment with bivalves by Kvitek (1991). Therefore, gulls have only been intoxicated by brevetoxins, saxitoxins, domoic acid, anatoxins, mycrocystins, while shags and cormorants, as stated by Landsberg et al. (2007), are believed to be the most affected, perhaps due to their relative abundance, feeding habits (fish,

primarily pelagic) and costal habitat, particularities that together with the absence of avoidance behaviour promote the intoxication phase and early detection of cases when compared with other species.

Although HABs are historically natural occurring events (Creekmore 1999;Daranas, Norte & Fernández 2001） where wind, oceanic currents, and other climatic elements spontaneously alter the density and distribution of the microorganisms and factors, enabling or not the occurrence of such phenomena (Sellner et al. 2003); HABs currently are regarded as recurrent phenomena, in expansion, persistent in certain sites and gaining a more dramatic expression in terms of observable frequencies, durations and intensities, predominantly when human involvement and its activities are taken into consideration (Creekmore 1999;Sellner et al. 2003;Landsberg et al. 2007;Tiffany, Wolny, Garret, Steidinger & Hurlbert 2008;Anderson 2009). This artificial presence is essentially due to the existence and/or accumulation of several pollutants with origins set in numerous aquatic and marine ecosystems throughout the world creating the optimal physical, biological and chemical conditions for the formation of such tides, especially when regarding phosphorus and nitrogen loadings (Creekmore 1999). With the advent of aquaculture and marine cultures, as stated by Sellner et al. (2003), new increases in the incidence of these blooms were and are foreseeable. Modifications in some aquaculture and agricultural practices, impoundments of rivers, human wastes (mines, industry, sewage… Buss & Bengis 2012), overfishing, ecosystem invasion by exotic species, ballast water discharges, global warming (Glibert et al. 2005) are additional influencing factors for the occurrence of HABs, in addition to long distant marine transportation or commerce systems that disperse these potential pathogenic organisms and worsens the global panorama (Creekmore 1999). In spite of this, sporadic major HABs are the ones that gather more attention, because, according with Shumway et al. (2003) quoting Anderson (1995), these are the source of the drastic mortalities seen in commercial species and exert important direct/indirect social and economic impacts (Anderson 2009). Nevertheless, the lack of efforts to confirm the involvement of these toxins in wild avian fatalities, despite the survival and dispersal/persistence strategies displayed by some toxin producing species that further hinders this aspect and potentiates the occurrence of HABs (e.g: cyst formation in suboptimal conditions among others detailed by Smith, Grant, Ferguson & Gallacher 2001;Sellner et al. 2003;Glibert et al. 2005;Landsberg, Flewelling & Naar 2009), knowledge regarding the causes of blooms is still limited. Thus, the determination of seasonal patterns (Creekmore 1999) or even geographical ones (as previously stated) is sometimes an impossible task and any attempt to predict such events is unrewarding.

2.2.6.2. Pathogenesis, Disease, Signs and Lesions.

Oral exposure is the main route of intoxication in birds, either by the direct contact with water, in the case of cyanobacterial toxins, or by ingestion of prey items that contain these toxins, chiefly fish and invertebrates in the cases of domoic acid, brevetoxins and saxitoxins (Creekmore 1999;Lowenstine 2008;Kreuder et al. 2002). The latter toxins can, in turn, suffer biomagnification/accumulation phenomena throughout the trophic chain (Creekmore 1999), however, although evidence of such finding is still limited in the case of cyanotoxins, these might also undergo a similar process (Buss & Bengis 2012). Biotoxins in these levels can be: further metabolized, retained differently in several tissues for long periods of time, excreted, degraded or bio transformed. As a consequence,

persistence of these toxins or microorganisms in the environment, well after a toxic bloom has dissipated, is a possibility, and latent stable sources of toxicity can endure in the food web, some potentiating or not the original intrinsic toxicity of the molecule and others suffering or not from its effects at natural doses (Lefebvre et al. 2007;Naar et al. 2007;Lowenstine 2008;Landsberg et al. 2009). In turn, in higher trophic levels, these conditions are the ones that guarantee the occurrence of lethal thresholds, which can be the cause of disease and mortality in several species (Blanco, Moroño, Franco & Reyero 1997;Sellner et al. 2003).

Immature or juvenile birds may be the most susceptible groups to this condition, because they tend to ingest toxic prey and suffer from this toxicosis more easily, possibly owing to inexperience in foraging for food, opting to capture and ingest dead, moribund or toxic prey as Kreudel et al. (2002) suggested and confirmed in their study of *P.auritus* (presumably intoxicated by brevetoxins). High levels of parasitism seen in these immature birds is another reasonable explanation for this increased susceptibility, as this finding is frequent in this age group and is associated to a compromised function of the immune system (Nisbet 1983;Van Deventer 2007). Taking into consideration a possible gender differential distribution of cases and/or susceptibility/sensitivity, pre-laying females appear to be more frequently affected, though no explanation was given for this finding seen in two species of terns (*Sterna hirundo* and *Sterna dougallii*) intoxicated by saxitoxins in Massachussetts in 1978 and documented by Nisbet (1983).

 Data concerning toxicokinetics, toxicodynamics and ecosystem impacts, on the word of Shumway et al. (2003) quoting Smayda (1990) and Shumway (1995) is still insufficient, although the study of several biotoxins, pathogenic mechanisms, affected species and effects have already been subject of attention, still prevails human and mammals studies and there have been relatively few experimental projects. Moreover, the pathogenesis is very diverse and varies with the toxin in question. Sometimes more than one system is affected and more than one type of alteration/lesion is seen. Clinical signs displayed by wild birds, although closely temporally connected with fatalities in association with the occurrence of blooms, vary greatly between different species or phyla and depend on numerous factors, hence and in agreement with Landsberg et al. (2007), systematized syndromes for these toxicosis are not described in wild birds, in opposition to what is seen in humans. Furthermore, these intoxications are normally acute cases where the manifestation of clinical signs, recovery and death are quick events, as was probably the case of the study of Gochfeld and Burger (1998), in which wild captured *L.argentatus* chicks were fed diced scallop muscles that probably contained a marine biotoxin (further attempts to identify several known biotoxins were inconclusive). In this case in less than an hour neurologic signs of paralysis and dyspnoea were seen (14 out of 20 gulls), in 2 hours 4 out of 20 birds died and after approximately 45 minutes, in severe cases, signs of recovery were recorded. Sub-chronic or chronic exposure to these phycotoxins is a possibility and might be an additional risk that can induce reproductive impairment, like the case of cyanotoxins in experimental conditions such as the one of Damkova et al. (2011). Considering the different toxins and information available, brevetoxins can lower the activation threshold of voltage-dependent sodium channels in nervous cells (sustaining depolarisation), they induce haemolysis, are anticoagulants and immunesupressors (like ciguatera toxins) (Lowenstine 2008;Shearn-Bochsler 2008). These toxins in the case of *Aythya affinis*, were responsible for: lethargy, weakness, reluctance or inability to fly or maintain the neck erect, diminished reflexes, excessive nasal/oral and ocular discharges (respectively

translucid and viscous), preening gland dysfunction, chalky yellow diarrhoea, dyspnoea, tachypnoea, tachycardia, dehydration and decreases in the body's temperature and blood´s pressure, documented by Forrester et (1977) on February and March 1974 in Tampa Bay. Whereas in a die-off of *P. auritus*, described by Kreuder et al. (2002) in the southerwest coast of Florida, severe cerebellar ataxia (truncal incoordenation, broad-based stance, hypermetric gait and head tremors), stimuli hyper excitability and positional vertical nystagmus were the clinical signs noted and probably are the result of the exposure to these toxins. Gulls have also suffered the effects of this group of toxins as is stated by Van Deventer (2007) (fig.75-77) given that the possible ingestion of brevetoxins in *Opisthonema oglinum* (fig.77) was sufficient for the manifestation of clinical signs of neurologic origin, like inability to right themselves, or a sub-lethal exposure was appropriate to induce alterations on the immune system or anomalous behaviour that were responsible for the death of a flock due to lightning strike (fig.75).

Figure 75 – Flock of *L.atricilla* and *Thalasseus maximus* dead in the beach of Siesta Key, Sarasota County, Florida 25/08/2005.
Figure 76 – *L.atricilla* retrieved from Tampa bay and killed after exhibiting neurologic signs and in distress.
Figure 77 – *Opisthonema oglinum*, recovered from their stomach and positive for high levels of brevetoxins .
Adapted from: Van Deventer (2007).

Domoic acid is an excitatory neurotoxin that binds to glutamate receptors, in the central nervous system, leading to neuronal excitotoxic death (Lowenstine 2008). Muscle tremors, side-to-side head movements, pouch scratching, anomalous flight, slightly extended wings, torticollis, toe clenching, opisthotonus, vomit and loss of righting reflex were signs reported as a result of this toxin effects in *Pelecanus occidentalis*, although in the same event *Phalacrocorax penicillatus* only lost the fear of human presence and manipulation (Creekmore 1999;Landsberg et al. 2007). Okadaic acid and dinophysistoxins inhibit protein phosphatases and are promoters of tumours (Shearn-Bochsler 2008), however this neoplasic potential hasn´t been proven yet in birds, as is declared by Landsberg et al. (2007). Freshwater toxins like microscystins and nodularins, phosphatases inhibitors, have the same potential for tumour formation as the former, but are also hepatotoxic (Shearn-Bochsler 2008).

The phycotoxins that actually are responsible for paralytic presentations (and reported to induce this sign) are the saxitoxins, anatoxin-a and anatoxin-a(s). The first by interacting with voltage-gated sodium channels preventing the transmission of the nervous impulse through blockage of sodium influx (Lowenstine 2008); anatoxin-a because they are post-synaptic cholinergic nicotine agonists that block these receptors in neurons and neuromuscular junctions, and anatoxins-a(s) because they are potent acetycholinesterase inhibitors (Onodera, Oshima, Henriksen & Yasumoto 1997;Shearn-Bochsler 2008). Vomit, yellowish or greenish faeces around the vent with protruding cloacas, paralysis (inability to stand or move wings) and sudden death were findings documented in saxitoxin intoxication and mortalities of several seabirds including larids, *L. argentatus,* and terns, *S.hirundo*. These signs were also documented in *P.aristotelis* with the addition of loss of equilibrium and motor coordination,

pupil constriction, green to brownish faecal matter (Nisbet 1983;Creekmore 1999;Shearn-Bochsler 2008). According to Landsberg et al. (2007), the clinical presentation of anatoxin-a ressembles the saxitoxin presentation and normally other findings can be detected, because cyanobacterial blooms are mixed events of species and toxins.

Rapid recrudescence of clinical signs of phycotoxicosis has been documented by Kreuder et al. (2002) in particular in brevetoxicosis and was defined as an event either owing to a second exposure after release or a delayed manifestation of the first exposure.

Many of these toxins exert their effects mainly in an electrical/chemical level, and so gross or microscopically diagnostic lesions are not found or/and described. When present they are: nonspecific (multiorgan hemossiderosis - brevetoxin; necrosis and haemorrhage of the skeleton muscle - domoic acid), inconsistent, and/or aren´t severe enough to cause death, although toxins that produce hepatoxicity cause haemorrhage, degeneration and necrosis and acute or chronic exposure to biotoxins dictates different body scores and degrees of fat deposition (Forrester et al. 1977;Creekmore 1999;Kreuder et al. 2002;Landsberg et al. 2007;Skocovska et al. 2007;Massango et al. 2010).

2.2.7. Heavy Metals – Lead and Mercury.

Heavy metals are a vast group of elements that can be important to the metabolism (some participate in several enzymatic reactions) or in some cases are not essential and are deleterious. Despite this distinction, these two kinds can be extremely toxic when in certain circumstances the organism's "control mechanisms" are compromised and toxicity arises. The cases of lead and mercury poisoning are examples of intoxications that occur in wildlife due to the effects of non-essential heavy metals and represent a good marker of environmental contamination of utmost importance nowadays, given the age of progress lived in these past decades. Such trace elements in the wild fauna have serious consequences and can be accounted responsible for individual impacts due to disease, death or even reproductive disorders (Lewis & Schweitzer 2000). In terms of population dynamics, these are also capable of detrimental effects, leading to serious declines, as is the case of lead and the *Gymnogyps californianus*, among several other cases of endangered/threatened species (Mateo 2009;Taggart et al. 2009;McLelland, Reid, McInnes, Roe & Gartrell 2010;Burgess, Bond, Hebert, Neugebauer & Champoux 2013). These intoxications are known as invisible diseases and although reported since the nineteenth century (at least clearly in the case of lead), are currently, still implicated in mortality events (Rodríguez et al. 2010), some with clinical presentations including neurologic signs such as paresis/paralysis. Their importance is heighten at the present time, because worldwide they are diseases of difficult management due to inconsistencies in regulations seen in the case of Plumbism and conflicts of interests hinder resolutions, even when several studies to present alternatives to this problem are presented (regulation for the use of lead ammunition in hunting sites and/or with the purpose of shooting birds just in the European Union varies from country to country, in the same nation by region or even by species); and the need for a global and uniform approach/intervention in the case of Mercury (Irwin 1972;Brewer, Fairbrother, Clark & Amick 2003;Fisher, Pain & Thomas 2006;Mohapatra, Nikolova & Mitchell 2007; Mateo 2009;Lambert, Evers, Warner, King & Selin 2012). Thus, these are possible differentials for the syndrome in question and, in a way, given the multitude of existing compounds, are here detailed as representatives of the deleterious effects developed after exposure to contaminants with different pathogenesis (i.e. bioaccumulation versus no accumulation).

2.2.7.1. Lead.

2.2.7.1.1. Pathogenesis.

It is the most common intoxication by heavy metals in birds and according to Samour (2000) is probably the most frequent intoxication worldwide. Plumbism or lead intoxication is caused by the absorption of perilous levels of lead into several tissues of the organism (Friend 1999b), leading to disease or mortality events. The exposure to this contaminant differs from species to species, in accordance with: the habitat where it dwells, the technique exhibited while foraging for food, and even the attractiveness that some possess for human purposes (e.g. hunting). This exposure is mainly due to 2 situations. The first occurs by the ingestion of anthropogenic material containing lead: bullets/pellets/disintegrated pellets; solder; cable sheathing; steel strips; batteries; stained glass or lead fishing weights/sinkers (Irwin 1972;Daury, Schwab & Bateman 1993;Ochiai, Hoshiko, Tsuzuki & Itakura 1993). Other possible sources include mine and smelting wastes, especially in waterfowls and passerines as seen by Blus, Henny, Hoffman & Grove (1995) and lead-laden paint chips, which were responsible for an epizootic mortality of *Diomedea immutabilis*' fledglings in 1982-83 in Hawaii, Midway Atoll (Sileo & Fefer 1987;Work & Smith 1996). The ingestion of these examples is normally seen as attempts to forage for food or grit, whereas the second case is the result of the consumption of prey/carcasses/discarded carcasses or viscera with lead embedded in the flesh or contained in the gastrointestinal tract by predatory or necrophagous birds (Friend 1999b;Almansour 2004;Friend, Franson & Anderson 2009;Mateo 2009). Several other authors suggest alternatives for lead intoxication in meat-eating birds, like the ingestion of carcasses and prey that already have Lead incorporated or bio accumulating on several of its tissues, but such cases are unlikely reported (Eisler 1988 citing Custer, Franson & Pattee 1984, Pattee 1984 and Stendell 1980;Gordus 1993 citing Benson, Pharaoh & Miller 1974;Custer et al. 1984). Also unlikely is the relationship between embedded shots and lead poisoning in the same bird, as on the word of Eisler (1988) is not yet very clear, but apparently this lead has a lower biologic availability and may not cause acute cases of toxicity (Guillemain et al. 2007 and Martin et al. 2008 quoted by Helander, Axelsson, Borg, Holm and Bignert 2009), as was verified in an experiment of Sanderson et al. (1998), where no detrimental effects were documented. A third type of exposure, which is also not so commonly described, is, possibly, due to lead derived from human activities like industrial and urban pollution, that disperses by and/or is incorporated in air, soil, water and ultimately in the food chain (Eisler 1988;Kalas, Steinnes & Lierhagen 2000) potentially bio concentrating in the form of alkyllead compounds in aquatic food items or sediments, that could be ingested by birds and causing intoxication (Eisler 1988;Blus et al. 1995;Blus, Henny, Hoffman & Grove 1993 cited by Buekers, Steen Redeker & Smolders 2009). Vertical transmission could be another plausible route to consider for intoxication purposes (Burger & Gochfeld 1995a).

Hence, all levels of the trophic chain can be affected by the presence of lead as is stated by Eisler (1988). Several different ecosystems can be affected, but the most important and frequently documented include regions with intense hunting activities, where waterfowl or other type of game birds can ingest lead or get shot during winter and suffering the disease on the first case, or being crippled in the second, are the ideal targets of predators and untimely, in due course, are the reason of secondary intoxication of the latter (Eisler 1988). However, and in turn, species considered opportunistic or that forage for food in heavily travelled roads, landfills or other disposal sites, mineral

prospection sites, smelters, refineries, or metal industries are also at increased risk of exposure to lead and suffer from this disease (Eisler 1988;Burger & Gochfeld 1995b).

Normally these outbreaks are not an "once in a time event" since the presence of carcasses with lead pellets or even the pellets alone in the environment can ensure an uninterrupted cycle of new cases of intoxication that could prolong for years (Friend 1999b). This may happen when this heavy metal becomes ubiquitous in the ecosystem, despite new legislation for interdiction of the use of lead shots, and intoxication may develop as was probably the case reported by Lagerquist, Davison & Foreyt (1994) in swan species in Washington between1986-1992. These outbreaks aren't also a "once in a place" events, because migration of numerous avian species is another reason that could substantiate the fact that, although there are regions with limited use of lead ammunition, the dispersion of birds to other regions may enhance the risk of exposure (Wheeler & Gates 1999;Degernes et al. 2006). Therefore, specific patterns for geographical distributions of lead intoxication cases are of difficult accomplishment. Also, in agreement with Friend (1999)b, dispatch of carcasses is another hindering factor for this analysis, given that the retrieval of such animals is influenced by the attractiveness of the region for vigilance and reports, and so   wintering grounds and the ones used in the spring migration are usually chosen (Lagerquist et al. 1994), the aquatic ecosystems with intense fishing or hunting or even upland or lowland shooting sites, in the case of terrestrial species, are the ones also elected (Ferreyra, Romano & Uhart 2009;Pain, Fisher & Thomas 2009 quoting both Elliott et al. 1992 and Wayland and Bollinger 1999). Additionally, the economic/recreational importance of the affected species is another factor that influences the retrieval of sick and/or dead birds (Friend 1999b) and likely influences the perception of the ecology of this intoxication and other components of its pathogenesis. Deaths can occur throughout all the year, however the exposure is more likely to take place in the hunting season, and during spring migration (Franson, Petersen, Meteyer & Smith 1995;Friend 1999b). Winter can also be an important season for exposure when the daily diet is enriched in certain types of food like cereals and grains (Wheeler & Gates1999).

Numerous species are afflicted by this condition, but waterfowl species are the most frequent, by far the most studied, but also the most hunted (Friend 1999b;Carpenter et al. 2003 quoting Sanderson and Bellrose 1986). Even though the expression of the disease in terms of observed frequency decreases with specialization of the food habits and the incorporation of a larger percentage of fish in the daily diet, these species are also the most affected because they tend to feed in the bottom of shallow lakes where lead can be easily retrieved (Lagerquist et al. 1994). However, that does not preclude other species from suffering this intoxication (Wilson, Oyen & Sileo 2004). And, by this way, aquatic birds, particularly marine like gulls (*L. argentatus*, *L. californicus* and *L. glauscescens*) have a low risk for exposure and occasionally are victims of mortality events (Friend 1999b). Similar situation is seen in the case of upland gamebird, nonetheless in Europe these have already suffered from lead poisoning in regions of high frequency of hunting (Friend 1999b;Lewis & Schweitzer 2000;Mateo 2009). In the case of predatory wild birds the case is different, but altogether similar to what has been said, being the more commonly reported as ill or dead: eagles (greater number of scientific papers published, but nocturnal birds like *Asio otus*, *Bubo bubo* and *T. alba* can also be casualties), and obligatory or occasional necrophagous like *Gyps fulvus* and *Aegypius monachus* are also affected (Friend 1999b). And so any bird, on the word of Pattee, Wiemeyer, Mulhern, Sileo and Carpenter (1981) and Beyer, Spann, Sileo and Franson (1988) (quoted by Carpenter et al. 2003) is susceptible

to the effects of lead, but the concentration of lead accumulated in the organism, the signs and behaviours displayed and the lesions presented are distinct, either between different species or within the same species, and several factors influence these aspects, both individually or in relation with the environment, like the ones about to be discussed.

The different concentrations in tissues or deleterious effects of lead on wild birds depend, individually, on: the degree of absorption of the metal and its bioavailability (Custer et al. 1984); species; physiological condition; diet (Rocke & Samuel 1991; Friend 1999b;Rodríguez et al. 2010); possibly genetic predisposition (Pattee et al. 2006); age, juveniles are more susceptible owing to different foraging behaviour, for instance the previously mentioned fledglings of (Work & Smith 1996), or superior food intake rate like the case of juveniles *B. canadensis* in the study of Wheeler and Gates (1999) citing the hypothesis of DeStefano, Brand, Rusch, Finely and Gillespie (1990); and stress (Rocke & Samuel 1991;Lagerquist et al. 1994 quoting Pain and Rattner 1988; Pattee et al. 2006). Gender, it is a little controversial, because field studies and experimental tests indicate either high susceptibility to exposure and high concentration of lead in tissues in males, like the ones of Rocke and Samuel (1991), Gangoso et al. (2009) and Rodríguez et al. (2010); or females, for instance *L.argentatus* in the study of Burger & Gochfeld (1995); or equally both as stated by Munoz, Hacker and Gesell (1976) and Helander et al. (2009), to name a few. However some of these authors indicate that physiological differences in the absorption and accumulation of this metal in post breeding or breeding females, particularly its incorporation in bones and excretion in eggs or different techniques used to search for food or chosen habitats to dwell while foraging, are plausible reasons for the difference seen in gender susceptibility and severity of the disease. The environmental factors that influence the outcome of the exposure and absorption are the ones that enable them and include: hunting intensity, number and size of the pellets, type of compound (Eisler 1988), availability of grit, firmness of the soil, water depth (Street 1983 cited by Eisler 1988) as well as climate alterations. This last one was responsible for an epizootic in flamingos species in 1992-93 and 1993-94 in El Fondo and Santa Pola where scarce rainfall, low water levels, presence of rice and hunters calling allowed this event to develop (Mateo, Dolz, Aguillar Serrano, Bellirure & Guitart 1997), a similar case was seen in a park in Massachusetts with the death of 200 *B.canadensis* in the winter of 1983-84 (Windingstad & Hinds III 1987).

2.2.7.1.2. Pathology – signs and lesions.

Lead intoxication is normally a chronic disease and the die-offs usually result in inability of necrophagous species to cleanse the area (Franson & Smith 1999). Nevertheless, sub-lethal doses have been implicated in decreases in reproductive functions, anaemia, immunosupression, cardiovascular degeneration and lesions in the peripheral and central nervous systems, compromising survival (Pattee, Wiemeyer, Mulhern, Sileo & Carpenter 1981 cited by Carpenter et al. 2003;Rocke & Samuel 1991;Wheeler & Gates 1999 citing Buerger, Mirarchi & Lisano 1986; O'Halloran, Meyers & Duggan 1989,Franson 1986, Karstad 1971 and Hunter & Wobeser 1980). Acute exposure/intoxication is rare (Eisler 1988). Lead after the ingestion, mechanically and chemically is reduced to lead toxic salts on the gizzard (Eisler 1988 citing Street 1983) and in accordance with Dieter and Finley (1979) cited by Blus et al. (1995) negatively exerts its effects in every organ, inhibiting several enzymes indispensable to every cell, alters the parenchyma and function of organs like kidneys, bone, nervous

and hematopoietic systems as well (Scott, Hwang, Jurkowitz and Brierley 1971 cited by March, John, McKeown, Sileo & George 1976). This heavy metal is also the basis of biochemical, histological, neuropsychological and reproductive abnormalities and a cause of foetal toxicity and teratogenic manifestations (Boggess 1977;Nriagu 1978;De Michelle 1984 all quoted by Eisler 1988). Lead accumulates in bone, kidney and liver tissue and only small amounts are found in muscle tissue (Eisler 1988; Wojcik 1980, Coleman, Elder, Basu & Koppennal 1992 and Kalas & Fjolstad 1995 cited by Kalas et al. 2000). In sensitive avian species, survival was hindered at oral doses of 50 to 75 miligrams Pb2+ for each kilo of body weight (bw) or 28 mg organolead/kg bw, whereas reproduction was compromised at dietary levels of 50 mg Pb /kg, with evident signs of poisoning at doses of at least 2,8 mg organolead/kg bw (Eisler 1988).

The clinical signs found are typically characteristic of chronic presentations (Gill & Iangelier 1994 quoted by Pain et al. 2009) and may only appear following the first week after ingestion, with the deaths beginning in 2 and 3 days or even 2-3 weeks afterwards (Friend 1999b; Sanderson et al. 1998 and Lagerquist et al. 1994, these citing Pain e Rattner 1988; Lewis & Schweitzer 2000 citing Pain 1992 and Butler 1996; Wilson et al. 2004). These signs are unspecific, diverse and include behaviour alterations associated with the neurobehavioral development, fine motor function, endurance, performance and learning ability, locomotion, begging behaviour, growth, thermoregulation, individual recognition, depth perception and balance in the case of chicks of *L. argentatus,* on the word of Burger and Gochfeld (1995a/b, 2004, 2005a/b); overall reducing survival and hindering it. Physical, peripheral and central neurological, gastrointestinal, kidney, reproductive and haematological deviations, expressed and described as initially inaptence, loss of appetite, polyuria, weakness and aqueous bright green diarrhoea or bile-stained faeces are other signs documented (Bates, Barnes & Higbee 1968;Irwin 1972;Eisler 1988;Mateo et al. 1997;Friend 1999b;Nakade et al. 2005;Rodríguez et al. 2010;Pikula et al. 2013). Ataxia, reluctance/inability to fly (or bad technique: small distances flown and anomalous landings) and stance disequilibrium, associated with impaired locomotion while running away, in spite of alertness and normal mental status, are seen following the previous signs described, although in the case studied by Rodríguez et al. (2010) in captive *A.platyrhynchus* a reduction or even absence of response to several stimuli was noticed. With the progression of the disease, birds: cannot fly; exhibit paresis/paralysis of the wings or legs, when the wings are affected the bird tends to keep them close in a "roof shaped" position over the dorsum, but with the progression of the intoxication the wings are drooped and remain abducted, as was the case of the albatrosses in 1982-83 reported by Sileo & Fefer (1987); and some convulse (Wobeser 1969;Irwin 1972;Eisler 1988;Friend 1999b;Brewer et al. 2003;Nakade et al. 2005;Rodríguez et al. 2010). In the disease event described by Sidor et al. (2003) in *G.immer* it was noted panting and dyspnoea accompanying head tremors, but it can also be seen beak discharges (Friend 1999b).

In general, birds can be suspects of lead poisoning when they do not flee when approached, don´t initiate migration at the same time as the other inmates and may stay behind. Or in the case of the hunting season are confused by humans as birds shot and/or crippled (Friend 1999b).

Death usually results of secondary infection, predation, famine or misadventure (Pattee et al. 1981;McLelland et al. 2010), as was probably the cause of 1% of 400 *Cygnus boccinator* and *Cygnus columbianus columbianus* that died due to collision with power lines possibly in the course of a lead induced encephalopathy, as suggested by Degernes et al. (2006).

Necropsy findings are also unspecific and normally allude to emaciation by chronic disease (weight loss, low pectoral scores and lack of fat deposits), which is accompanied by: green staining of the feathers of the vent (aqueous bright green diarrhoea); impactation and/or dilatation of the oesophagus and/or proventriculus; bile-distended gall bladder (dark or bright green); the yellow kaolin lining of the ventriculus is stained, appearing darker than usual or discolorated to green by bile like the gastric contents (it can also be denoted hyperkeratosis, Ochiai et al. 1993 and Wilson et al. 2004); liver and kidney may be greenish to grey; greenish to blue-gray slate discoloration can be seen in the intestines, as well as a moderate to severe congestion (Locke & Bagley 1967;Bates et al. 1968;Wobeser 1969;Irwin 1972;March et al. 1976;Windingstad & Hinds III 1987;Eisler 1988;Ochiai et al. 1993;Blus et al. 1995;Mateo et al. 1997;Friend 1999b;Ochiai, Kimura, Uematsu, Umemura & Itakura 1999;Lewis & Schweitzer 2000;Brewer et al. 2003;Carpenter et al. 2003;Wilson et al. 2004;Nakade et al. 2005;Degernes et al. 2006;Rodríguez et al. 2010;Pikula et al. 2013). Pellets or lead fragments may be found in the lower oesophagus, ventriculus or proventriculus, sometimes deformed by chemical or mechanical action, or in muscular masses or subcutaneous tissue. This finding isn't a proof of intoxication by lead, but its absence doesn´t foreclose this toxicosis, since regurgitation may happen (Locke & Bagley 1967;Bates et al. 1968;March et al. 1976;Blus et al. 1995;Friend 1999b;Lewis & Schweitzer 2000;Nakade et al. 2005;Pain et al. 2009). Anaemia and concordant signs of pale mucosae and conjunctiva, pale organs and atrophy is present and indirectly is due to the presence of lead and subsequent intoxication (Bates et al. 1968;Nakade et al. 2005;Pikula et al. 2013). In the particular case of swans, their heads appear swollen or puffy-like due to cephalic oedema that is seen in this disease (Windingstad & Hinds III 1987;Friend 1999b). In acute presentations, good fat deposits are seen and almost no muscle atrophy or lesions are detected (Sileo & Fefer 1987;Windingstad & Hinds III 1987;Franson & Smith 1999;Sidor et al. 2003;Degernes et al. 2006). Microscopic lesions of fibrinoid degeneration/necrosis of the arterial's medium tunics, is considered a typical lesion in waterfowl cases of lead intoxication (Karstad 1971 cited by Franson et al. 1995), but was also found in other species like bald eagles (Pattee et al. 1981), nevertheless was absent in the observations of Franson et al. (1995) of *Somateria fischeri* and *Somateria mollissima* in Alaska between 1992-1994. Skeletal muscle necrosis, myocardial necrosis/fibrosis (Franson et al. 1995), hemosiderin accumulation in Kuppfer cells (Bates et al. 1968;Sileo & Fefer 1987;Carpenter et al. 2003;Sidor et al. 2003), hepatocellular atrophy and necrosis (Sileo & Fefer 1987;Pikula et al. 2013), nefrosis and epithelial acid fast positive intranuclear inclusions in the renal tubules (Locke & Bagley 1967;Bates et al. 1968;Irwin 1972;March et al. 1976;Sileo and Fefer 1987;Carpenter et al. 2003;Nakade et al. 2005;Pattee et al. 2006;McLelland et al. 2010l) are other findings observed. However and as previously stated, these are not patagnomonic lesions, particularly the renal inclusions, as in other studies, Pattee et al. (1981);Ochiai et al. (1993);Sidor et al. (2003), these findings were the less frequently reported, perchance owing to a short exposure time to a large concentration of this pollutant in a sudden onset of intoxication - the levels of lead far exceeded in these cases the values described in the literature (Locke et al. 1967). Neurologic lesions found and documented in lead intoxication events/experiments include demyelination of the peripheral nerves (Degernes 1995), perivascular and perineural brain oedema and spongiosis of the white matter of the cerebellum, brain and medulla oblongata (Carpenter et al. 2003;Pattee et al. 2006).

2.2.7.2. Mercury.

Mercury, according to Franson (1999) and Boening (2000), is a non-essential and toxic metal for vertebrates and invertebrates and like lead exists naturally in several ecosystems. It occurs in several redox stages, but in low concentrations in the soil and sediments suffering natural processes of global recycling (volcanic emissions, weathering rocks, water evaporation, atmospheric transport and deposition in soil and water, forest and grass burns, animal secretions, water currents and floods), and so, is found in the soil, water and air. However, it is also released in considerable quantities by human activities in these mediums (Franson 1999;Boening 2000;Ochoa-acuña, Sepúlveda & Gross 2002;Clarkson & Magos 2006;Mohapatra et al. 2007;Berry & Ralston 2008). In these two last centuries, this artificial input almost doubled the natural ones (Arcos, Ruiz, Bearhop & Furness 2002 quoting Nriagu 1989, Thompson 1996, Cossa, Martín, Takayanagi & Sanjuan 1997 and Monteiro, Granadeiro, Furness & Oliveira 1999), corresponding to an increase of up to 2/3 folds in global deposition (Driscoll et al. 2007 cited by Seewagen 2010). It is mainly due to human activities like: industries that manufacture or use mercury compounds (chlor- alkali, electric equipment, paints, wood pulping, Boening 2000); the incineration of residues including medical wastes; the combustion of fossil fuel; the activity of nonferrous metal smelters and cement plants; discharges of industrial units, waste water and sewage facilities into aquatic ecosystems; mine prospection activity and its residues; acid rains; forests burns; and use of fungicides or other mercury based products in agricultural practices - e.g.waste sludge and mercury treated seeds (Franson 1999;Ochoa-acuña et al. 2002;Mohapatra et al. 2007). This increment is specially felt in the coastal and estuarine regions, but also in the Great Lakes and even in the Mediterranean sea (Koster, Ryckman, Weseloh & Struger 1996;Arcos et al. 2002;Marvin, Painter & Rossmann 2004), primarily in highly urbanized and industrialized sites, but also in nearby regions. This additional input of mercury, in a certain manner not natural owed to these humans practices, but also due to legacy sources or historical point sources - remnants of these activities that allow the perpetual contamination by persistence of mercury compounds and their remobilization in the biosphere (Lambert et al. 2012) - has the potential to suffer active biological phenomena of bio concentration, bioaccumulation and bio magnification in the aquatic and marine trophic chains, heightening the importance of this intoxication in wildlife (Franson 1999). Additionally, the conversion, although natural but nowadays augmented by human interference (Chan et al. 2003) of its inorganic form to organic by methylation in these ecosystems (Clarkson & Magos 2006), is another risk for animals that dwell in these environments, since the former is converted into a more toxic form - easily absorbed and with a longer half-life (Boening 2000;Clarkson & Magos 2006). And so, taking into consideration these aspects, the emergence of this disease, its pathogenic mechanism and clinical presentation and also the fact that gulls are birds that abide in these ecosystems and currently in propinquity to places of intense human presence, intoxication by mercury is a plausible differential for paresis/paralysis.

2.2.7.2.1. Pathogenesis.

Wild bird are expected to be victims of this intoxication, because they are found inhabiting these environments or even in close proximity up to several kilometres (Jakson et al. 2011). It is while they are in these ecosystems, when foraging for food, that birds ingest water or prey with high

concentrations of this element, either directly while preying or by means of discards and offal by fishing vessels (Monteiro et al. 1998;Arcos et al. 2002). Normally, the risk is increased when the food items collected are stationed in higher levels of the food pyramid, which are also the longed lived ones and tendentiously the most inefficient ones in the depuration of this metal (Clarkson 1992), all characteristics that promote the phenomena already disclosed, as are examples fish particularly demersal or mesoplagic species, which are the most hazardous. Other birds or even mammals can also be a source of mercury to predatory avian species that ingest them mercury-enriched (Burger & Gochfeld 1997;Franson 1999;Kojadinovic, Bustamante, Churlaud, Cosson & Le Corre 2007).

Since it can be considered a global problem, as stated by Klaassen, Amur and Doull (1986) (quoted by by Boening 2000), in view of the fact that the atmospheric lifetime of mercury is long (meaning deposition reflects local, regional or even global influences) and water currents, wind and erosion allow for dispersion, the contamination of the food web is geographically pervasive (Clarkson & Magos 2006;Arctic Monitoring and Assessment Program [AMAP] 1997 cited by Lambert et al. 2012). And in remote places like Arctic and Antarctica high levels of mercury in biota are detected without local or regional sources that justify these values (AMAP 2002 cited by Lambert et al. 2012;Wilson, Steenhuisen, Pacyna & Pacyna 2006 quoted by Seewagen 2010). Accordingly, a geographical pattern of disease events doesn´t exist and increased frequencies are expectable in northern latitudes (Franson 1999;Ochoa-acuña et al. 2002) or certainly in countries going through exponential economic, industrial and/or agricultural growths. Because this disease depends on foraging skills and bird movements between or within these problematic regions, seasonality is also not recognized. And so large scale mortalities events are rare and reports of scattered causalities are the usual occurrence (Franson 1999).

The most affected species are therefore the ones that are found in these ecosystems (higher environmental bioavailability) and simultaneously the ones that owing to their feeding habits (and typical prey) and their higher positioning in the trophic chain, are at greater risk to ingest and accumulate the highest concentrations of this heavy metal. Other factors that warrant the highest levels of mercury, and consequently a higher probability for the individual to suffer the detrimental effects of mercury intoxication, include: taxonomical differences in metabolism or excretion of mercury, season, habitat, exposure timing, migratory habits, age, genre, body size, life span, moulting patterns (one possible route to elimination of mercury) and prey composition (Braune & Gaskin 1987;Burger 1995;Burger & Gochfeld 1997;Goutner & Furness 1997;Monteiro et al. 1998;Wolfe, Schwarzbach & Sulaiman 1998;Arcos et al. 2002;Burger 2002;Jagoe, Bryan Jr., Brant, Murphy & Brisbin Jr. 2002;Ochoa-acuña et al. 2002;Evers et al. 2005;Burger et al. 2007;Kojadinovic et al. 2007;Zolfaghari, Esmaili-Sari, Ghasempouri & Kiabi 2007;Goodale et al. 2008;Eagles-Smith, Ackerman, De La Cruz & Takekawa 2009;Zolfaghari, Esmaili-Sari, Ghasempouri, Baydokhti & Kiabi 2009;Frederick & Jayasena 2010;Blévin et al. 2013). Although, for each case some correlate more strongly with the results achieved in several studies, like is the case of Arcos et al. (2002) that indicated that seasonality of feeding habits and habitat distribution were the main reasons for the highest concentrations detected in *L. audouinii,* while breeding, and *L. cachinans michahellis* during the rest of the year, while feeding of fishing discards specifically in these periods of time. Furthermore, Hifner, Hobson and Elliott (2011) claim that the interactions/influences of these factors are complex and of difficult characterization, so a careful analysis of each situation is needed. Considering other factors like age, adults normally have

higher concentrations of mercury in their tissue than juveniles, almost 3,6 to 10,6 times higher in fish-eating adults when considering juveniles in the study of Evers et al. (2005), because chicks eliminate the excess during feather growth and also because they consume smaller prey items (less contaminated) or accumulate for shorter periods of times (Spalding, Frederick, McGill, Bouton & McDowell 2000a;Evers & Clair et al. 2005 cited by Goodale et al. 2008). Nevertheless, in species that change diet with age, for instance on the word of Lindsay and Dimmick (1983) from animal-based to plants, the pattern can be inversed (Hoccut & Dimmick 1971 cited by Lindsay & Dimmick 1983). In respect to gender, gender differences were found in the values of mercury accumulated in *G. immer,* by Evers et al. (1998) cited by Chan et al. (2003), with males displaying higher levels when compared to females owing to differences in the size of the prey (larger in the first). But then again, avian species that exhibit niche partitioning during forage and the fact that eggs are also another route of depuration of this pollutant, as seen in the work of Braune and Gaskin (1987) with feathers of adult *L.philadelphia*, these are additional plausible explanations for this gender discrepancy (Evers et al. 2005). But then again, these aren´t common or significant to every bird species or age, like adults of *L.argentatus* (feathers) in the observations of Burger & Gochfeld (1997) and juveniles and immature *L.philadelphia* (several organs) by Braune and Gaskin (1987).

Accordingly and as Franson (1999) states, the species more commonly or likely to be documented as being intoxicated are: loons, wading birds, pelicans, cormorants, mergansers, gulls, terns, raptors and gallinaceous birds. Yet aquatic/marine species tend to be more frequently reported due to particularities of the aquatic cycle of mercury, mainly the potential for a rapid movement of the contaminant in this medium compared with the inland and the easiness of accumulation seen in the sediments of these habitats (Thompson 1996 and Fisk et al. 2003 both cited by Poissant, Zhang, Canário & Constant 2008;Burger & Eichhorst 2005 cited by Tsipoura et al. 2011). The project of Zolfaghari et al. (2009), to determine the concentrations of this contaminant in 100 animals of 27 species of 14 families in south western Iran in 2005, is a clear evidence of this fact. In this study, aquatic birds were the ones with the highest values (with the *Laridae* family in the top positions with the highest concentrations measured in terns and *L. canus*). Nonetheless, the trans-positioning of aquatic mercury to terrestrial food chains in contiguity is possible, as well as its direct incorporation in terrestrial ecosystems, as a result of atmospheric deposition, floods or legacy sources, and so several other species, other than aquatic or marine (and others than the ones hereby alluded), can be influenced by this metal (Evers et al. 2005;Brasso & Cristol 2008;Cristol et al. 2008;Jakson et al. 2011).

After ingestion, this metal is absorbed in the intestinal compartment, transported by blood and accumulates in tissues like liver, kidney and muscles (Monteiro et al. 1998;Spalding et al. 2000a). Mercury is primarily a neurological toxin as said by Wolfe et al. (1998), it can penetrate the blood-brain barrier and lesion the brain and spinal cord, thus is responsible for a central nervous system dysfunction, although its presence and other exerted effects in other systems and organs are likewise found and could be significant. Nevertheless and on the whole, the outcome of this intoxication depends on several of the factors aforesaid along with: the species; redistribution in plumage during feather growth; dilution effect by tissue growth in younger birds; elimination in eggs and excreta; possibly a demethylation reaction that occurs in some species (mostly seabirds with long lives and slow moulting patterns that have higher proportion of inorganic mercury in the liver), and additionally

the presence of selenium in the diet - agent of detoxification (Braune & Gaskin 1987;Thompson & Furness 1989;Burger & Gochfeld 1997;Burger 1993,Kim, Murakami & Tatsukawa 1996 and Monteiro & Furness 2001 all quoted by Bond & Diamond 2009;Wenzel, Adelung & Theede 1996;Kim, Saeki, Tanabe, Tanaka & Tatsukawa 1996;Cuvin-Aralar & Furness 1991;Scheuhammer et al. 2007;Thompson 1996,Fisk et al. 2003 and Thompson & Furness 1989, all quoted by Poissant et al. 2008).

2.2.7.2.2. Clinical presentation and necropsy findings.

The majority of descriptions concerning this intoxication result from experiments conducted in controlled environments, normally feeding trials with a subset of wild birds captured in order to assess the effects of different concentrations of mercury ingested on several health parameters. From such studies it was concluded that mercury can affect growth, development, behaviour, metabolism, appetite, tissue and plasma biochemistries (Eisler 1980) by interaction with elements of the nervous, circulatory (like anaemia and altered phagocytosis) and endocrine systems (Spalding et al. 2000;Wada, Cristol, McNabb & Hopkins 2009;Frederick & Jayasena 2010;Jayasena, Frederick & Larkin 2011). The presentation usually seen is of an insidious nature were chronic exposure causes an emaciation syndrome with several sub-lethal effects that can compromise the survival of wild birds (altered behaviours) and stability of several populations (reproductive failures) (Franson 1999;Chan et al. 2003). Acute disease is rare, since the dose of methylmercury to induce neurological impairment (5mg/Kg in diet) is normally higher than the concentrations present in nature (Chan et al. 2003) and when in a controlled experiment, normally leads to death within 1 hour or less, with very few signs (Franson 1999).

The signs reported include: lethargy (hypo reactivity and hypo activity); lack of efforts to forage/hunt and other aberrant behaviours - anomalous incubation routines, reduced food intake and others at 0,5mg/Kg in diet (Chan et al. 2003); ruffled/fluffled feathers; higher wing area asymmetry; weight loss; progressive weakness of legs and wings; drooping eyelids; incoordination/ataxia; difficulties to fly/walk/stand (exaggerated positional response of wings and other proprioceptive deficits Spalding et al. 2000a); tremors; wing- drop and paralysis. These clinical presentations culminate in reproductive failures (fecundity/hatching/fledging rates are affected even with minimal doses of 0,1 mg/Kg in diet Chan et al. 2003); and debilitation or death (Heinz 1979;Eisler 1980;Franson 1999;Spalding et al. 2000a;Ochoa-acuña et al. 2002;Poppenga & Tawde 2012).

Necropsy findings in wild birds frequently are only related to emaciation, other gross lesions are rare (Franson 1999). In laboratory experiments these include demyelination, neuron shrinkage, necrosis and haemorrhage of meninges in the cerebellum in ducklings of *A.platyrhynchos* fed 3ppm wet weight of methylmercury with the protocol of Heinz and Locke (1976). Peripheral lesions in sciatic nerves characterized by degeneration with loss of myelin, inflammation and atrophy and wallerian degeneration in the brachial nerves among other lesions, as well as neuronal degeneration in the central system (midbrain, cerebellum, cerebrum and spinal cord) were findings described by Spalding et al. (2000b) in captive nestlings of *Ardea albus*.

Other documented affected organs, gross and microscopic lesions comprise: the Fabricius' bursa, thymus and spleen (all with cues of atrophy and immune cell depletion); bone marrow (cellular density decreased); endocrine and exocrine glands (vacuolation and disruption); lungs (perivascular oedema

and mucoid distension of globlet cells); kidneys (expansion of the Bowman's capsule and vacuolation of the proximal tubule epithelia); liver (vacuolation of cells and periportal infiltrates) (Spalding et al. 2000b). Altogether these lesions are uncommon in nature and not specific of mercury intoxication, or even consistent among themselves.

2.2.8. Pesticides.

The compounds used daily, by mankind, capable of causing disease in wild birds are numerous and among the several existing molecules, the examples of the ones that produce paresis as a clinical sign are also diverse. Since an exhaustive description of these kinds of intoxication would surpass the purpose of the present study the author decided to analyse only the paresis/paralysis as a result of intoxication by pesticides, and particularly the ones more commonly identified/implicated in these kinds of afflictions (when considering others for instance, molluscicides), like the anticholinergic specifically the organophosphates and carbamate pesticides (Martínez-Haro et al. 2007a).

Organophosphorus (OP) and carbamate (CA) pesticides are/were used worldwide mainly as insecticides, herbicides, nematocides, acaricides, fungicides, rodenticides, avicides and as bird repellants (Glaser 1999). Their use suffered an increase after the ban on organochlorides (OC) in the 60's and 70's (Rattner & McGowan 2007;Poppenga & Oehme 2010), because of the persistence of the latter in the environment and subsequent consequences (Grue, Gilbert & Seeley 1997;Corson, Mora & Grant 1998). This increment was also due to some features presented by these two groups of pesticides: short half-life in the environment (from days to months), high sensitivity to chemical decomposition (elevated temperatures and/or pH) and additionally, in accordance with Stickel (1974) cited by White, Hayes and Bush (1989), a rapid effect; which in conjunction with the theoretic inability for these compounds to accumulate in the food chain, represented a minor risk in cases of secondary intoxication (Elliott, Langelier & Wilson 1996;Glaser 1999;Whitney 2004;Poppenga & Oehme 2010). In spite of recent restrictions conditioning the use of OP and CA pesticides (Poppenga & Oehme 2010), these are applied in various situations with numerous purposes: in agriculture on cultivated fields, pastures, orchards and forest; in residential and urban sites for more ornamental intents (parks, golf courses – Kendall, Brewer & Hitchcock 1993); in pest control; as formulations such as pour-on on livestock to prevent production and economic losses owing to parasitism (Henny, Kolbe, Hill & Blus 1987;Henny, Blus, Kolbe & Fitzner 1985 quoted by Blus & Henny 1997); among other examples, for instance Martínez-Haro, Vinuela and Mateo (2007a) denote deer hunting regions. However, situations of inadequate or malicious use of these pesticide (e.g. use of baits) were and are still frequently pointed out as the cause of disease and death of wild animals, either directly in the case of certain species or indirectly in others, despite restrictions imposed in terms of use and commercialization of specific active principles (Stone, Overmann & Okoniewski 1984;White et al. 1989;Flickinger, Juenger, Roffe, Smith & Irwin 1991;Fleischli, Franson, Thomas, Finley & Riley.Jr 2004;Kwon, Wee & Kim 2004;Wobeser, Bollinger, Leighton, Blakley & Mineau 2004;Martínez-Haro et al. 2007b;Tennakoon, Perera & Haturusinghe 2009). It is because of these kinds of applications that intentionally, or not, birds are intoxicated, including seagulls. One case reported by Whitney (2004) indicates another possible source for intoxication, although rare, was probably the cause of disease and death of more than 800 birds, mostly *L.argentatus* but also affecting *L.marinus*, *L.delawarensis*, *Corvus corax* and

Figure 78 – Affected species.
Adapted from: Whitney 2004.

other species (fig.78). This event was the result of an accidental explosion of an illegally discarded canister containing fensulfothion in a landfill in the Lake Quidi Vidi area in April of 2000.

2.2.8.1. Pathogenesis.

According to Glaser (1999) several vertebrates and invertebrates can suffer intoxication by these pesticides, but avian species appear to be more sensitive to its toxic effects, particularly by these compounds that are more toxic than the previous OCs (Hill 1995 cited by Grue et al. 1997;Wobeser et al. 2004;Pisani, Grant & Mora 2008). Several groups of wild birds are documented as more sensitive to this kind of intoxication (Fleischli et al. 2004), but at the same time they display sensitivities and exposure routes that are distinct, and so the groups of aquatic birds, Passeriformes and Raptors are the ones more frequently distinguished. The exposure can be due to the ingestion of treated seeds, vegetation containing pesticide residues, dead or poisoned invertebrates; while in predatory or necrophagous birds the intoxication may result of the ingestion of intoxicated carcasses, baits or predation of sick animals, in what is known as secondary poisoning (Henny et al. 1987; White et al. 1989;Glaser 1999;Elliott et al. 1996;Wobeser et al. 2004). Other documented routes of exposure encompass inhalation and dermal/ocular absorption or even consumption of contaminated water by discharges or runoff/irrigation processes occurring on fields (Hunt, Hooper & Littrell 1995;Glaser 1999;Pisani et al. 2008). In addition, the congregation in the same habitat, on which are applied these compounds, by a high number of several species either in search for food or directly feeding, is the behaviour with the highest risk of exposure (Glaser 1999;Corson et al. 1998). Individuals of any age can be affected, though juveniles have a sort of duality in the way they suffer from its effects. Consequently these effects in young individuals can be: direct and detrimental, because younger birds ingest larger amounts of food by body weight and/or their metabolism is less developed for the detoxification of these molecules; direct and less deleterious because their nervous system are still underdeveloped for the effects to manifest (Hill 1992/1995 cited by Corson et al. 1998), or indirect, causing death as a result of negligence by disease or death of the parents, as Cox (1991) states by citing the example of a colony of *L.atricilla* in Texas wherein adults and part of the offspring where intoxicated as a result of the ingestion of insects from a cotton field (where insecticides were used) and the remaining young lost their body condition and died by parental disregard.

The kind of molecules applied can be immensurable, however, on the word of Cox (1991), cases of intoxication are infrequently documented (Martínez-Haro et al. 2007b). Nevertheless, poisoning events tend to exhibit a certain seasonality, given that these products are short-lived in the environment and therefore the occurrence of these cases is linked with the application of these molecules (Glaser 1999). Nonetheless, the adverse effects can persist from one season to the next like what Elliott and collaborators (1996) reported. In this special circumstance the environmental conditions, namely the pH and humidity of the delta region of Fraser in British Columbia in Canada in the winter of 1990, maintained the viability of the pesticide applied on the previous spring, and so the number of

casualties by this intoxication in this time frame was justified. Another factor to consider is the adverse weather conditions, as additional adjunct elements that can disperse and concentrate pesticides in other sites far from where they were originally applied (sometimes in the correct manner and with the correct formulation and concentration) and this is a possible explanation for fatalities found in wild birds (Zinkl, Jessuo, Bischoff, Lew & Wheeldon 1981).

The pathogenic mechanism by which these compounds exert their negative effects is associated with their biding to acetylcholinesterases and subsequent inhibition of the latter. In some organophosphates this takes place after activation of part of the dose in the liver and brain, therefore, and inhibiting other enzymes, some can induce cases of delayed neuropathy (ataxia leading to paralysis) within 2 to 3 weeks after a single or repeated exposure (Baron & Johnson 1964;Grue, Gibert & Seeley 1997). While in the case of the carbamate pesticides the effect is direct (Glaser 1999;Thompson, Langton & Hart 1995;Pisani et al. 2008). Since these enzymes are important in the synaptic transmission of nerve impulses in the central and peripheral nervous systems, when inhibited, in acute cases, there is a decrease of their activity and the conduction/transmission of the impulse is hindered due to overstimulation (uninterrupted) by the presence of the neurotransmissor, acetylcholine, resulting in a set of neurological signs amongst which it is described paresis/paralysis (Grue et al. 1997;Glaser 1999;Pisani et al. 2008).

2.2.8.2. Pathology, Signs and Necropsy lesions.

Several factors can influence the clinical presentation of pesticide intoxication and on the word of Glaser (1999) age, gender, dietary deficiencies, diminished fat reserves, poor physiological condition and/or life phases of high energy demands, like migration or increased metabolism; augment the vulnerability of these birds to the toxic effects of the organophosphates and carbamate compounds, combined with differences in behaviour, types of formulation of the compound or routine/scheme of pesticide application (Thompson et al. 1995;Martínez-Haro, M. et al. 2007b).

Nevertheless, the clinical presentation found doesn't diverge greatly from species to species and normally death results of respiratory failure due to airway constriction, decrease ventilation owing to paralysis of the intercostals or direct depression of the respiratory centre in the brain (Rattner & McGowan 2007).

On the majority of the die-offs, the first sign reported is the presence of several dead birds (frequently the only one seen), however and analysing the scene of the mortality event, clues of other possible peri-mortem signs are noted, like the disturbance of the vegetation by the convulsion state of the living bird. Additionally and usually in close proximity to the carcases, the source of the problem is identified, given that birds don´t usually disperse greatly from it as is the case of predatory/granivorous or insectivorous birds (Stone et al. 1984;Glaser 1999;Kwon et al. 2004). The ones still alive normally display: lethargy (Kwon et al. 2004), tremors (Stone et al. 1984), paresis/ paralysis (Kwon et al. 2004) and convulsions (Zinkl et al. 1981;Stone et al. 1984;Kwon et al. 2004). Other signs enumerated by Glaser (1999), although unspecific, are caused by the overstimulation of the parasympathetic nervous system during the intoxication (Degernes 1995) and can be equally seen and noted, and include: hyperexcitability/induced tranquillity, ataxia (also reported by Zinkl et al. 1981 and Elliott et al. 1996), myasthenia, opisthotonos, dyspnoea/tachypnoea (supported by observations of Zinkl et al. 1981 and Stone & Okoniewski 1984), vomit, defecation, diarrhoea, spasmodic contraction of the anal sphincter,

blindness, miosis (also seen by Elliott et al. 1996), mydriasis, ptosis, exopthalmia, lacrimation, polydipsia, crop distension (e.g. raptors – Elliott et al. 1996), epistaxis and piloerection.

Figure 79 – Ill *L. argentatus*. Adapted from: Whitney 2004.

Whitney (2004) documents in the aforementioned die-off, that the affected *L. argentatus* (fig.79) were alert, but, although simultaneously incapable of controlling their bodies, defended themselves with their bills. It was also reported: lethargy, wing-drop, paresis/paralysis and vomit.

In cases where the exposure is acute, but in a sub-lethal level, effects on thermoregulation, food consumption and reproduction can be seen and include, respectively: hypothermia, anorexia/altered foraging behaviour and altered hormone levels/reductions in clutch and litter size/alterations in behaviour (Grue et al. 1997). In some sub lethal cases, the severe onset of clinical signs, concordant with pesticide poisoning, is transient and rapidly replaced by a quick recovery and the overt clinical status of the bird tends to stabilize as Lehel, Laczay, Déri, Darin and Budai (2010) described in their experiment with sub lethal doses of a CA pesticide in broiler chickens.

Regarding the necropsy examination, since the alterations provoked by these chemicals are inherently biochemical, gross or microscopic findings are hardly found or identified (Kwon et al. 2004). The birds at necropsy are usually in a good physical condition, with abundant and well developed fat deposits, as well as a good score of the development of all the musculature (White et al. 1989;Flickinger et al. 1991;Augspurger, Smith, Meteyer & Converse 1996;Glaser 1999). Specific gross or microscopic lesions are not found or otherwise when they exist are minimal (but likewise unspecific), as is the case of congestion of the intestinal wall or even haemorrhage (Zinkl et al. 1981;Stone et al. 1984;Flickinger et al. 1991;Glaser 1999). In some cases congestion and excessive fluid in the lungs can be observed indicating respiratory insufficiency, but without pathognomonic signs of the presence of pesticides (Stone et al. 1984;Glaser 1999). Traumatic lesions, e.g. acute focal haemorrhage of the liver (Augspurger et al. 1996) are documented in terminal convulsions (Franson & Smith 1999). It is also frequent the presence in the upper gastro-intestinal tract of freshly ingested grains or seeds in waterfowl, passerines and cranes or feathers/skin/flesh or portions of larger carcasses in predators and necrophagous (Zinkl et al. 1981;Stone et al. 1984; Flickinger et al. 1991;Elliott et al. 1996;Kwon et al. 2004). This finding or dye or both is a conspicuous proof of poisoning, especially if recent and if the contents are uniform and in large quantities (Glaser 1999), it indicates, as well, the possible source of the pesticide and is characteristic of acute intoxications (Franson & Smith 1999).

2.2.9. Nutritional imbalances.

Taking into account the category nutritional disorders, and as could be inferred previously, wild birds when compared with the commercial ones have a lot of liberty in the selection of what to eat, though they depend more of food availability, which in turn is strongly influenced by innumerable natural factors and some, unfortunately, nowadays more and more anthropogenic. Another singularity of this kind of birds is associated with the practical impossibility/difficulty in supplementation or correction of nutritional deficiencies or excesses that may ensue, similar situations do not take place in aviary farms, where every single factor is monitored and controlled and the corrections are applied, and so cases related to this category in domestic birds are acknowledged and result in a vast and explicit

literature of every single disease that can arise when there are nutritional disorders, being rare descriptions of these kind of diseases in the group of wild birds.

2.2.9.1. Paresis and Nutrition.

When considering signs like paresis and paralysis the way little nutritional deficiencies or excesses can lead to these is extremely variable. They can be caused by: an increase in the susceptibility to infections that directly cause this clinical presentation; a decrease in the organism's antioxidant capacity in the presence of internal or external free radicals, and so the homeostasis and integrity of several cellular components and enzymatic processes are compromised; or a heighten or lower anomalous influence in countless steps in several metabolic reactions, whose purpose was to assure the best defence conditions against external pathogens and the correct function of all the body's systems and organs, which in these cases may not be achieved. These situations are all likely to be attributable to imbalances concerning certain nutrients like vitamin A (Vit.A); Complex B vitamins; Vit. E and selenium; Vit. C and caretonoids (Wobeser & Kost 1992;Chew 1996;Garland & Pritchard 2009). The paresis can also be a result of "more direct" effects, particularly if the deficiency affects some of the nervous system elements, such cases are illustrated in the B hypovitaminosis (Swank 1940; Johnson & Storts 1988;Cai, Finnie & Blumbergs 2006) and in deficits of vit.E (e.g. encephalomalacia); or the locomotor system, examples are the role of the Vit.D- Calcium –Phosphorus triad, Vit.E and its relation with selenium and cystine (e.g. Nutritional miopathy), Zinc/Manganes/Folic acid (absence of at least one is the cause of condrodystrophia), among several others (Klasing 2008;Garland & Pritchard 2009). Similar effects can also be seen in other organs/tissues in close proximity to these two systems and thus could render an analogous paresis/paralysis presentation.

The deficiency or excess of one nutrient, despite capable of alone causing disease (hence the various specific illnesses documented of the absence or excess of a particular nutrient) is rarely reported, frequently they act together (e.g. the functional complementary relationship between Vit. E and selenium - Burau 1985 and Garland and Pritchard 2009 citing numerous authors) or they are affected by one another and these mechanisms are not fully known, like the case of Vits. A and E, when given together orally and reported in *Spheniscus humboldti* (Crissey, McGill & Simeone 1998).

2.2.9.2. Food and nutrients.

Wild birds obtain their nutrients in the most variable ways, so the deficiencies/excesses tend to occur in periods when the needs are the greatest, when adverse conditions are present and it´s difficult to find food, or the normal or ideal food item is absent, thus making wild birds search and ingest alternatives with low or high quantities of what its considered normal and healthy (Honour, Kennedy, Trudeau & Wobeser 1995b). In spite of some nutrients being apparently produced by the organism, like vit. D and C, in the case of commercial birds and according to Klasing (2008), Garland and Pritchard (2009) these two vits. need to be complemented or supplemented in the diets established (Vits. D and C, respectively), since in some stages of production it is required, but is it possible that such needs are also found in the wild bird populations, while as a result of different stressors. The acquisition of the proper portion of the various nutrients needed, which can be already the ideal in the food item, can be impaired by the presence of other substances in it, and so the problem derives not

from the nutrient concentration *per se*, but from the existence of substances that can act directly on the individual and hinder the role that a good nutritional equilibrium has in animal health. There a several examples of these situations one of them described in commercial birds indicates that the occurrence of aflatoxins and ochratoxins interfere with the vit. D metabolism (Waldenstedt 2006), another is the case of Riley, Rinchard, Honeyfield, Evans and Begnoche (2011) in which it is stated that the presence of thiaminases in *Alosa pseudoharengus,* in the Great Lakes was responsible for a thiamine hypovitaminosis in the *Salvelinus namaycush*, with repercussions in egg-laying and mortality or sub lethal effects in newly hatched fry. From these results one can expect, possibly, that other trophic levels can be influenced as well, including wild birds.

Another cause for nutritional disorders associated with food is probably one of the most important and progressively more preoccupying. It's linked to alterations provoked by human activities on the environment and consequently in the various food items normally ingested by birds. Their nutritional status, which greatly depends on the ecosystem where they are original, can be negatively enriched, due to the use of fossil fuels, agriculture practices, industrial activities, residue management plans and soil prospection to name a few (Lemly 2004). The detailed activities are responsible for the discharge of several elements in many kinds of ecosystems like selenium, copper and zinc in concentrations that are probably the origin of perturbations in trophic chains and subsequently are the cause of disorders and toxicities, essentially when selenium is considered, as by far is the most commonly documented. This is clear in a study of Hamilton, Buhl and Lamothe (2002) where the goal was to detect and quantify a few trace elements in the BlackFoot River Watershed in Southeast Idaho in June of 2000. The results obtained revealed high concentrations in the food chain, particularly of selenium, with correlation between the more elemental trophic levels (sediments, aquatic plants and aquatic vertebrates in more than a few places), bioaccumulation, with dispersion by water currents, and that justified several past selenium intoxication cases in the local fauna.

2.2.9.3. Pathogenesis.

The pathogenic mechanism intrinsic to these kinds of diseases is one of the most complex, not only because nutrients can perform quite a few functions, but other factors associated with: the micronutrient in question and its potential interaction with others in excess or in deficit; the bird's diet and magnitude of the imbalance; the previous diet and the organism storage capacity of the nutrient in question (adipose tissue or liver for liposoluble compounds); bioavailability; the duration of the disorder; preferences/ability to select other alternatives; time of the year (winter versus spring – Albers, Green & Sanderson 1996) and species/age/gender/reproductive status; interfere with the clinical presentation, lesions observed, and the prognosis of these illnesses.

Theoretically, and taking into account the above, nutritional disorders can afflict all species, the clinical presentations can vary, and everywhere in the world this disease category can take place and be seen.The clinical signs related to the nutritional disorders that elicit paresis or paralysis in general can be initially imperceptible like the case of vit. A hypovitaminosis, in which, the first changes are in the immune system (Friedman & Sklan 1989 cited by Wobeser & Kost 1992). Some clinical presentations are unspecific, such as the ones caused by vits. B_2 and B_6 hypovitaminosis (Garland & Pritchard 2009). In others, the whole clinical picture depends on the age, gender, reproductive status of the bird and this is evidently seen in calcium deficiencies: females exhibit osteoporosis, osteomalacia, the

production is affected (and hatchability) and consequently the reproductive success rate decreases; in adults it is possible to observe bone deformations and the chicks have skeleton, beak and claw malformations, fractures, secondary paralysis and growth problems (Wallach & Flieg 1969 and Jones 1999 quoted by De Matos 2008;Klasing 2008;Garland & Pritchard 2009). In a variety of these illnesses it's difficult to see a sign or lesion because of the storage capacity that prevents the precocity in detection of such manifestations, or these only come into being in places only accessible by means of a necropsy. In the necropsy examination it's possible to identify pathognomonic lesions, for instance in vit.A deficiencies the mucosa of the respiratory, digestive and reproductive tracts suffer a keratinized, stratified squamous metaplasia and is grossly seen as white plaques (Wobeser & Kost 1992;Honour et al. 1995b;Jungherr 1943 cited by Honour, Trudeau, Kennedy & Wobeser 1995a;Scott & Dean 1991 and Wobeser 1981 cited both by Wobeser & Kost 1992); or not, an example is the toxicity by selenium that was responsible for the death of birds in the Keterson National Wildlife Refuge in California. The lesions detected in this wildlife refuge were many and included: muscular atrophy, loss of feathers and body fat; and various gross and microscopic alterations in the lungs, liver, heart, spleen and kidneys (Ohlendorf et al. 1988 cited by Albers et al. 1996).

Given the multitude of possible differentials for the paretic syndrome and since it is not the purpose of this dissertation to accomplish a profound discussion of only one differential (i.e. nutritional), the author decided to choose, in order to analyze the effect of nutritional diseases in wild populations, the recent study conducted in the North of Europe, regarding a possible B_1 hypovitaminosis as a cause of paralytic syndrome in Larids, which exactly corresponds to the aim of this thesis.

2.2.9.4. Thiamine.

The Vit. B_1, also known as thiamine, belongs to the vit. B complex, is hydrosoluble and is an essential micronutrient, functioning as an enzymatic cofactor in several important reaction in the body – fundamentally in the energy metabolism including the amino acid and fatty acid pathways (diphosphorylated form) - and is crucial in the correct function of the neuronal membranes in its triphosphorylated form (Balk et al. 2009).

2.2.9.4.1. Domestic birds.

In the current literature regarding commercial birds the nutritional deficiency of Vit. B and others, and according to Julian (2005), are well documented, especially when the locomotor apparatus of chickens and turkeys is considered. As stated by Klasing (2008) in the cases where there is a decrease of thiamine in the organism, birds tend to develop polyneuritis within 3 weeks since the deficiency period has started, chicks can display this lesion before the completion of the second week of age, and is always more sudden in development in the youngest. Additionally, in the study of Gries and Scott (1972) clinical signs of lethargy and head tremors where observed in chicks with 3 and 4 days that evolved to ataxia, inability to stand and opisthotonus or neck flexion and death; similar presentations were described in adults by Klasing (2008). This author states that as a chronologic sequence the first sign to be visible and accounted for is anorexia, followed by weight loss, ruffled feathers, leg weakness with paresis and paralysis (that starts at the digital flexors and ascends to the leg muscles, wings and neck) and then stargazing is seen associated with complete inability to rise or maintain a

correct recumbency. Other signs related and reported comprise: subcutaneous oedema, cyanosis, hypothermia and a progressive decreasing in the respiratory rate. Considering the gross and microscopic findings commonly identified in birds suffering from this deficiency, they encompass: cardiac atrophy with marked dilation of the right side (in particular the auricle); adrenal hypertrophy, gonadal atrophy, as well as stomach and intestinal atrophy (Klasing 2008). The duodenal crypts of Lieberkuhn are found dilated, full with necrotic and desquamated cells and debris, and the mucosa is modified and almost absent (as a result of a decrease in mitosis and desquamation). Another lesion detected in such cases is cytoplasmatic vacuolization with the formation/presence of hyaline bodies in the pancreas' acinar cells (Gries & Scott 1972;Klasing 2008). Nevertheless and in spite of the neurologic presentation, descriptions of alterations on the nervous system (in this case brain, medulla and sciatic nerves) are rare. As suggested by Gries and Scott (1972) the absence may be due to the fact that biochemical alterations are fatal before they are "expressed" and evident as tissue modifications, and because the majority of the research is done in a context of acute deficiency and not chronic where certainly it can be easily identified, like what Swank (1940) found with White Karneaux pigeons of 6 to 8 weeks. In this experiment the birds where divided into 4 groups (starvation and vit. B_1 deficiency; acute vit. B_1 deficiency; chronic vit. B_1 deficiency and control group-starvation), stabilization/recovery was attempted by administration of the vit. at fault (only on groups 2 and 3) and histological lesions in the central and peripheral nervous systems were analysed. Swank found granular degeneration of the axis' cylinder and also a beginning of a myelin stealth fragmentation in the most distal and with the highest calibre nervous fibers. These alterations where associated with muscular weakness and chromatolysis in the more chronic cases of B_1 deprivation.

2.2.9.4.2. Wild birds.

Balk and collaborators (2009) indicate that this deficiency can be the cause of a fatal idiopathic paralytic syndrome found in several wild birds, in the region of the Baltic Sea in the last decades, apparently documented since 1982 and linked with a reproductive failure. The authors believe that, possibly, this is one of the reasons to explain the population declines of many birds noted until now - the estimated numbers for mortality attributed to this syndrome in the South of Sweden was 451 out of 837 specimens found dead or dying that belong to 28 of the 36 species studied). Balk et al. (2009) suggest that this condition might result from an agent acting directly on the affected animal and/or by an insufficient trophic vit. B_1 transfer trough the food chain.

This study comprises the years 2004 to 2007 and three species with different life cycles (*Larus argentatus*, *Sturnus vulgaris* and *Somateria mollissima)* and uses a vast array of hypovitaminosis markers (activities of thiamine-dependent enzymes like Transketolase in the liver of pulli, the latency of them and the concentration of the various forms of B_1 in eggs, pulli and adults). Clinical signs presented upon observation and the success in the rehabilitation of sick birds by administration of the adequate vit. in deficit, orally or intramuscularly, were also investigated. The clinical signs documented by chronological order indicates that adult *L. argentatus,* initially have difficulties in maintaining the wings folded correctly against the body when in recumbency - "hanging wings"; loss of the ability to fly while they can still walk; complete loss of voice; appetite loss while the ingestion of water remained; laborious breathing and progressive loss of strength in the legs – attempts were made to move by the use of the beak and wings as if crawling; complete paralysis of the wings and legs (fig.80); strength

loss in the beak; diarrhoea; force feeding could result in vomit; opisthotonus – "star-gazing" (fig.81) and tremors. In the final stages, ataxia, catatonia, seizures and at last death were observed (this last one arises 10 to 20 days after the loss of the ability to fly). This paralysis was also seen in individuals of the species *L. marinus* and *L. canus* among other species excluding larids.

During all the course of this syndrome Balk et al. (2009) noticed some ophthalmic signs that, although no systematically documented, were seen: "squinting eyes" and iris pigmentation changes, appearing as black spots (fig.82) that were independent of the phase of the progressive paralysis and resulted in this *retinitis pigmentosa*.

Figure 80 – Paralyzed *L.argentatus* (screenshot of video).
Figure 81 – Specimen with opisthotonus and hanging wings.
Figure 82 – Ocular signs of iris dispigmentation in *L. argentatus*: a e b are two individuals of the Baltic Sea and c and d are seagulls from Iceland with incipient alterations highlighted by arrows.
Adapted from: Balk et al. (2009).

According to laboratorial tests, the levels of enzyme activities measured were lower than the ones from a control population of Iceland (where the disease was probably non existent) and the latencies where higher than those of the control. These results and the signs reported conjoined with the success in the recovery of sick birds (adults and pulli) are highly suggestive of hypovitaminosis, although other differentials were not explored and no necropsy of these birds was made.

2.2.10. Diagnosis, Treatment, Immunity, Tolerance and signs of Exposure.

The diagnostic workout in every single one of these illnesses requires a thorough collection of the clinical history, a complete physical examination and in cases of death a comprehensive necropsy evaluation. However, the diagnostic test for each disease is not the same and the treatment options may differ as well between these diseases. Therefore, a compilation of the commonly recommended diagnostic approaches and treatment options is present in table 2. In some of these ailments previous exposure, development of immunity against the harming pathogen and other mechanisms are advantageous and may bestow additional protection in future re-exposures. These are also detailed in the aforementioned table.

Table 2 – Diagnostic approaches, treatment options and potentially advantageous mechanisms against the effects of future exposures of the diseases in study.

	Diagnosis	Treatment	Immunity/Exposure/Tolerance
Newcastle Disease	Faeces or swabs of trachea or cloacae and/or tissue samples are used for viral isolation and subsequent characterization of the pathogen (OIE 2009).	No treatment (OIE 2009).	Antibodies were detected in eggs and migratory birds (Wobeser et al. 1993; Kuiken et al. 1998;Schelling et al. 1999 cited by Camenisch 2008; Farley et al. 2001) Prior exposure to other APM serotypes may protect/alter the course of infection by APMV-1, proved at least in chickens (Nayak et al. 2012).
Salmonellosis	Faeces, cloacae swabs or tissues are collected for bacteria isolation or serum is obtained for serological diagnosis in cases of prior or current infection (OIE 2010).	Antibiotics are selected after appropriate antibiotic sensitivity testing (Daoust & Prescott 2007).	Inconclusive as to vertical transmission of antibodies in this group of birds (sub-chapter 2.2.2.1).
Aspergillosis Converse (2007a)	Positive serology, culture or cytology of respiratory washes, endoscopy and radiography can be used in ante-mortem cases; whereas cytology and histology examination for presence and culture for identification are indicated in dead birds.	Amphotericin-B, Itraconazole or Fluconazole are used alone or in combinations, topically or systematically.Surgical debridement of lesions in restricted cases is advisable.	Body of knowledge is still limitated and not definitive.
Sarcocystosis Greiner (2007)	Necropsy or biopsy for the presence of *Sarcocystis* sp. in muscles and/or histological sections of several organs/tissues is the method described to diagnose this condition.	Trimethoprim-sulphamethoxazole and pyrimethamine or trimethoprim-sulfadiazine have successfully been used in control in psitacines	Unknown
Botulism	Mouse protection bioassay with blood or tissue samples is the common approach (Poppenga & Tawde 2012).	Antitoxin administration (especially in waterfowl), drenches, cathartics, laxatives are used to counter the toxin or expel it. Food and fresh water in a sheltered room is made available. Fluid therapy may be required with vitamin supplementation (Rocke & Friend 1999;Neimanis & Speck 2012).	Immunity is short-lived and might not protect in future exposures (Neimanis & Speck 2012).
Algal toxicosis	Is achieved by concurrent spatial and temporal association with the bloom, detection of the toxin producing organism in the gastrointestinal tract, detection of toxin in clinical samples (urine, serum..) or habitat in conjunction with lesions concordant in ill animals (Skocovska et al. 2007; Lowenstine 2008; Puschner and Roegner 2012).	Unknown: rescue, symptomatic /support therapy with the administration of activated charcoal and use of mineral oil; which are recommendations when there is a suspicion (Degernes 1995;Sonne et al. 2012a).	Avoidance of toxic prey after previous exposure prevents new cases (Kvitek 1991/2005).

Table 2 (continuation) – Diagnostic approaches, treatment options and potentially advantageous mechanisms against the effects of future exposures of the diseases in study.

	Diagnosis	Treatment	Immunity/Exposure/Tolerance
Lead and Mercury	Lead: Whole blood lead concentration of >20 micrograms/dL is indicative of toxicity and 50 or more confirms intoxication. Lead concentration in tissues such as liver and kidneys can also be used; on a wet weight, levels of 3 parts per million to 6ppm are indicative of toxicity while values of 6 or more are confirmatory of disease (Samour 2000;Poppenga & Tawde 2012) Mercury: High tissue concentrations (>20ppm) in respect to the concentration of selenium and presence of compatible signs or lesions must be equated (Franson 1999;Poppenga &Tawde 2012).	Lead: Gastric lavage, laxatives, cathartics or endoscopy/surgery to expel or remove remaining lead. CaNa$_2$EDTA, succimer, Dimercaprol, D-penicillamine, for chelation purposes, are administered. Supportive and symptomatic care when needed includes: fluid therapy, seizure control and vitamin supplementation (Samour 2000;Poppenga & Tawde 2012). Mercury: chelation therapy, selenium and vitamin E supplementation may be used in the case of Mercury intoxication (Poppenga & Tawde 2012).	Tolerance to lead intoxication after survival to high doses may develop, at least in mallard ducks (Rodríguez et al. 2010). Presence of selenium and demethylation are advantageous in cases of exposure to mercury (sub-chapter 2.2.7.2.1)
Organophosphorus and Carbamate poisoning	Is reached when depressed brain or blood cholinesterase activity (>25% for exposure and >50% for confirmation) and analysis of gastro-intestinal contents or other organs/tissues for the presence of pesticide or residues are made. (Fleischli et al. 2004)	Unknown or symptomatic/supportive: food, rehydration and maintained of body temperature, surgical removal of gastric contents in acute cases and use of atropine and/or pralidoxime chloride (2-PAM) till resolvance of signs (Zinkl et al. 1981;Elliot et al. 1996; Degernes 2008).	Gradual exposure leads to compensation and tolerance to the deleterious effects (Mineau & Tucker 2002).
Thiamine deficiency (Balk et al. 2009)	Measurement of decreased thiamine levels and dependent enzyme activities in several tissues.	Administration of a thiamine solution, either orally or intramuscularly.	-

3. Paretic Syndrome in gulls (Laridae) in the south of Portugal.

3.1. Main Objectives.

In view of the previous literature revision, a vast array of diseases can elicit a paretic presentation in birds, the pathogenesis is also extremely diverse and a multitude of species can be affected. In spite of the importance that some illnesses can have in the dynamics of certain wild bird populations, or conservation projects, or even in selected cases in other animals, as far as the author's concern no study of this particular syndrome was published or is available in Portugal for the Algarve. Thus, the present dissertation is a preliminary study with the following objectives:

- Compilation of the data recorded, findings seen in RIAS and results of ancillary diagnostic tests, in order to compose a detailed description of this syndrome for the period of time comprehended between 2009 and 2012;

- Characterization and statistical assessment of the association of the presence of this syndrome with the different affected age classes and species;

- Assess possible relations between cases of paretic syndrome and respective geographic distribution with specific potential problematic areas or activities in Algarve;

- Evaluation of the efficacy of three treatment protocols in the outcome of the rehabilitation of paretic gulls and their value as tentative diagnostic methods;

- Comparison and discussion of the findings in consideration of the studies published pertaining to paretic or paralytic conditions in birds in order to: ascertain a probable cause for this syndrome in Algarve, recognize the limitations of this study and propose viable options for future investigations.

3.2. Materials and Methods.

3.2.1. Description of the area in study.

Portugal belongs to the Iberian Peninsula and is the westernmost country of Europe (fig.83A Ap. 2.1). Surrounded by the Atlantic ocean on its West and South fronts and Spain in its Northern and Eastern boundaries, this country is composed by a continental part, in proximity to Spain (approximately 89.084,3 square kilometres), and the two archipelagos, Madeira and Açores (3123,1 km^2). Algarve, the area in study, is one of the 5 major continental regions of Portugal (fig.83B Ap.2.1). It is the southernmost one, with approximately 4996 km^2, ranging from 37º31'44'' N 36º57'42''S to 08º59'49''W -07º23'35'' E, EPSG 4326:WGS 84 (Instituto Nacional de Estatística, [INE] I.P. 2010a). This is the only continental region composed of only one district, Faro, but like the remaining regions, is comprised of several municipalities each subdivided in a set of civil parishes, respectively 16 and 85 (fig.83C Ap.2.1). There are two major mountain systems: Caldeirão and Monchique (maximum altitude 577 and 902 meters, respectively in Loulé, São Brás de Alportel and Tavira - the former and Monchique - the latter), which in conjunction with corkoak and holm forests, strawberry trees and rockrose scrubs characterize the northern part of this region. In more southern latitudes, Mediterranean woods, traditional agricultural areas and pastures are found. Whilst in the southernmost portion of this area, closer to the sea: streams, riparian galleries, marshes, estuaries, saltpans, coastal lagoons, reedbeds and coastal pine forests dominate. On the other hand, the coastline also presents itself with a grand variety of landscapes from steep rocky scarps, in its most western limits, to cliffs intersped by small beaches and estuaries, in the south, and to sandy beaches and long stretches of dunes in the east

and towards Spain, fig.84-92 (Turismo do Algarve 2012a/b). There are three major mainland rivers/streams: Ribeira da Quarteira, Arade and Guadiana rivers (respectively their sources are in Serra do Caldeirão, first two, and Spain, while the mouths are in Quarteira, Portimão and Vila Real de Santo António - V.R.S.A.), however Algarve's hydrographical basin is of approximately only 2.643 km ^{2} - one of the smallest for the 5 continental regions when taking into consideration the two major rivers, Guadiana and Arade, and respective tributaries (INE, I.P.2010a).

Figure 84 – Mountainous relief of Serra do Caldeirão.
Figure 85 – Mediterranean woods in Cerro da Cabeça.
Figure 86 – Almond tree blossoming.
Figure 87 – Riparian vegetation in Carrapateira's stream.
Figure 88 – Cerro do Burros' saltmarsh.
Figure 89 – Estuary of river Arade.
Figure 90 – Ria Formosa.
Figure 91 – Vilamoura's Reedbed.
Figure 92 – Karst features at Marinha beach.
Adapted from: Turismo do Algarve (2012a/b).

The typical climate throughout Algarve is Temperate Mediterranean (in the Köppen-Geiger's system is Csa mesotermic), where dry seasons coincide with hot summers and rain is centred in small periods in the colder seasons (Peel, Finlayson & McMahon 2007;Claro & Pereira 2009). Even though its general classification is Csa with an annual average air temperature of 18,5ºC at least in Faro in 2009, and though the occurrence of precipitation is not frequent (INE, I.P. 2010a), according to Instituto Português do Mar e Atmosfera (2012) in part of the western littoral the climate is instead rated as Csb, the summers are still dry, but only warm and the maximum temperature gauged for the hottest month is inferior to 22ºC (Peel et al. 2007).

Given this climate and the close proximity to the ocean with additional influences of the Mediterranean sea and North Africa, Algarve has a set of ideal conditions that aid in the production of several products, some with a good representation on the country's productivity and/or are quite important and others are typical of this region and renowned throughout the country and worldwide. As is clear in figure 83D in appendix 2.1 of the land use, a part of Algarve is used for agriculture practices. This region contained in 2009 only 4% of the agricultural holdings of the country, which occupy approximately one third of this region's surface, only 3 % of what is used in all the continent and 2% of the Portuguese utilized agricultural area [UAA] (INE, I.P.2011b). Several types of products are grown (cereals, fresh fruits among others), but are mainly in rainfed regimes with a tendency towards an increase in the intensification of the exploitation and use of irrigation techniques, particularly in permanent cultures (INE, I.P.2011b;NEMUS-HIDROMOD-AGRO.GES [N-H-A.G] 2012). Permanent crops of citrus fruits, like oranges and tangerines, are the ones that occupy greatly this extension of the Portuguese territory and contribute significantly to the respective country's production numbers (INE.IP.2010a;INE.IP.2011b). Vineyards are also found in this zone, some grant end products of Protected Designation of Origin and Protected Geographical Indication merit, but are of lesser

representation when compared with other regions (INE, I.P. 2010a), as is the case of olive grooves and other cultures, mainly inland when considering the year 2009 (INE, I.P. 2011b). Animal production is another activity seen in this region normally in extensive regimes, but bearing in mind other districts of Portugal, Algarve´s productivity, the number of holdings in activity in this region, number of heads and the number of livestock units per UAA are quite under the highly productive ones of Norte, Centro and Alentejo, for the same year (INE, I.P. 2010a;INE, I.P. 2011b). Another sector of importance in this region, particularly given its location, is fishery. Nominal catch landed in the region for 2009 was 27.271 tons (18,8% of Portugal's) with the *Scomber japonicus* (7.687t), *Sardina pilchardus* (4.765t) and *Octopus vulgaris* (3585t) being the species caught more frequently and in higher numbers in the seaports of Lagos, Portimão, Olhão, Tavira and V.R.S.A. (INE, I.P. 2010a). Taking into consideration the production in aquaculture facilities, particularly located in Portimão, Lagos, Faro, Olhão and Castro Marim, in 2009 a total of 3.581t of the country's total 7.979t were produced in Algarve, but only in marine and brackish waters, chiefly in extensive systems, with *Ruditapes decussatus* being the most significant species representing more than 50% of the national productivity (INE, I.P. 2010a; INE, I.P. 2010b; INE, I.P. 2011a;NEMUS-ECOSSISTEMA-AGRO.GES [N-E-A.G] 2012;[N-H-A.G] 2012). In contrast, the manual catch of crustacean and molluscs is still a traditional and common practice of great importance for the local communities, particularly in Portimão, Lagos, Faro, Olhão, Tavira and V.R.S.A. (N-E-A.G 2012;N-H-A.G 2012). Due to its geography, geology, climate and traditions, Algarve is also an idyllic place for the production of salt and almost 90% of the total produced in this country has its origin in this region, which translates to 65.349 tons in approximately 1.110 hectares (86% of the continent), fairly 2.253 tons per saline in 2009, principally in the Ria Formosa-Tavira section and Castro Marim (INE, I.P. 2010a;INE, I.P. 2010b;N-E-A.G 2012;N-H-A.G 2012). The industrial sector was and is incipient and little significant, less than 3% of the Portuguese industrial companies and enterprises were based in Algarve in 2009 (INE, I.P. 2010a; INE, I.P.2011c;N-H-A.G 2012). The manufacturing division only granted in 2009, 0,5% of Portugal's turnovers with low percentages of importations and exportations, less than 0,1% (INE, I.P.2011c). This sector is located chiefly in the municipalities of Portimão, Albufeira, Silves, Loulé, S.B.de Alportel, Faro, Olhão, Tavira and V.R.S.A. This economic sector, although formerly characterized by fish industries (preparation and conservation of fish, crustaceans and molluscs), is now devoted to a larger extent to the agro-industrial divisions of food production (Lagos, Portimão, Lagoa, Loulé, Faro and Olhão), wood and cork industries (Silves, Loulé, S.B.de Alportel, Faro and Olhão) and manufacture of other non - metal mineral products (Loulé, Faro and Olhão) fig.92 (INE, I.P.2011c;N-H-A.G 2012). Other important industries are mainly located in Loulé like the case of cement, ceramic, grinding stone and construction materials; typography in V.R.S.A., as well as in Faro and Loulé; carpentry for construction, mainly in Faro and naval construction units in Lagos, Faro and V.R.S.A. (INE, I.P. 2011a;N-H-A.G 2012). Mining and quarrying industries, although present in this region, only represented 2,36% of Portugal's volume of businesses in the industry sector for the year of 2009 (INE, I.P. 2011a). The only mine still in exploration is located in Loulé (rock salt), however it is also in this municipality and the adjoining, S.B.de Alportel, that the greater part of the active quarrying enterprises and companies are situated, extracting mostly sand, clay and limestone, fig. 92 (N-H-A.G 2012;Direcção Geral de Energia e Geologia 2013). Still in the industry sector in Algarve there are two power plants, one hydroelectric in Lagos and an alternative, a thermoelectric, in Silves (N-E-A.G

2012;N-H-A.G 2012). Nonetheless, bearing in mind the principal economic activities, their gross value added (GVA) and total employments rates in 2009 disclosed by INE, I.P. (2012) on basis of 2006, the primary and secondary sectors only represented approximately 19,8% of the regional GVA and employed only 27% of the population. It is in the third sector where the highest values are found, particularly in the accommodation and food divisions, which in conjunction with wholesale and retail trade, real state activities and construction (others associated with the first, N-H-A.G 2012) are the ones with the highest GVA and number of employees in this region (53,7% and 52% respectively). In fact, just the first activity corresponds to almost 15% of the national GVA for the same category (INE, I.P. 2012). These together indicate the true touristic vocation of this region, especially for foreigners that are pursuing the "sun and sea" binomial and more recently other complements, like golf or nautical sports or business tourism (in 2009, 1.708 million foreigners were guests in hotels, touristic apartments and other infrastructures and almost 9.280 million slept at least one night in these establishments) (Comissão de Coordenação e Desenvolvimento Regional do Algarve - 2008;Claro & Pereira 2009; INE, I.P. 2010a;N-E-A.G 2012;N-H-A.G 2012). In fact this phenomena, international and national tourism, exhibits a certain seasonality, expressive in the warmer months of spring and summer (Claro & Pereira 2009), chiefly in littoral counties such as Lagos, Portimão, Lagoa, Silves, Albufeira, Loulé and Tavira (INE, I.P. 2010a and N-H-A.G 2012). These municipalities are also, in part, the ones regularly occupied by the resident population. Accordingly, in Algarve in 2009, the mean population density was of 86,9 inhabitants/km^2 (115,4 inhabitant/km^2 was the country's value), but the municipalities of Portimão, Lagoa, Albufeira, Faro, Olhão and V.R.S.A. were the heavily populated ones with over 200 inhabitants per km^2 (INE, I.P. 2010a). People, in this region, tend to aggregate in these southern coastal municipalities, instead of the northern inland counties, because of this recent touristic development and also the direct/indirect effects of historical events and traditions like the exploitation of marine resources (N-H-A.G 2012). This particular pattern of density is also, to some extent, due to the growing population of foreigners that decided to live in this region and these municipalities. A tendency extremely important, as in accordance with INE, I.P.(2010c), Algarve is the only region where effective growth rates were the highest between 2004-2009 (0,91% in 2009 for Algarve versus 0,1% for Portugal) owing to, in a large part if not all, the migratory growth rates, which are higher than other regions, including Portugal as a whole (0,89% in Algarve and 0,14% in Portugal for the year of 2009).

3.2.2. Seagulls admitted in RIAS.

RIAS, a wildlife rehabilitation centre[2], received from the first of October 2009 to the end of 2012, a total of 780 specimens of seagulls, either as dead or alive admissions. Debilitated and deceased gulls were admitted to this centre after being rescued/retrieved from coastal or inland areas by common citizens, wildlife rehabilitators and state institutions like SEPNA, a nature and environment protection service provided by the Guarda Nacional Republicana (GNR), and ICNF personnel. Upon reception in this centre, a detailed clinical evaluation was performed, blood and faeces were collected (when possible) and the weight registered. All this data was logged under an alpha-numerical code for record identification that was assigned in association with a simpler code in a ring that was placed to identify

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2 Detailed information regarding RIAS and the training period of the author is in appendix 2.2.

the animal throughout the rehabilitation process in the centre. These, in addition to data collected regarding: specie identification (taking into consideration all the variants in the phenotype that are specie-characteristic, chapter 1); age in 5 categories – Undetermined, Chick/Pulli, Juvenile, Sub-Adult and Adult, based on the phenotipic features detailed by Olsen and Larsson (2004); gender (only determined after necropsy examination); date of admission; date when found (and also when was retrieved, when these were distinct); location where the gull was found (local, civil parish, municipality and district); status (collected dead; euthanasia; in rehabilitation; died within the first two days of admission, after two days of the beginning of the rehabilitation or after one month; transferred; escaped or released); suspected cause of admission ("Accidental capture", "Illegal capture", "Illegal captivity", "Orphaned", "Fall from the nest", "Pillage", "Oiled/Soiled Feathers", "Drowning", "Intoxication/Poisoning", "Disease", "Debility/Innutrition", "Predation", "Trauma", "Shot", "Snare/Trap/Net/Fishhook", "Electric shock", "Dazzled", "Collision with a structure or a power line", "Run over" or "Unknown") were also recorded in a digital database. In the course of the physical examination and collection of the clinical history, the veterinary staff performed a thorough examination of the birds, especially with the intent to exclude/minimize cases where a rightful cause or confounding factor could explain the paretic/paralytic presentation. The clinical signs seen, other diagnostic examinations/techniques used (and results attained), necropsy findings, daily treatment plans (including nutrition management) or any observation of clinical importance gathered during the handling in the clinic (administration of pharmaceuticals or weighting) or when the bird changed enclosures (from admission to release: recovery room inside animal transportation boxes; recuperation chambers; exterior pen with access to an artificial lake fig. 95-98) or even during feeding, were also logged.

Figures 95, 96, 97 & 98, Rehabilitation facilities (left to right) – Recovery room, March 2012. Screenshot of video of paretic gull in transportation box in the recovery room, January 2012. Recuperation chamber, March 2012. Artificial lake pen, July 2012. Originals.
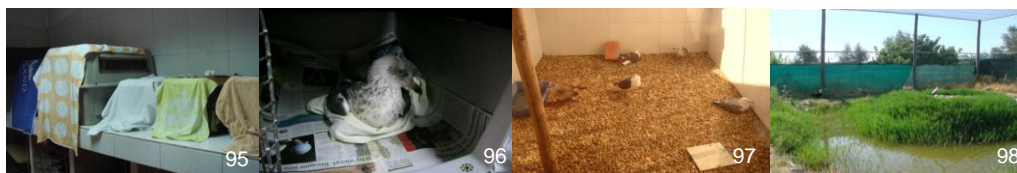
3.2.3. Paretic seagulls.

Birds were classified as having the paretic syndrome specific clinical signs if they exhibited (or has been recorded) paresis/paralysis or hyposensitivity/hyporreflexibility and/or displayed combinations of the following signs: muscular weakness, wing-drop, ataxia, diarrhoea and flaccid cloacae, without clinical evidence of another cause for these signs. From the 780 admissions, 148 gulls (still alive) were received in this centre with the present syndrome and were previously classified in the classes "Disease", "Intoxication", "Debility" or "Unknown" of the set already mentioned. Rescued gulls were released once they were fully ambulatory, were able to fly, feed and fend for themselves, and with the recovering of an optimal body score and weight of approximately 740 +/- 137,8 and 216 +/- 19,8 grams, respectively in larger (*L.fuscus*, *L.argentatus* and *L.michahellis*) and small species (*L.melanocephalus and C.ridibundus*) for the period of time of the study (RIAS 2011/2012a/2013).

3.2.3.1. Physical examination.

The physical examination was performed promptly, in a systematic order and was thorough. It included the evaluation of the: mental status to stimuli (sound, light..), as normal/alert, depressed/conscious - when depressed activity, but still alert to the involving environment and stimuli, or depressed/unconscious - when the gull was completely oblivious; body score, in a subjective scale of 0 to 5 (evaluated by the development of the pectoral muscular mass and consequently the degree of easiness to palpate the carina of the keel, being 0-imcompatible with life,1-caquetic,2-emaciated/very thin,3-thin,4-normal and 5-fat); dehydration degree in 5%, 7%, 10%, 12% and 15% classes (accomplished through the assessment of the degree of hydration in the skin, glistening of mucosal surfaces, vein´s turgidity and the observation of the eyes); presence of excreta (absence, normal or diarrhoea) and some of its features like colour, viscosity and smell; cloacae characteristics, such as normal appearance, flaccid, hardened, prolapsed, excreta in the interior or exterior; state of the plumage and presence of stress lines; and evidence/kind/location of external parasitism (absence, mild, moderate or severe). Additionally, a written description of clinical signs or lesions found in the neuromuscular system, nervous system, respiratory apparatus, cardio-vascular system and sensorial organs, encompassing the head, body, wings and legs were registered in the same document. From the descriptions of the clinical signs, some, particularly the ones of the neuromuscular system, required a unification and systematization of the findings, and in this way the analysis of these recordings resulted in the creation of new classes. Wing-drop, was systematized in degree of severity in the classes: not observed, mild – incompletely retracted when in sternal recumbence; moderate – visible while the animal was standing and in recumbence when the wings were held far from the body and in a lower position; and severe. Muscular weakness, when present, was categorized in three sets: not observed, posterior weakness and generalized – when weakness was general and beak strength was decreased. This sign was further organized in four classes of severity (mild, moderate, severe or not observed). Ataxia, when reported, in accordance with the area where incoordination was mostly seen, was divided in three groupings: posterior, generalized and not observed. Paresis, according to the definitions presented in the literature review in chapter 2, was rearranged in two different categories, degree of severity and generalization (legs or generalized). Paresis was classified as: mild, if several attempts were made to stand and the bird manage to remain standing on its feet, but exhibited some deficits like loss of equilibrium; moderate, when it couldn´t stand, displayed serious difficulties in the elevation of the body and gait maintenance, sometimes the bird stood on its tarsometatarsi; and severe, when they tried to move and stand, but couldn´t and so they remained in sternal recumbency. Paralysis, as complete immobilization, was characterized in 2 classes: generalized or leg paralysis, depending on the observation or description of complete body immobilization or only affecting the legs (the bird never attempted to rise).

3.2.3.2. Collected samples and diagnostic tests used in the centre.

Samples collected in the centre and herein reported include only the animals that were received during the training period. Samples of blood and faeces were the only analysed. Blood, when possible, was drawn from the ulnar or medial metatarsal veins (depending on the hydration degree of the bird). The samples were collected for blood cell count and evaluation of the presence and abundance of

haemoparasites after Diff-Quick ® staining. Dejects were collected upon arrival and/or during the first part of the rehabilitation process, while gulls were kept in the recovery room, but only fresh samples were submitted to parasitological analysis – direct observation and the flotation techniques. The procedures applied in these parasitological studies are RIAS's protocols and are described in detail in Ap. 2.3. The results of such procedures belong to an unpublished parasitological study of which intent was the determination of the prevalence of various types of parasites in several animal families conducted between September 2011 and February 2012, by two students of Biology in the course of their training period.

3.2.3.3. Necropsy.

Every seagull that died while in rehabilitation in RIAS or was initially admitted dead was identified with a paper tag placed in one of its legs with a description of the following: identification code, species, common name, date of admission, date of death, suspected cause of death and any important additional information that could be useful in the necropsy examination. Subsequently, the bird was positioned in a plastic bag and frozen in one of three freezers until necropsy (kept at approximately - 20ºC). In cases of recent death, necropsy could and was performed in the same day without the use or need for this kind of preservation, as was the case of seven in the database and one in the author´s training period. However, the common practice was to immediately freeze the carcass as a result of the high number of admissions and/or the high number of animals in the centre that required attention, and also because between September 2011 and the year 2012 a positive response for external collaboration or extra funding for more complex clinical diagnostic approaches for this condition was awaited for. Syndrome gulls and other gulls (mainly humanly killed due to severe traumatic lesions of impossible resolution) were submitted to necropsy with the intent to describe possible specific lesions of this syndrome. In the case of the author, the inclusion of these unaffected animals had the objective to compare the findings with the best alternative of a negative control that was available and served, in a way, to keep in mind the specific anatomical particularities of this group of birds. Forty one animals whose rehabilitation process or admissions were accompanied and frozen carcasses of cases from 2010 to 2012 were examined by the author, by in large outside of the official training period in July of 2012. Additionally, recorded data of another 41 specimens from October 2009 to December 2012 were included in this analysis. Several species and age classes were evaluated and sex was determined by direct visualization of the gonads, if carcass condition allowed a positive identification.

Bearing in mind the first forty one gulls, the first step of these necropsy examinations corresponded to the identification of the case and allocation of it in the group of the syndrome presentation or negative control, subsequently it followed the thawing. The necropsy technique used didn´t differ greatly from the ones described by Ritchie et al. (1994); Samour (2000); Work (2000); Davis and Morishita (2001); Munson, Friend and Franson (2001); Australian Government – Department of the Environment and Heritage (2006); Rose, Newman, Uhart and Lubroth (2006) and Buckles et al. (2012) of which this is an adaptation. Only the order and some steps were modified and/or simplified for more expediency in terms of agility and systematization in the implementation of a high number of examinations. These alterations were also undertaken in view of a number of anatomical particularities of gulls and the clinical presentation of the ones considered paretic, namely a superior cranium resistance to its opening and the careful examination of the vertebral column and principal nerve groups and

articulations, respectively as is detailed in Ap. 2.4. Throughout the whole procedure, every discrepancy from the normal expected findings, summarized in table 4 of Ap. 2.4, was documented both in a written report and by photographs. Ultimately at the end of these evaluations, the carcasses and removed organs were frozen and kept in a separate freezer while waiting to be collected and sent for incineration.

3.2.3.4. Carcasses and samples submitted to other institutions for more complex ancillary diagnostic tests.

Nine dead frozen seagulls were sent to the Faculty of Veterinary Medicine, University of Lisbon for additional tests that could not be performed in RIAS.The ancillary diagnostic procedures that could be performed by this institution in this case were: anatomo-histopathological analysis by the Service of Pathological Anatomy and respective Routine Laboratory; in accordance with the findings in the necropsy examination, samples (intestine and faeces of the large intestine) were collected for the Microbiology and Immunology Routine Laboratory (search for aerobic, strict anaerobic pathogens and *Salmonella* specimens) and for the Parasitic Diseases Routine Laboratory (flotation technique was used to identify Protozoa and/or Helminths). Liver and stomach content samples were also collected during these necropsy evaluations for the Pharmacology and Toxicology Laboratory for toxic analysis of pesticides i.e. Carbamates, Organophosphate, Molluscicides and Strychnine (which are the usually used in the country and also the ones detected in cases of intoxication) and heavy metals i.e. Lead and Copper (contaminants that could be the cause of this paretic presentation, given the species in study and where they could dwell). Two blood smears were also asked and submitted for parasitological analysis. The necropsy, toxicology, bacteriology and parasitological examination procedures are routine and standard practices of these laboratories, for a detailed description of those, descriptions and references for the methods are present in Ap.2.5. Carcasses, sample identification and techniques used in each specimen are in table 5 in the same appendix.

Additionally, propositions for: extra fundings and/or authorization to send more carcasses/samples and/or pursue other differentials with other diagnostic tests, were also attempted with this institution and others.

3.2.3.5. Therapeutic protocols.

Three protocols were attempted between September 2011 and the first three days of March 2012 in 50 gulls (one was dispensed because when admitted in the centre was already standing, walking and eating alone). These protocols were based on therapeutic protocols already existing for the suspected causes for this syndrome (table 2). Protocol F consisted on oral and subcutaneous Fluid therapy; protocol C was protocol F with the addition of the oral administration of activated Charcoal (protocol already in use in the centre) and T comprised F and the administration of Thiamine, intramuscularly (a meticulous explanation of these protocols is in Ap.2.6). The doses and volumes were calculated in accordance with the maintenance volume, weight of the bird and its dehydration degree, and the only criterion for the selection of either protocol was the availability of charcoal or thiamine in the admission moment. Seventeen gulls received activated charcoal; twenty nine were in the group of protocol F and in four, thiamine was injected intramuscularly. Initially, paretic seagulls were kept in the recovery room, individually in animal transportation boxes covered with newspaper and with free access to clean

water, and every day food was presented to stimulate feeding behaviour (fig.95-96). They remained in this room while they couldn´t stand or walk without deficits and couldn´t feed alone. Survival and an improvement in the paresis or paralysis (number of days until full standing/walking positioning without deficits, noted while feeding and on clinical rounds) were the variables considered to better evaluate the efficacy of the treatment and possibly indicate the best protocol for this paretic syndrome, and tentatively determine its rightful cause.

3.2.3.6. Statistical Analysis.

Data regarding code number, year, species, weight, sex, age class, date of admission and finding, place where the bird was found, civil parish, municipality, district, status, suspected cause of admission, signs, therapeutic treatment applied, and necropsy findings from October 2009 to December 2012 were logged in a spreadsheet of the Software Microsoft Office Excel 2007 © and all the descriptive statistical analysis were made in this software. Statistical tests were performed in the Software R © version 2.15.3. developed by R Foundation for Statistical Computing, available online in open-source (R Core Team 2013). Chi-square test for independence was used to determine significant differences between variables "Age class" and syndrome, as well as "Species" and syndrome. Differences between the severity of the signs identified in the physical examination and variables "Age class" and "Species" were also assessed by this method and Fisher's exact test was used when expected frequencies were lower than 5 or 1. The prognosis was also evaluated with this test, taking into consideration the three therapeutic protocols and the outcome. The relationship between the geographic distribution by municipalities of the proportion of paretic birds and density of economic activities and services per municipality, factors that could have been affecting the development of this disease (i.e.Utilised Agricultural Area/km^2; livestock density: bovine, goats, sheep, swine, horse, birds and rabbits/km^2; density of a number of agriculture and/or livestock holdings/km^2; mines either active, abandoned or in recuperation/km^2; and landfills and wastewater treatment plants/km^2), was studied with the Software R ©, namely by scatterplot matrix analysis and by determining the Spearman's rank correlation coefficient for each association, with data acquired: in the INE website for the year of the last agriculture census, 2009 (www.ine.pt), from the Laboratório Nacional de Energia e Geologia (LNEG) and obtained from the companies that oversee the waste and water management sectors, specifically landfills and wastewater treatment plants, in this case Algar-Valorização e tratamento de resíduos sólidos, S.A. and Águas do Algarve, S.A., respectively.

Differences, in all tests, were considered significant, while using a two-tailed test, at a level of $p<0,05$.

3.3. Results and discussion.

3.3.1. Detailed description of the Paretic Syndrome and possible association between variables.

3.3.1.1. Yearly distribution of the cases.

The number of cases per year varied for the time interval in study (fig.99). The year 2011 was the one with the highest number of admissions (56,8%), and from 2009 till 2011 there was a marked increase, with the year 2012 being only represented by 10,8% of the total. One could possibly state, from these numbers, that there was an effective increase in the number of animals that suffered from this syndrome in the first three years, however some particularities may compromise such assumption. The centre started its activities in October of 2009, therefore and since the retrieval of these animals is a passive process, depending on citizens and external institutions, the sensibility of these for the work developed in this centre and/or the short time span could possibly justify the low number of cases in the year of 2009. The increase in the number of cases from 2010 to 2011 could be due to an actual increase in the number of such cases, but could also be due to a greater awareness of the personnel of RIAS to this particular combination of signs, given that it was the second full year of activity in which the same presentations were seen and a pattern was beginning to be suspected. This awareness is extremely important, given that in 2011 state institution that are the link between the common citizen that normally finds these animals and RIAS, like SEPNA, due to budget cuts, partially stopped retrieving debilitated animals (RIAS 2012a), which could and possibly decreased the number of cases that entered in the centre in 2011 when compared to 2010, and could hinder the high percentage of cases presented in the first. This contingency could clearly explain the low relative percentage of paretic gulls admitted in the subsequent year. In 2012 this retrieval problem was more drastic because in this year these external organizations stopped almost completely (RIAS 2013). Therefore, the low percentage found in this year could more strongly be due to this situation than a actual decrease in the number of ill birds, as numerous phone calls were received by RIAS of people claiming to have found gulls with this presentation (RIAS 2012a), but unfortunately could not be collected, either because they were far from the centre (RIAS doesn´t have a budget for rescue dislocations, hence the arrangements made with the aforementioned institutions who declined this responsibility), or probably given the economic crisis in progress in Portugal (INE 2010a/ 2011a/2012;N-H-A.G 2012) the person who found the animal could not deliver it.

Figure 99 – Temporal distribution of relative frequencies of the admission of paretic syndrome cases from October 2009 till December 2012, in years n=148.
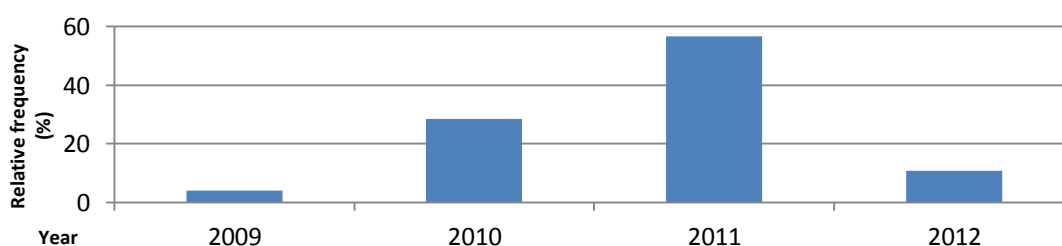
3.4.1.2. Affected Species.

The centre, since its opening, received several species of gulls: *L. fuscus, L. michahellis, L. argentatus*, *L. melanocephalus*, *C. ridibundus*, *L. marinus* and *L. audouinni*, either alive or dead. However, only the first five species exhibited the clinical signs considered specific for this presentation, the remaining were 4 *L.audouinii* (1 "Trauma", 1 "Debillitated" and 2 "Unknown"- 1 already dead) and a deceased *L. marinus*. The species that frequently exhibited paresis were *L.michahellis* and *L. fuscus* (0,47 and 0,49 respectively), however proportionally *L. melanocephalus* and *L. argentatus* were the species in which the syndrome presentation was most representative as a cause of admission (over 25%: 0,25 and 0,33, respectively), but since few were rescued in total (4 and 3, respectively) attempts to determine a more susceptible species may be misleading (fig.100). Contrariwise is the case of *L.fuscus,* as one quarter of the admissions were presentations of paresis.

Figure 100 – Distribution of the absolute frequencies of syndrome and proportion of cases of syndrome/total by species, 2009 - 2012, Syndrome n=148 Total admissions n=780.
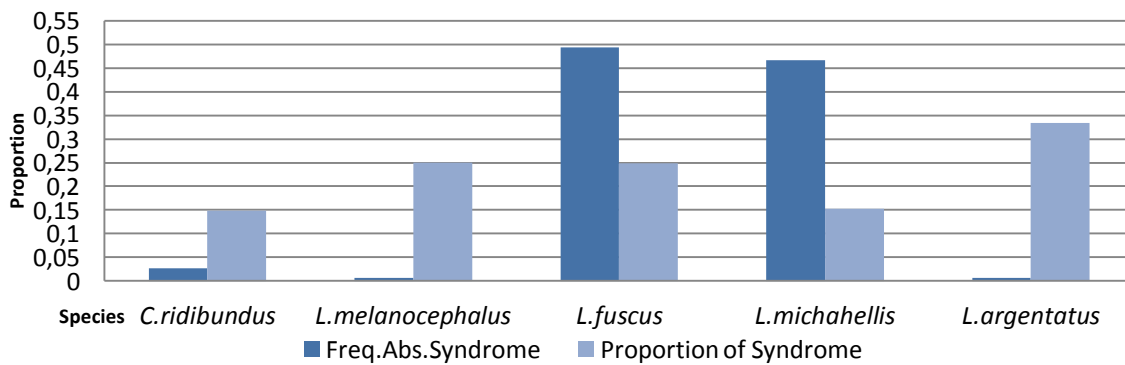
To determine if significant differences were observed between the different species and the presence or not of paretic syndrome in the admission examination, only 505 cases were considered of the total 780 admissions of gulls. Seagulls whose cause of admission was unknown or not determined (n=18) were excluded, as well as cases of birds that didn´t have a description of the findings acquired during the physical examination (n=194) and seagulls already dead (n=63). These exclusions were done in order to prevent the analysis of gulls that, although in categories of unknown cause of admission (they didn´t exhibit any evident sign that could classify them in other categories) or gulls without physical exam results or dead (uncertainties as to the primary cause of disease or death were present) could be cases that belonged to the specific groupings established and bias the results obtained. In this analysis, the cases were evaluated in a two way contingency table with the variable "Species" organized in only 3 subcategories, "*L.michahellis*", "*L.fuscus*" and "other gulls", due to a small number of representatives of species *L.argentatus*, *C.ridibundus*, *L.melanocephalus* and *L. audouinii*, that would complicate the analysis. The null hypothesis tested, of no association of these two variables, was rejected and a possible association between this variable and the presence of this syndrome exists, with the species *L.fuscus* being probably the most affected (p=0,02 for the test value 23,4383 with 2 degrees of freedom).

3.3.1.3. Age classes of the affected.

Taking into account the distribution of cases of this syndrome by the different age classes and the proportion of cases of syndrome in relation with the total admissions for each category (fig.101), no chick or pulli apparently exhibited the signs described when they entered the centre, because they were either classified as victims of trauma, were in captivity, orphaned, fell from their nest or were debilitated. Undetermined individuals were gulls whose age was difficult to assess and some displayed a presentation of paresis (f=0,03). The groupings juveniles, sub-adults and adults were the ones with the highest absolute frequencies (f=0,28; 0,35 and 0,35 respectively). However, when considering the proportion of cases of syndrome, sub-adults was the one in which the number of admission of paresis, in comparison with the total, including other causes, had the highest number (f=0,32).

Figure 101 – Distribution of the absolute frequencies of syndrome and proportion of cases of syndrome/total by age classes, 2009 – 2012, Syndrome n=148 Total admissions n=780.

Age	Chick/Pulli	Juvenile	Sub-adult	Adult	Undetermined
■ Freq.Abs.Syndrome	0	0,28	0,35	0,35	0,03
■ Proportion of Syndrome	0	0,17	0,32	0,19	0,06
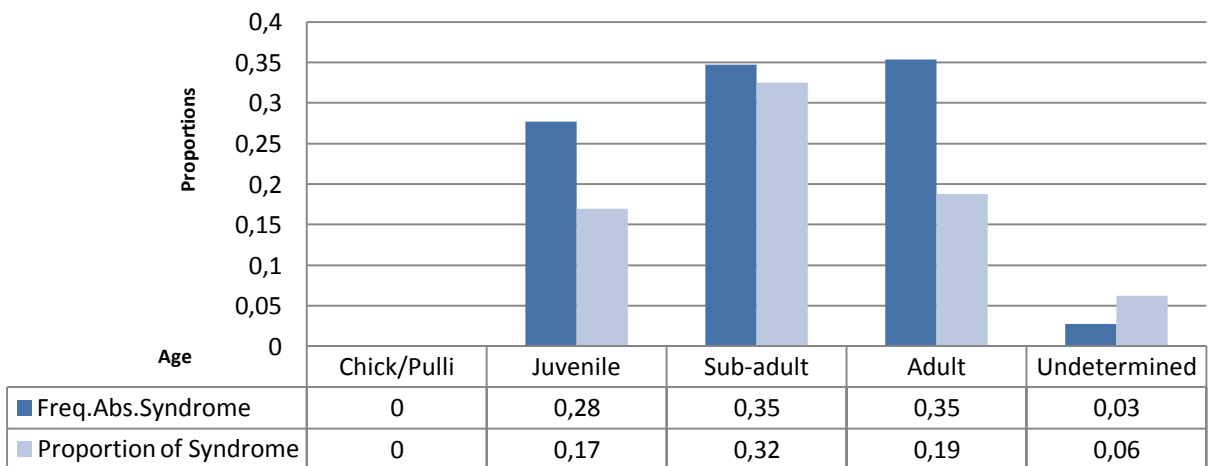
When assessing significant differences with this variable, only 477 records were considered. The same criteria of exclusion of the same cases as in the variable Species were used and the ones whose age could not be determined (n=28) were also rejected, because of the same reasons aforementioned. Accordingly, there is enough evidence to reject the null hypothesis of no association and age class might be associated with the clinical presentation of paresis. In this way, the class sub-adult is probably the most affected (p= 0,00005 for a test value of 49,2727 with 3 degrees of freedom).

3.3.1.4. Month of admission of the paretic.

For easy comparison, the dates of admission of paretic gulls in RIAS were organized by months (fig. 102).

Figure 102 – Nº of cases with syndrome by month of admission from 2009 to 2012, n=148.

Months 2009-2012
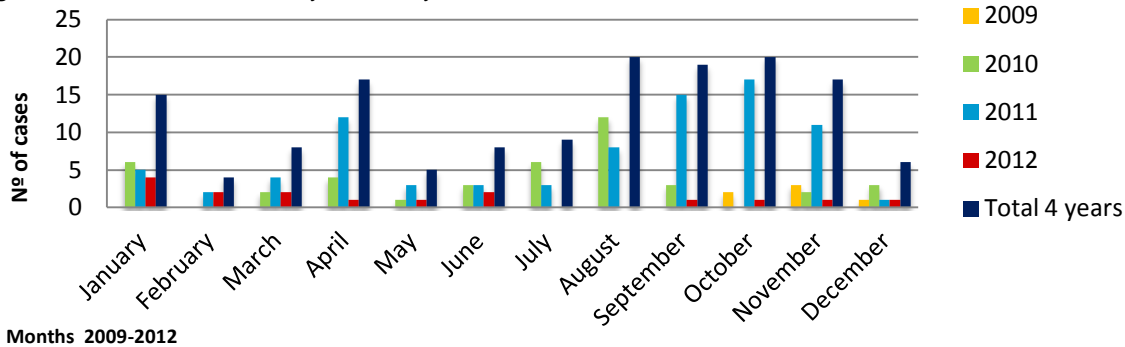
The year 2009, only represented by the last three months, had the lowest number of admissions of paretic gulls, as was previously discussed. However, comparing the remaining 3 full years, a distribution pattern is difficult to asses, as in the year 2010 the majority of gulls considered paretic were admitted in January and in the warmer months of summer, but in 2011 the highest numbers were seen later: in April and at the end of summer and during autumn. In 2012, it is difficult to evaluate this variable, as already explained, cases did occur but retrievals were irregularly accomplished and no record was kept of the phone calls regarding the sightings of such birds that didn´t enter the centre. However, an increase in the number of such phone calls was noticed towards the last months of 2012, and comparing the number of gulls received in 2011 with the ones of 2012 this retrieval problem translated in a reduction of the total numbers of *L.michahellis* and *L.fuscus* admittances for various reasons of 41% and 56%, respectively (RIAS 2013). Nevertheless, the distribution here depicted, at least for the years 2010 and 2011, could be due to the etiologic agent responsible for this syndrome, but could also be very likely influenced by the seasonal migratory patterns of some of these species and resident status of others, as indicated by Catry et al. (2010), which would result in an increased exposure to the cause of this disease of a larger number by gulls and/or aid in their detection and rescue (fig.103 Ap.3.1.). Knowing that wintering or passing by migrants species like *L.fuscus, C.ridibundus* and *L.melanocephalus* normally breed in northern latitudes and may winter in Portugal or in other southern regions, the high number of cases with paresis, apparently, were admitted in between these two biologic events or during their wintering period in Algarve which could justify, in part, the high number of paretic cases in these time intervals. Additionally, the number of cases rescued during the breeding period in migratory species, particularly *L.fuscus* can also be justified by the common presence of immature, non-reproductive or late adults in this region (Catry et al. 2010). The case of the *L.argentatus* is altogether different, as it was found in a month that cannot be explained by a normal migratory pattern. Therefore, either this is a rare finding, or the larid was extremely debilitated to continue its trajectory and remained in Portugal, or is a vagrant, or untimely the identification of this specie is incorrect (it was an immature gull and as previously stated, in the review, an accurate differentiation may be difficult). The seasonality of tourism is another possible factor that may be affecting the number of seagulls collected in the hotter months, as it enhances the probability of finding these animals and may, in part, be a possible explanation/adjunct for the distribution peak seen in the resident specie *L.michahellis* in warmer months.

3.3.1.5. Municipality of origin of the paretic seagulls admitted.

Seagulls, either paretic or not, were retrieved from 14 out of the 16 municipalities in the period of time delimited by this study (fig.104). It is clear that the number of gulls rescued from the more distant regions was the smallest and also none, either paretic or not, was recovered from more inland municipalities, Monchique and Alcoutim (fig.83 Ap.2.1). Perchance, these findings could be due to the fact that these birds can be considered marine birds, as previously stated in part 2 chapter 1, particularly on the word of Schreiber & Burger (2001). Thus, upcountry regions may not be their usual habitat and so the lack of retrievals from such places could be justified. On the other hand, distant counties like Vila do Bispo and Aljezur (distancing more than 90 Km from the rehabilitation centre, fig.83 Ap.2.1), in view of the present economic crisis and consequent severe monetary constraints (impending transport to RIAS) and also a possible unawareness of the work developed in RIAS when compared with nearer towns, are most likely to have the lower number of admissions, despite their close proximity to the ocean and extensive coastline (fig. 83). Furthermore, when compared to more eastern municipalities the coast of these counties can be of difficult human access, as it is defined to a greater degree by its steep cliffs and rocky scarps, as previously stated in subchapter 3.2.1.and this aspect potentially further limits the discovery of sick animals, including paretic cases if present. Municipalities like Portimão and Loulé, for instance, had both the highest numbers of cases of syndrome, but it was also from these two (and the ones adjoining them) that RIAS received a greater part of its gulls, perhaps such results are in consequence of the close proximity of some of these areas to RIAS, but factors like: the extent to which partial and complete unretrieval by external institutions affected these areas; the higher chance for finding, reporting and retrieving sick birds in these heavily populated municipalities by tourists and residents; different distribution of seagulls in these locations and subsequent differential densities; or even the presence of a higher number of disease causes in these municipalities, are additional features that could influence this prospect and should be taken into account before drawing a conclusion pertaining to a possible problematic zone, where a pathogenic agent could be causing this paretic syndrome.

Figure 104 – Nº of cases with syndrome and gull totals recovered from the various Municipalities of Algarve between 2009 and 2012, Syndrome n=148, Total n=780.
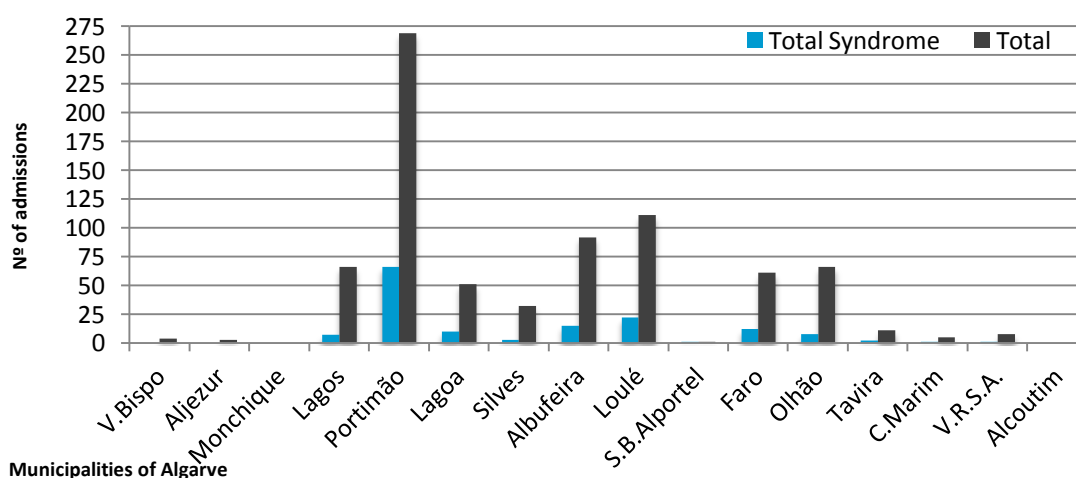
Comparing the three full years and part of the year of 2009, the distribution per municipality of syndrome gulls doesn´t differ greatly, being the county Portimão the one with the highest retrievals of paretic gulls and for each year the next municipality with highest recoveries was respectively Loulé

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(2009), Albufeira and Loulé (2010), Loulé (2011) and Faro/Loulé in 2012. Apart from Loulé, these are all neighbouring coastal municipalities of the latter. Thus, bearing in mind the countless variables that could influence the geographic distribution of paretic syndrome cases and considering the proposed list of differential in part 2 chapter 2, particularly the detailed description of a restricted group of diseases, the association of this distribution (proportion of paretic gulls in each municipality) with a set of variables was analysed to measure its association by the Spearman's rank correlation coefficient. These variables were selected, because they could partake in the pathogenic mechanism of several of these illnesses, like, for instance poultry densities and circulation of APM-1 viruses, agriculture holdings and pesticide use, mine activity or legacy and bioavailability of hazard compounds, landfills and wastewater treatment facilities as points of gathering of high number of birds, but also as sites where optimal sets of physical, chemical and biological factors are reunited for the development of specific conditions, as was previously stated in the case of *Salmonella* or Botulism (subchapters 2.2.2. and 2.2.5).

In this manner and in accordance with table 6 herein presented and table 7 in Ap. 3.2, there were only seven significant associations.

Table 6 – Spearman's rank correlation coefficients of 8 of the associations tested between municipality distribution of proportions of syndrome and density variables.

	Agriculture and livestock holdings	Vegetal specialized holdings	Olive grooves	Diverse permanent crops	Livestock specialized holdings	Commercial birds density	Waste water treatment facilities	Landfills	Syndrome proportions
Agriculture and livestock holdings	1	0.9912	0.3038	0.7196	-0.4176	0.6500	-0.0221	-0.0128	0,5067
Vegetal specialized holdings	0.9912	1	0.2950	0.7049	-0.4706	0.6265	-0.0250	0.0256	0,5304
Olive grooves	0.3038	0.2950	1	0.5447	-0.3422	0.1209	-0.6125	0.1026	0,5052
Diverse permanent crops	0.7196	0.7049	0.5447	1	-0.5695	0.6976	-0.1885	-0.1279	0,5723
Livestock specialized holdings	-0.4176	-0.4706	-0.3422	-0.5695	1	-0.2735	0.3547	-0.0460	-0,5170
Commercial birds density	0.6500	0.6265	0.1209	0.6976	-0.2735	1	-0.0912	0.1764	0,4711
Waste water treatment facilities	-0.0221	-0.0250	-0.6125	-0.1885	0.3547	-0.0912	1	-0.1714	-0,5426
Landfills	-0.0128	0.0256	0.1026	-0.1279	-0.0460	0.1764	-0.1714	1	0,4584
Syndrome proportions	0,5067	0,5304	0,5052	0,5723	-0,5170	0,4711	-0,5426	0,4584	1

They were proportion of syndrome gulls with variables: density of agriculture and livestock holdings, vegetal specialized holdings, cereal/oilseed and protein crops, number of olive groves per km^2, density of other permanent crops, livestock specialized holdings and distribution of waste water treatment plants. Other variables (e.g. industries among others) could not be studied, because data for its analysis wasn´t available online, or upon request. In other cases, variables like waste management cannot be fully studied as information can hardly be inventoried. As an example, in the Algarve there are only two landfills, one in Portimão and the other in Loulé (exactly in the two municipalities where the highest retrievals were found, fig. 104) however, although the 20 official municipal garbage dumps were closed and have remained sealed since 1998 (ALGAR 2012), numerous illicit refuse tips are occasionally seen and some are the focus of several voluntary efforts of common citizens to mitigate and cleanse these sites, as well as are a reason to protest by the locals. These clearly constitute

additional attractive sites for the gathering of birds and are potential areas for the development of disease. These should have been assessed when considering the problematic of waste management in relation to this syndrome, but unfortunately were and are of difficult identification for the purpose of this analysis.

A careful analysis of these results is required in view of the particularities that characterize these animals and that were in full detail introduced in chapter 2.1. of the literature review. Seagulls, in a general sense, are sinantropic birds that nowadays co-habit with humans in more artificial environments, and so their presence in certain settings is now considered a constant finding and that it is why, although this statistical analysis indicates that the geographic distribution of paretic gulls may be associated with high concentration of specific crops and activities, one must ponder that these factors probably are also representative of attractive places to forage for food, especially by gulls. Therefore, these values are possibly an indication of a preference for such places and not a suggestion of a possible location where the pathogenic agent or factor for this condition is present, as again the total of gulls received by RIAS was the highest in these regions and information regarding the presence of diseased birds in exactly these locations is not available. Moreover, pest control measures applied in these sites do not include these birds (e.g. wastewater treatment plants), or this action is not a priority, or information of this aspect is unavailable upon request to at least disregard this point (presence of gulls in these settings). Another issue that was not assessed and probably is influencing this analysis is the fact that variables were only associated with proportion of syndrome and as can be seen in the rank correlation coefficients (table 6), some do have a high value among themselves, either because they constitute a part of the category, like is explicit in the case of the vegetal specialized holdings and animal and agriculture holdings and some, although different, may exhibit the same geographic distribution due to optimal conditions for production or perchance for historical reasons, as is the case of vegetal specialized holdings, olive grooves and the variable diverse permanent crops. Therefore and in addition to the low number of paretic cases that were included in this test (n=148), the p-values close to the significance level and the lack of other variables, some associations might not be true associations and simply coincidences may have had a role in this outcome.

The proportion of paretic gulls was positively correlated with some of the agriculture holdings (total, olive grooves and diverse permanent crops – crops that were not included in other categories), which could be viable places for the contact with potential pathogenic or toxic agents. Nonetheless, ideally, the surface area used by each type of culture/livestock per km^2 in each municipality would be the correct approach to understand if there is a strong correlation pertaining to the geographic distribution of the cases of paresis, as it would be a more realistic index for the probable exploitation of these settings by these species per municipality and subsequent contact or exposure or development of the diseases in discussion. Nevertheless, the data necessary for such analysis is not readily available, although attempts to acquire such information were made, none was successful.

The low significance of some of the p-values is also another aspect to consider as it may indicate that another approach should have been considered. Instead of the use of the 16 municipalities, civil parishes or the sites where the birds were found could be useful in the determination of more accurate levels of significance and correlation for these variables and others like landfills and waste water treatment facilities. For instance, in the previous table, although not considered significant, a high

correlation was observed with landfills and density of commercial species of birds (p=0,0741 and p=0,0655, respectively). The association with density of nowadays inactivate mine exploitation sites of Manganese and Barium (for both p=0,0515) is another similar case. Unfortunately, such data for this analysis is rarely gathered, as repeatedly the animals have been admitted in the centre with this information missing from the forms used by the collecting institutions or it was incorrect, an aspect that has been a struggle of this wildlife centre and has been recently addressed with more training sessions of the operatives (RIAS 2013).

3.3.1.6. Clinical presentation, necropsy findings, ancillary tests and associations.

The signs observed and registered in all paretic syndrome gulls are here resumed (table 8).

Table 8 – Clinical signs seen and reported in paretic seagulls and respective relative frequencies, between 2009-2012, n=148. N.O. stands for not observed.

Clinical Signs		Relative Frequency (%) n=148
Mental status	Normal/Alert	39,2
	Depressed/Conscious	58,8
	Depressed/Unconscious	2
Body score	1	8,1
	2	81,8
	3	10,1
Dehydration (degree)	5%	25
	7%	69,6
	10%	5,4
Excreta (presence and type)	Faeces	10,8
	Diarrhoea	43,9
	N.O.	45,3
Cloaca (appearance)	Normal	20,9
	Flaccid	70,3
	Excreta in the exterior or interior	8,8
Infestation (degree)	Absent	79,7
	Mild	19,6
	Moderate	0,7
Wing-drop (degree)	Mild	2
	Moderate	10,8
	N.O.	87,2
Weakness (extent)	Posterior	1,4
	Generalized	48,6
	N.O.	50
Weakness (degree)	Mild	0,7
	Moderate	46,6
	Severe	2,7
	N.O.	50
Ataxia	Posterior	4,7
	Generalized	8,8
	N.O.	86,5
Paresis (extent)	Posterior	69,6
	Generalized	8,1
	N.O.	22,3
Paresis (degree)	Mild	4,1
	Moderate	71,6
	Severe	2
	N.O.	22,3
Paralysis (extent)	Posterior	1,4
	Generalized	2
	N.O.	96,6

Table 8 (continuation) – Clinical signs seen and reported in paretic seagulls and respective relative frequencies, between 2009-2012, n=148. N.O. stands for not observed.

Clinical Signs		Relative Frequency (%) n=148
Dyspnoea	Mild	2,7
	Moderate	25,7
	Severe	2
	N.O.	69,6

From the data organized and systematised the majority of gulls received in RIAS, between October 2009 and 2012, were alert or a little depressed (98%). Nevertheless, they were extremely aggressive even when paresis was generalized, severe and to some extent a noted muscular weakness was apparent, especially denoted as a decrease in the strength applied when fending (fig.105). Body score and dehydration degrees were problematic: gulls in general were extremely thin and dehydrated, but few (8,1%) or none were classified in the life-threatening levels, 1 and 15% respectively. Temperature was rarely measured, as little clinical information could be provided by this procedure and the additional stress or the associated risks were avoided (Harrison & Ritchie 1994). Upon reception and on the first physical examination, in 45,3% of the cases, the presence of droppings or evidence of diarrhoea was not documented. Normally, when these birds reached the centre on transportation boxes or card boxes, the journals and the feathers were soiled hindering the possibility to distinguish faeces from diarrhoea and evaluate some of its features. In some cases like the one of fig. 106 the number of animals received per box was too high to distinguish this feature, and very few (8,8%) had cues of its presence around or visibly inside the cloacae to further asses this sign. When present, diarrhoea was characterized as having a greenish colour, with an intense odour and a pasty-like consistency and could be responsible, in part, to the dehydration status of these birds. Flaccid cloacae was a recurrent finding, 70,3% of the admissions presented this sign. Regarding external parasitism, still in the general clinical approach of the debilitated gull, very few cases of lice and ticks were detected (n=30). In cases that were positive for this condition, the pathogens weren´t specifically identified.

When considering the signs that were reported as a written description, it became imperative to standardise the set of findings recorded, although a certain degree in subjectivity in the description was always present and made it difficult, including some of the author's own written statements. Wing-drop was a sign less frequently documented and probably in the 87,2% that didn´t display deviations from the normality in the upper body, this sign could be present, but due to the various attempts to rise manifested by these birds or the reluctance to move from the position adopted when they fell in these situations, it could be easily disregarded or of difficult detection, given that 73,6% of gulls had moderate/severe paresis, particularly of the lower body portion (fig.107).

Figure 105 – Adult paretic *L.fuscus'* aggression state, January 2012 (screenshot of video). Original.
Figure 106 – Admitance of several juvenile and sub-adult paretic gulls in a card box, July 2010. RIAS**.**
Figure 107 – Adult *L.michahellis* moderate paretic standing on its tarsometatarsi, January 2012 (screenshot of video). Original.

Taking into consideration the sign muscular weakness, this is probably one of the most difficult signs to evaluate, since it is difficult to determine to what extent the paresis is influencing or is influenced by

Figures 108 & 109 – Adult paresic *L.fuscus* trying to stand and reluctant to move after several attempts, lateral views January 2012 (screenshots of video). Originals.
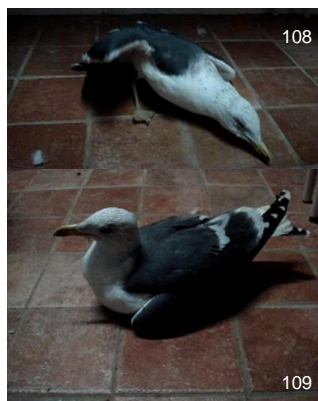
this sign, and so 50 % of the cases were reported as not having it, but when generalized it was easily seen maybe more due to a decrease in the strength of the beak than as a result of an observation of the legs, as previous stated. Ataxia (here defined by a wide gait, misplacement of the feet, anterior buckling of the knee and/or staggering) is similar to the case of Wing-drop and could have been unnoticed, because undoubtedly is more easily detected when the animal is in motion than, possibly, when trying to stand, as was frequently the case. The greater

Figures 110 & 111 – Adult paresic *L.fuscus* trying to stand and reluctant to move after several attempts, dorsal views January 2012 (screenshots of video). Originals.

part of the 148 admissions was paretic and it was more commonly reported a posterior and moderate paresis (69,6%;71,6%, respectively). The typical picture seen in the training period was of a paretic gull making an effort to stand while resorting to the wings, fully functional, and beak, as seen in figures 108 and 110, or refusing to move (fig. 109,111). A puzzling result shown in table 9 is the percentage of 22,3% that represents animals that upon the physical examination were not documented as having paresis, and since the theme of this dissertation is paresis the inclusion of such animals would be an inaccuracy. However, this last percentage corresponds to the ones that were paralytic, fig.112 (paralysis was considered as a progression of the disease); plus the ones with hyporreflexability or hyposensibility, and also the ones that exhibited the other signs that were considered specific to this syndrome (e.g.diarrhoea, flaccid paresis of the cloacae among others explicit in the material and methods section) that were seen to linger during the rehabilitation process in the centre and from the few clinic histories that could be gathered of birds that recuperated the ability to stand and walk prior to admission. The cases displaying these signs were counted as belonging to individuals with the paretic syndrome, also because these signs were never reported, or seen, in other gulls in these combinations. Dyspnoea was another sign found in these animals, in particular a moderate presentation, 25,7% (fig.113). When comparing the severity and extent of this clinical signs with variables "Species" and "Age class" to establish if there were significant differences, by the use of the

chi square test of independence, Fisher's exact test had to be used and there wasn´t evidence to reject the null hypothesis, of no association for the key clinical signs. Nonetheless, "Species" versus "Extent of weakness" and "Age class" and "Body score" were significant (after omitting animals whose age was classified as undetermined and grouping species with less representation), table 9 Ap.3.3 (Fisher's exact test p=0.03836 and 0,04102 respectively). Other signs found in these 148 animals, although not so consistent - no more than three cases per finding, were: respiratory stridor; regurgitation; pale mucosa; soft tissue traumatism of legs, keel and wings, as well as possible wing luxations (because of the efforts to walk and stand or the prolonged recumbency); vascular congestion of the foot webs; bilateral conjunctivitis; inflammation of the third eyelid (figure 114); epiphora; arrhythmia and peri-cloacae oedema.

Figure 112 – Paralyzed adult *L.fuscus*, March 2012.
Figure 113 – Dyspnoeic paretic sub-adult *L.fuscus* (screenshot of video), January 2012.
Figure 114 – Detail of the plaque on third eye lid of a sub-adult *L.fuscus* paretic gull, November 2011.
Originals.

Analysing the data in the digital records and the findings of the necropsy examinations conducted by the author, seventy three specimens were included in this diagnostic examination of the paretic syndrome. Of these seventy three, thirty one gulls were admitted in 2010, thirty six were from 2011 and six were received in 2012. Considering the categories "Specie" and "Age class" this number is represented by two adult *C.ridibundus*, thirty one were *L.fuscus* (3 of undetermined age, 5 juveniles, 11 sub-adults and 12 adults) and forty were individuals of the species *L.michahellis* (1 undetermined, 11 juveniles, 11 sub-adults and 17 adults). These animals were rescued all over the years 2010 to 2012 and from several municipalities of Algarve (from Vila do Bispo to V.R.S.A.), including Lagos, Portimão, Albufeira, Loulé, Faro, Olhão and Tavira; even though Portimão was the municipality most represented with 30 cases retrieved from this zone and Loulé was the second one with 15 specimens (the others did not reach the dozen cases each). These distributions and the number of cases in study in this ancillary diagnostic technique depended highly on the number of cases whose information was logged in the database, but also on the selection of the carcasses to open made in July 2012. Given that in both situations the decomposition degree was a determining factor and some exemplars were rejected, because records, carcasses or observations made while in the course of the necropsy (particularly dead admittances and birds of 2010) indicated/exhibited signs of advanced decomposition that would hinder the correct description of anomalous findings that could explain the pathological mechanism in progress in this disease. Other factors contributed to this set of selected cases like the high or low number of admitted seagulls of determined species or age classes, municipality of origin and clearly the ones that died during the therapeutic protocols. Nonetheless, in a certain manner this assortment is a reflexion of what was seen and herein previously documented: species most affected, age groups potentially more sensitive and hypothetical problematic municipalities of provenance.

In the course of the organization of the data and during the period established to do the necropsy evaluations there was a need to categorize the findings in classes of days spent in the centre: zero days – dead admissions and also gulls that stayed less than 24 hours in the centre, 1 to 2 days – category already present in the database, 3 to 7 days - a week, 8 to 30 days, and more than a month – already existing classes. These rearrangements were necessary as certain lesion patterns were apparent and could be deriving from this variable, and also because the therapeutic protocols differed temporally. Thus, a table of the observations reported in gulls considered as exhibiting signs of the paretic syndrome is in Ap.3.4. The grand range of the selected animals was chosen in order to try to understand the clinical presentation seen and potentially assess and describe the macroscopic lesions present in these paretic gulls. In view of the fact that signs of possible opportunistic infections or captivity lesions were seen in some, which could be confounding factors for this analysis, records and necropsies of dead gulls were used to study and describe any possible macroscopic deviations from normality. Sixty three dead seagulls were admitted in RIAS between 2009 and 2012, of these, in 4, traumatic lesions could be and were identified as the primary cause of death, but for the remaining in the tag and corresponding digital log the suspected causes of admission were either "Unknown", "Disease", "Intoxication" or "Debilitation" before a necropsy was attempted. Since no background clinical history was present to corroborate the initial classification of these wild birds, from the 59 remaining, the ones that were highly suspected of having the syndrome were the ones with signs of gastrointestinal distress presented in the form of faecal/diarrhoeal material adherent to the cloacae and feathers and the absence of any other suggestive clue for any other affliction, at least grossly visible (n=21). This stipulation allied with data regarding the local where the bird was found ill (chiefly Portimão) were the main restrictions for the selection of the cases, although certainly a margin of error exists in these assumptions. Additionally, the inclusion of animals that died past a month after entering the centre was more on a basis of curiosity, since their rehabilitation was closely accompanied during the training period, and questions, as to why animals that fully recovered died, as well as others whose complete rehabilitation was a strenuous process, were present and entailed explanations. Therefore, this situation represented an optimal opportunity to evaluate if this syndrome could be due to pathogenic agents or substances that required time to develop lesions visible to the naked eye and provide preliminary indications of the resistance of these animals to captivity while in rehabilitation, or possibly if a depression of the immune system resulted and aided in the establishment of opportunistic diseases.

By analysing the aforementioned table, the existence of external lesions that were previously seen in the physical examination were also recorded in the necropsy. This lesion pattern, affecting the beak and the distal portion of the limbs (n=7), could be consistent with the recurrent attempts that paretic gulls do to rise up. Subcutaneous vascular congestion was also noted in a high number of cases in the first four categories (n=29), but instead of being limited to these peripheral zones was generalized and sometimes this finding was accompanied by vestigial or small quantities of fat deposits with also engorged vessels (n=19). All 5 body scores were identified, however the highest were seen in animals retrieved dead or that spent less time in rehabilitation, as is clear in part by the amount of fat deposits present in these animals that was considered moderate; while towards the last two groupings the lower scores of 1, 2 and 3 were the only documented. Feathers soiled with normal faecal contents or diarrhoea was a frequent finding (n=29) and likely could be due to the impaction and distension of the

cloacae, which was recurrently found replete with excreta/diarrhoea and gas, and its walls were accordingly extremely thinned (fig.115,116). Feathers soiled, peri-cloacae accumulation of excrements, with the observation of flaccid cloacae upon admission in ill birds, in accordance with Cole (2007), are indicative of cloacae dysfunction and enteritis as what was seen (fig.117). The remaining gastrointestinal tract had lesions of oedema, vascular congestion and was, as well, distended with a yellow-brownish fluid-like faecal material and gas in the majority of these observations (n=29), in what could be a tentative diagnosis of a gastroenteritis and named in the centre, in association with the clinical signs, GEPIC, from the Portuguese, literally translated, GastroEnteritis and Paretic Idiopathic syndrome of the Charadriiformes (fig.117). In another 29 seagulls, although with evidences of early or moderate decomposition, this lesion pattern was also found but only documented in gulls in the initial phases of this process (n=3). Stomach contents were rarely seen and sporadic findings of food items were reported (chicken flesh or fish given in the recovery room, n=4), which is in agreement with the few that could eat alone, seeing as these animals when admitted, the majority, tried to eat but few could accurately and with ease ingest solid food items, either because they were weak or the movement was difficult and paresis could be influencing this low success. In the case of gulls of higher classes of days spent in rehabilitation, digestion might have eliminated any evidence of food. Cases of ingestion of foreign bodies were present and a glass fragment was found in a dead gull (despite its presence, additional related lesions were absent), as well as a finger fragment of a latex glove in an alive admission, possibly ingested in one of the episodes of aggression manifested during the physical examination. Taking into consideration the gall bladder, the only repeated finding (n=8) was enlargement of this organ due to distension by the presence and accumulation of bile, whereas various lesion patterns were found in the liver: hypertrophy and/or vascular congestion (n=4), necrosis (n=1), atrophy (n=2) or the organ was uniformly pale (n=1).

Constantly, gross findings of congestion and haemorrhagic presentations were seen in severe cases, in which several organs were affected simultaneously: lungs (n=15), kidneys (n=5), heart (n=16), celoma (n=4), endocrine organs (n=5), gastrointestinal tract and annex (spleen, n=5). They were found mainly congested, hypertrophied and/or haemorrhagic (particularly the lungs) and in some birds, collections of unclothed blood were seen in the celomic cavity (n=5), gastrointestinal tract (n=2), respiratory tract (n=2), pericardic sac (n=2), which in conjunction with the generalized vascular subcutaneous congestion are strongly suggestive of a systemic disease.

Figure 115 – Feathers soiled.
Figure 116 – Cloaca distended, thinned walled and replete with fluid faeces.
Figure 117 – Inflamation of the gastrointestinal tract, July 2012.
Originals.
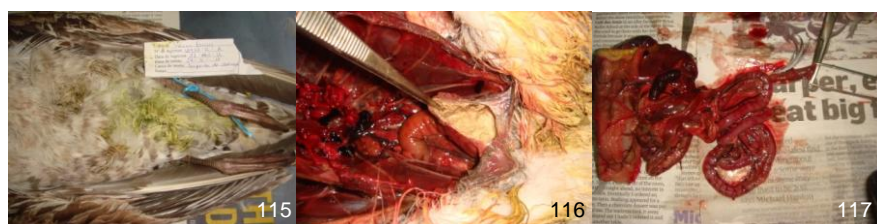
However the cause of this syndrome is difficult to assess since in these animals other combinations were seen like pale dilated hearts (n=8), unclothed blood in the hearts chambers (n=3), myocardium

atrophy (n=3), white precipitates in the pancreas (n=1) or pale pancreas and spleen (n=6) among several. In 6 of these 58 gulls, opacity of the air sacs was found (n=2), which in one case was associated with point like lesions scattered in the liver, but could also be associated or not with similar pointy/nodular lesions in these compartments of the respiratory tract (n=4). Whereas, in at least 4 other gulls it was possible to describe a group of lesions highly suspect of aspergillosis (laboratorial confirmation was not made). In this last group haemorrhagic and congestive presentations were seen allied with alterations on the transparency of the air sac´s walls and presence of nodules or masses that could be found with various shapes/sizes, but with an elastic-cartilaginous consistency, caseous appearance and some with a superficial grey-green-whitish velvety texture scattered on the surface or inside of the clavicular, cranial and caudal thoracic, and/or abdominal air sacs. In the lumen of organs like the syrinx, trachea and bronchi similar nodules/masses or plaques were found, sometimes associated with the same lesions in other organs and tissues such as: lungs, celomic cavity, heart, gastrointestinal tract and kidneys and the bird usually had the lowest body scores.

Taking these findings into consideration the question is whether in all these cases the cause was present primarily and was responsible for the paretic syndrome, or additional pathogens were acquired or developing during the period of disease, in liberty or in captivity in the centre, were masking the original lesions and/or can be the rightful causative agents for the paresis. Seeing as some animals did present these lesions on admission (e.g. signs of opacity in the air sacs and nodular lesions were seen as early as the first days in the centre, in dead admission and probably in the group of the 1-2 days); and others, particularly the ones with almost a month or over a month of rehabilitation, didn´t recover completely like was the case of an adult *L.michahellis* (V0588/11/A) that exhibited all the signs described (in this case moderate leg paresis, generalized moderate weakness and flaccid cloacae) and whose wing-drop never disappeared (fig.118). This gull, on protocol C, in one week was capable of walking and within a month upon entering the centre was already in the lake, but since both wings where still held in a lower position (after another month) and this wing-drop was beginning to be more severe on the left side of the body, he returned to the clinic where a possible shoulder luxation was suspected and diagnosed in the physical examination. The gull was again confined in the recovery room with the wing immobilized for another month and meloxicam (0,1ml of Rheumocan ®) was daily orally administered. With the swelling gone and the shoulder in the correct position, treatment halted but the same wing-drop remained. In the last days, he progressively started to display difficulties in breathing and eventually died. This larid, upon the necropsy examination, had huge granuloma like lesions in the cranial celoma, particularly on the right side and shoulder, and a small nodular formation with congestion and haemorrhage on the left shoulder, which were in accordance with the clinical signs observed. Plaques/nodules in the trachea, bronchi and intestines, both with a velvety-like texture on the surface, where also found, as well as hemorrhagic lungs and air sacs, kidney hypertrophy, free blood in the lumen of the trachea, gastrointestinal tract and in the celomic cavity; a presentation deemed compatible with aspergillosis (fig.119-121).

Figure 118 – Gull V0588/11/A in the recuperation chamber after confinement (screenshot of video), November 2011.
Figure 119 – Cranial celoma of the same gull with granuloma like lesions (black arrow) and signs of congestion and haemorrhage (white arrow), December 2011.
Figure 120 – Detail of the lumen of the trachea/bronchi with plaques and free blood.
Figure 121 – Stomach and cranial portion of the small intestines with focal haemorrhages and a plaque with vegetative growth.
Originals.
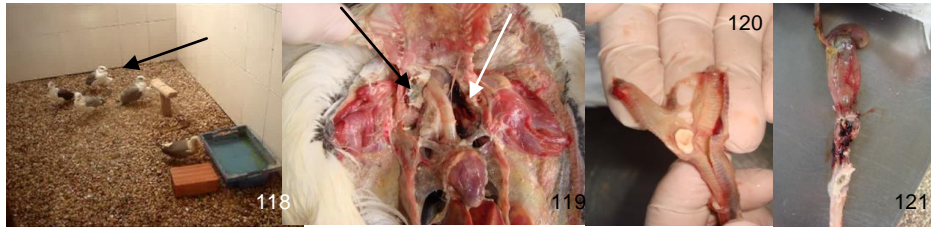
Other point to consider is the case of the gulls that fully recovered, but died abruptly as is an example individual V0038/12/A. This bird was an adult *L.fuscus* with the same clinical presentation as the former, but was on protocol T. He recovered in two days the ability to walk and in one week started eating alone, nonetheless on the seventeenth day started head pressing and was frequently found on his back, in the recuperation chamber, without any kind of awareness of his surroundings. This seagull was extremely listless and dyspnoeic and was admitted again in the recovery room, where cortisone was given intramuscularly (0,26ml of Hoscortisona ®) and fluids were administered, but the animal died shortly after (30 minutes). Upon the necropsy procedure the kidneys were congested and with a roughened surface, opacity of the air sacs allied with small yellow nodular like lesions was found, the myocardium was congested, the liver enlarged exhibited a scattered pattern of yellow pin point lesions that extended to all the parenquima, the gall bladder was full and the cloacae distended with faecal matter (fig.122-124). The rapid onset of the disease and death in an apparently healthy gull was considered suggestive of a secondary disease.

Figure 122 – Subcutaneous oedema, detail of the pin point pattern on the liver and air sacs of gull V0038/12/A.
Figure 123 – Opening and exposure of the celoma cavity extent of the lesions.
Figure 124 – Left kidney with a wrinkled appararence; February 2012.
Originals. Note: blood is an artefact of the accidental severing of a few vessels, during the procedure.

Parasites were found in various organs of these birds (n=6), particularly in the small intestines and air sacs (only one case), but were only classified as either cestodes or nematodes on the records. The lesions seen and recorded in association with these parasites were usually of congestive or haemorrhagic nature. In one case 30 specimens of cestodes were counted in the duodenum and in another no more than 6 nematodes were observed in a thoracic air sac. However, in one gull the presence of digested blood in the ventriculus and upon removal of the cuticle, circular haemorrhagic lesions in more profound layers of this organ were strong indicators of the presence of parasites in this part of the gastrointestinal system. The presence and effects of these pathogenic agents together with the gastroenteritis frequently seen apparently results in a more severe presentation, since in only two cases was documented a considerable muscular atrophy (more severe than the one usually

accompanying the low body scores registered in other birds) and these corresponded to two of this parasitized animals.

Chronic lesions indicative of a traumatic nature in the skeleton in early life (in two gulls: one with callous bone in the scapula and other in the elbow) or malformations like the one seen in the pelvis (Fig.125 sub-adult

Figure 125 – Skeletal malfomation in the insertion of the right limb in the pelvis, July 2012. Original.

L.michahellis V0597/11/A) were also found, but could hardly be consistent with a presentation of paresis/paralysis. They were unilateral, already healed, no alteration of adjacent structures was noted and no form of compensation was seen in life (i.e. an asymmetrical atrophy/hypertrophy of a specific set of muscles as a result of a dislheveled gait and stance, or abnormal positioning of the limbs, or even due to irregular locomotion). Injuries in joints were seen and documented, like the cases of intertarsic capsule and tendinous shealfts distension of both limbs (fig.126) and left shoulder purulent synovitis with active haemorrhage of the left wrist (this haemorrhage was probably a secondary lesion of the first due to the wing drooping). These two situations could be causes for the paretic syndrome or a confounding agent, given that in the second (an adult *L.michahellis* V0606/11/A) gross lesions of generalized involvement of multiple organs was logged, similar to what was previously described and already considered; but in the first (V0100/11/A) a collection of lesions with no involvement of the gastrointestinal tract were seen, although diarrhoea was present upon admission. Seagull V0100/11/A was a sub-adult *L.fuscus,* that when examined post-mortem had: white opaque pointy-like lesions of less than 0,5 millimetres in diameter scattered in the visceral surface of the liver and on pale and hypertrophied kidneys with engorged vessels (fig.129). Similar alterations, but in the form of nodules of 0,5 to 2mm were also found in the celomic cavity and air sacs. The pericardium had several adhesions to the epicardium, which was thickened and almost completely covered by an adherent white and dry layer of a friable nature (fig.127,128). Hypertrophy and congestion of the spleen, as well as adrenal hypertrophy were additional observations made. This *L.fuscus* was admitted paretic and dyspnoeic, like the remaining, died after one week of ingression and didn't respond to enrofloxacin (¼ of a pill of Enrox ®) and Rheumocam ® (0,12 ml) given daily orally. Nevertheless, this lesion pattern and clinical history are more in accord with visceral and articular gout, as was what was suspected. This disease can manifest as a consequence of dehydration or may be due to a functional impairment or damage of the kidneys owing to a primary or a secondary disease, such as lead intoxication or blockage of the ureters by cloacae impaction (Ritchie et al. 1994, Harrison & Lightfoot 2006); ailments that could be present or be a cause of paresis.

Figure 126 – Distension of articular capsule and tendinal shealth.
Figure 127 – White and dry layer of friable consistency adherent to the heart and main vessels.
Figure 128 – Details on the heart.
Figure 129 – Pale and enlarged kidneys with small pin-point lesions scattered through the organ, March 2011. RIAS.

In only 54 larids was possible a gender discrimination, either because the remaining were still not fully developed, or the corpse was severely altered by the decomposition and the effects of freezing and thawing. Decomposition probably took place already in the wild, in the case of dead admittances (there isn't information regarding the time of death and number of days till collection, but the high number of carcasses with organs in the early or even advanced stages of decomposition was high in this group as is clear in table 10). But it could surely progress rapidly in RIAS in the other specimens, as a consequence of the already stated gastroenteritis and probably was accelerated by the warm environment kept in the recovery room when gulls died over night in the colder seasons. Freezing is not the ideal method for preservation of tissues and so decomposition, although impeded, did not halt as are examples some specimens of 2010 that were opened in 2012 or the ones of the training period in the same time-frame. The thawing was also other factor that probably affected the carcasses, as in July 2012 it occurred overnight in one of the hottest months of the year. Notwithstanding, 25 birds were males and 29 were females and none exhibited inflammatory processes, lesions, malformations, neoplasias or any anomalous physiological reproductive response that could affect the neuromuscular apparatus and provoke a paretic or paralytic condition.

The analysis of the nervous and muscular systems, excluding the traumatic lesions already disclosed, did not reveal any abnormal alteration, although in accordance with RIAS' database the cranium was rarely examined (only one time). Nevertheless, the elements of the peripheral nervous system were evaluated and no abnormal observation was made. During the necropsy procedures of the author, lesions were absent in the two systems and the muscular division, in spite of evidences of early and advanced cases of autolysis of the brain.

Lesions and pointers of the effects of the therapeutic protocols were seen and in some of the already discussed specimens could have impaired their rehabilitation process. A dark thick fluid, congruent with active charcoal, was sometimes seen throughout the gastrointestinal tract in gulls that were in protocol C and died in a week after retrieval, always in the absence of ulcers that could account for the presence of a similar fluid (n=2). This presence could probably be the cause of death in one sub-adult *L.fuscus* gull (V0679/11/A) that in the necropsy examination had distended cloacae with active haemorrhaging and oedema. This outcome could have been due to the aggregation of small particles of charcoal that dried and were grazing the mucosa, a finding also documented. These spherical bodies could have led to the prolapse of this organ and the infection of the damaged tissues could explain the hyper acute mortality event witnessed. In less than 24 hours, this gull exhibited epistaxis/hemoptise associated with severe dyspnoea which culminated in death (blood afterwards was seen in the trachea and celomic cavity, the kidneys were hypertrophied and congested like the heart, fig.130). In a juvenile *L.michahellis*, V0585/11/A, death was probably due to aspiration pneumonia as a dark viscous fluid was noted in the trachea, air sacs and lungs of a severe dyspnoeic gull (fig.131,132). Other effects of the therapeutic protocols in use were less deleterious, but should be kept in mind because they could easily be considered as pathological observations, like the subcutaneous oedema of the inguinal region found in the early stages of the rehabilitation process (n=2), where preferably is performed the subcutaneous fluid therapy. But then again, the incorrect administration of fluids in this zone led in one case, juvenile *L.fuscus* code V0696/11/A, to the damaging of the skin and consequent invasion/proliferation of fly larvae, which didn't cause paresis as the gull was initially paretic, but may have contributed to her death (fig.133).

Figure 130 – Blood in the celomic cavity.
Figure 131 – Black viscous fluid in the lumen of the trachea.
Figure 132 – Dark fluid present in thoracic air sacs.
Figure 133 – Inguinal iatrogenic wound, July 2012.
Originals.

Even though a careful approach was considered in the selection of carcasses, the number of animals in early/moderate or advanced phases of decomposition was high, predominantly explicit in the gastro-intestinal compartment. Consequently, in these a full reporting of the discrepancies from normality was difficult and in some (n=16) gastrointestinal lesions were not considered given the elevated risk of them being artefacts of the decomposition. In two of these cases, alterations seen in adjoining organs like liver, lungs and kidneys, that also displayed the effects of this process, were likewise disregarded. Conversely, in other 6 gulls, findings were impossible to differentiate and none were recorded. Thus, these reported findings can be an undervalued estimation of the reality. The low number of cases included in this analysis; the fact that microscopic observation of lesions was impossible and no capable institution showed interest in pursuing this analysis in collected samples or before/after the freezing and/or necropsy evaluation; records were written accounts entered by different persons, with different background experiences leading to probably different degrees of sensitivity to this findings (as is clear in the various combinations standardized in table 10, Ap.3.4); and numerous lesion patterns were present with a possible relation to the time spend in captivity and simultaneous progression of the disease, are all hinder factors for a complete, accurate and representative description of this disease, and shouldn't be disregarded when considering the data here compiled.

Taking into consideration the ancillary tests that were performed in the centre with samples collected from paretic gulls between September 2011 and February 2012 (only data available), haematology and coprology studies were the only diagnostic techniques that were and could be executed. The number of samples was small in this evaluation and as is stated by the biologists, authors of this study, and bearing in mind what was experienced during the training period in this time frame, there are three major reasons for this (RIAS 2012a). The most important is the large influx of admissions that took place in this period, which required all the attention in the triage setting, handling and caring of the animals once in rehabilitation. As a consequence, efforts were made to manage the small team of available volunteers and trainees, as well as the limited stocked material needed for these techniques. Some seagulls were extremely stressed, debilitated and/or already dyspnoeic upon entering the facilities and so the physical examination was kept to the minimal and collection of blood samples or swabs of the cloacae, when were difficult or could augment and harm the initial well-being of the gull, were not repeated (case of blood samples) or were completely avoided (case of cloacae sampling). In the case of the coprology study, the low number of samples collected depended also on the freshness of the excreta and its degree of contamination. Essentially in this case, the collection was made upon entrance (where the problems already discussed in the differentiation and

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classification of this feature in the clinical examination were affecting this method) and during feeding time, or while cleansing the recovery room and boxes, always in a passive manner - waiting for the unforced elimination. In the haematology part of this parasitological study, fifteen gulls were sampled (7 *L.michahellis* and 8 *L.fuscus*) however only five *L.michahellis* and six *L.fuscus* were exhibiting the paretic syndrome. Of these only 3 *L.michahellis* were positive for *Haemoproteus* sp. (27,3% of paretic birds) and in one of them (9,1%) *Plasmodium* sp. was seen, gull V0588/11/A (RIAS 2012a). In turn, the two blood smears sent for the parasitology laboratory were positive for the presence of *Aegyptianella* like protozoa. In the coprology section seventeen gulls were examined and 13 were paretic, of these, five, were parasitized (38,4%) and eggs of the genera *Capillaria* were found (RIAS 2012a). Despite the low number of positive identifications of blood and faecal parasites and considering the potential that some possess to cause myositis, like *Haemoproteus* sp. (Atkinson 2007), the rare reports of clinical disease caused by these agents in wild birds and their presence in other gulls with different causes of ingression in the centre without the considered distinctive signs of the syndrome in study, may exclude their involvement as a primary cause in this ailment. Nonetheless, they can weaken their host by several means (Wobeser 2007), as was probably the case of these parasitized gulls as they displayed more severe clinical signs of this syndrome, a relation already apparent in the two gulls with intestinal parasitism reported in the necropsy examinations.

Regarding the carcasses sent to the Faculty of Veterinary Medicine of the University of Lisbon and the several diagnostic procedures performed and listed on sub-chapter 3.2.3.4 and Ap.2.5, only one specimen was not examined because it was already suffering autolysis and decomposition. Even though they were frozen and transported overnight, approximately three hundred kilometres separate Algarve and the capital and seeing as the delivery could only be completed in the following morning, the deterioration of the corpses, which had surely began in RIAS, was not hampered (they were wrapped in newspapers and kept in a styrofoam box with ice packs). Furthermore, the different analysis in the Faculty were not carried right away, as it is clear in the dates of the examinations, as they depended on the workload of the laboratories and the organization of the practical lessons for the subject of Pathologic Anatomy. Authorizations from the faculty to send the carcasses may also have influence this aspect, as it wasn't known when and how many birds could be accepted each time, and so, when this was consented, sometimes fresh carcasses were not available (explicit in the dates of death and submission). Thus, gull V0772/11/A was not analysed, difficulties in the interpretation of the findings of gull V0071/12/A and in some organs (liver and intestines) of others were felt and it was impossible to discern macroscopic lesions or collect samples of brain tissue for microscopic observations. Bearing this fact in consideration, in the first group sent, the 2 gulls, V0664/11/A and V0666/11/A, on protocol C, died by asphyxiation or respiratory insufficiency due to the administration of activated charcoal with an ensemble of findings identical to the ones documented previously (dark viscous or pasty fluid in the airways and severe oedema and congestion of the lungs); whilst the remaining were on protocol F or T. On the whole all were cachectic and their gastrointestinal tracts were empty, but V0717/11/A had a glass fragment on the stomach and was anaemic and V0664 had its spleen engorged and no other lesion was seen or additional samples were collected for histological observation. None of this set, tested positive for pesticides and in the samples collected for heavy metal determination, values of 1,21 parts per million and 1,74 ppm for lead were measured respectively in gulls V0664 and V0666; whereas 9,77ppm and 18,21ppm of copper were quantified,

with *L.michahellis* having the higher number. The cause of death of gull V0010/12/A, which had the typical clinical paretic presentation but developed dyspnoea in the centre, was inconclusive: alveolar oedema and congestion were noted, marked congestion of the intestines associated with congestion an focal points of haemorrhage in the involving fat was identified in microscopic sections; apparently no alteration on the liver was distinguished, but detrimental signs of the freezing process and autolysis were clear; the intestine sample collected for bacteriology analysis was negative for pathogenic agents and isolation of *Salmonella*; and in the toxicological tests no pesticide was detected. In the case of the last 3 gulls sent, only one, V0071/12/A, was positive for an organophosphorous compound, however its identification was not doable. This larid had congestion of the internal organs, which was more severe in the lungs, at least macroscopically. On the observation of histological sections, only the lungs were found congested with small foci of oedema and the kidneys had signs of recent necrosis (piknosis of the tubular cells), probably owing to a hypoxic agonic period. The remaining examined organs were difficult to study, given that freezing and autolysis were in progress. Taking into account the remaining two, V0761 may have died due to a bacterial pericarditis and the juvenile succumbed to a micotic pneumonia, seeing that the first had fibrinous pericarditis with bacteria in the serosa and pulmonary congestion and the latter had small foci of intense white in congested lungs that were micotic granuloma scattered between foci of consolidated parenchyma and serous aerosaculitis. Additional findings documented for these two birds include cachexia and the piknosis of the kidney's tubular cells, already described, together with distension of the excretory ducts by a thin mucinous material with few cellular debris (its relevance to the clinical picture is unknown).In gull V0761 catarrhal enteritis of a small portion of the intestines was also recognized. Parasitological examination of the gastrointestinal tracts and contents of the tested gulls was negative for the presence of these kinds of pathogens.

3.3.1.7. Outcome of the rehabilitation process in Paretic Syndrome gulls and results of the therapeutic protocols trial.

The outcome of the rehabilitation of these gulls was characterized as released into the wild or died within a defined time interval (fig.133). Gulls that escaped, in this case one that when placed in an outside larger enclosure managed to free itself, or were humanly killed in the centre (because they developed secondary lesions that didn´t heal completely and could hinder their survival in the wild and/or never fully recovered the ability to fly), were also classes considered. But even so, the majority of the cases either were released or died, with very few dying after the 30th day of admission (n=2).

Figure 134 – Outcome of the rehabilitation process of paretic gulls, between 2009-2012, with the category "Died" further divided in its different subcategories, n=148.
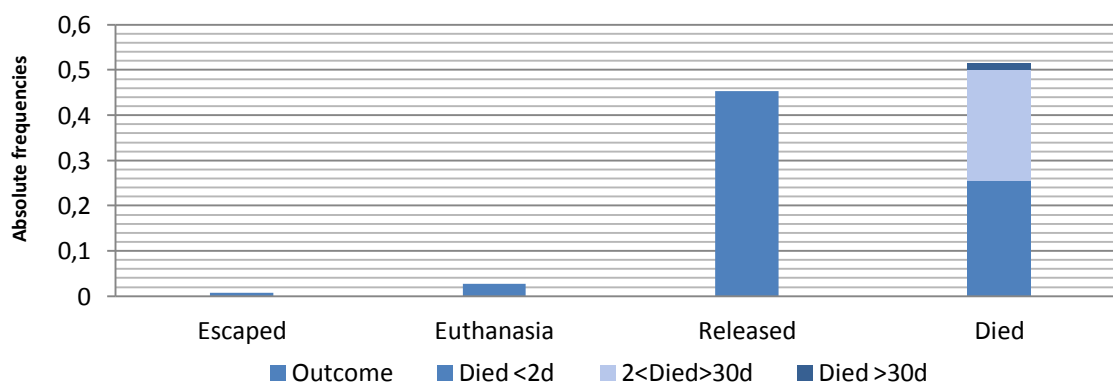
Regarding the therapeutic protocol trial where 50 birds were submitted to 3 different protocols. It was initially delineated that the variables to be assessed in this experiment were the outcome of the rehabilitation process and number of days till complete recovery of a functional standing stance and walking ability, however only the outcome could be evaluated. In view of the fact that gulls are not genuinely wild animals, given the closer proximity to more humanized habitats, they do not fear human presence to the same extent seen in other animals, where the common response is avoid contact or escape at all costs. Accordingly, when trying to determine a certain degree of improvement of the paretic condition, normally while changing boxes and providing water or food in the recovery room, frequently, the only response of the bird was attack without rising or moving from the position where it was initially found. Conversely and unfortunately, while tending other animals, sometimes the same animal previously in recumbency, was seen trying to stand or standing without any difficulties. Given that the contact or manipulation of these animals (and other species) is kept in a level of the minimal and indispensable, this variable was disregarded in this analysis. Nonetheless, the placement of gulls in the recuperation enclosures, after a possible complete recovery of the gait and locomotion in the recovery room, could be a good estimation of the duration of the recovery of this sign, but only 4 chambers were available and during the experiment period 3 were occupied by more pelagic species like northern gannets, owls or diurnal raptors and the one remaining, in spite of being used for gulls, sometimes required a management of the number of animals to preclude cases of high densities and agonistic behaviour, seen in at least one of the paretic gulls humanly killed due to severed wing tendons (table 10 Ap.3.4). For the statistical testing of the association of the different protocols used and the final outcome of the rehabilitation process and given the findings of chronic or captivity disease in the necropsies already discussed, cases that died after one month after the admission in the centre were excluded (n=2), because the direct involvement of the initial therapy instituted was thought to be minimal or even non-existent (although the hypothesis that indirectly it could influence the long term survival was not taken into consideration, is still a possibility). Larids, that suffered euthanasia, were also omitted (2 birds), because they also corresponded to cases of chronic or captivity afflictions. Thus, animals that died within 2 days of admission or before the 30th day of rehabilitation (the other 2 categories) were included and grouped, since protocol C in particular the administration of charcoal was limited to the first two days and protocol T required a weekly injection of thiamine (total of 21 for the three protocols). Additionally, gulls that were released into the wild were considered (25 gulls), as they were believed to be the best estimation of the success of these protocols, given the current circumstances. From this analysis there is a high chance that the observed results could have manifested if the null hypothesis, of no association, was true and consequently it cannot be rejected (p=0,7422 in the Fisher's exact test). This conclusion, nevertheless, also indicates that perchance a higher number of gulls may be required for a more representative and correct analysis of these two variables. Though the training period only lasted 6 months, previous data could be used, but the methods varied (several antimicrobial active principles were used and sometimes were combined with the use of anti-inflammatory drugs) and its inclusion could influence the proportion of cases included in either the three categories or new ones had to be added. Other influencing factors that could have affected this result or the number of cases available for the statistical analysis were: a period of shortage of activated charcoal and findings of pneumonia by aspiration of charcoal (these two conditioned the use of this protocol). The recent discovery of the

formerly cited study of Balk et al. (2009) in the course of this trial resulted in a low number of animals receiving supplemented thiamine (4 gulls) and constitutes another influencing factor. Furthermore, age and species specific responses to this treatment were not studied, but could also have influenced its results, as individuals of every age and of three species, *C.ridibundus, L.fuscus and L.michahellis*, were considered. Individual response to treatment and the real degree of paresis and weakness are other variables that could and certainly influenced this result and should be analysed carefully in other opportunities. Perchance, the future use of electronic equipment to digitally record their behaviour whilst avoiding the direct contact with these animals, after their proper acclimatization to the new environment in the centre, could be a viable option to circumvent these aspects.

3.3.2. Implications of these results in the comprehension of the Paretic Syndrome and the determination of its rightful cause in Algarve.

The clinical findings of moderate posterior paresis, flaccid cloacae, depression of the mental status and low body condition (body score of 2 and dehydration degree of 7%), were the most frequently reported in gulls considered as having the paretic syndrome upon admission in RIAS between 2009-2012, which could suggest a common aetiology. However these signs are not specific of a particular condition, as are also the lesions most recurrently herein reported, despite the risk of extrapolating findings from one species to a different one when baseline data for gulls is scarce. The lack of consistent and disease specific lesions, probably owing to the ongoing course of the disease (as various birds with different survival times were examined), but also to the possible involvement of confounding factors (e.g. effects of captivity on wild birds – traumatic lesions) or the probable development of opportunistic diseases, like the case of aspergillosis, or the signs of recent development of bacterial infection in the anatomic and histopathology findings, hinder the correct determination of an accurate lesion pattern for interpretation. Therefore, the lack of distinctive evidences of a specific disease with only the physical exam records and gross observations upon the necropsy procedure, cannot alone, yield an accurate diagnosis in this particular situation, which is normally the case when the determination of the cause of death is attempted with such absence of specificity (Franson & Smith 1999). Thus, a careful analysis of these findings and the results obtained in ancillary diagnostic tests, in respect to the diseases herein reviewed previously, the life cycles of these birds and the habitats where they dwell in this southern region of Portugal, is required to understand or at least discern a potential causative agent for this syndrome, as it will follow.

Considering the group of infectious diseases, firstly the case of **Newcastle disease**, for the years in study, it is unknown the carrier status of these birds in Portugal. No sample was collected and could be submitted to determine if this disease could be a reasonable cause of the paretic syndrome, or if these birds were carriers of APM viruses or were exposed. For the time span of this study, only four confirmed Portuguese cases of wildlife infection without clinical signs were reported to the OIE, in the course of its general surveillance plan. These were detected in the last semesters of 2010 and 2011 and in the first of 2012 and only in the latter was disclosed the specie affected, a rock dove (World Animal Health Information Database [WAHID] 2012). Considering the clinical signs described by Wobeser et al. (1993), Kuiken et al. (1998) and Glaser et al. (1999) loss of awareness was not present in the commonest form here documented, but ataxia and paresis with signs of compensation (use of beak and wings) were reported, which is similar to the description of gulls probably affected in the

aforementioned work of Glaser et al. (1999). The outbreaks described in the literature occurred in the breeding season, but could extent towards the summer, which is not fully concordant with the temporal distribution of the admittance of paretic gulls. However, Wobeser et al. (1993) documented in their study that affected birds could be discovered as late as October, in Canada. The description of the outbreaks normally indicates that mostly immature birds are affected and equally such finding is observed in this syndrome. Even though it was impossible to test for the presence of this pathogenic agent and given the economic importance that this disease has, the fact that some of the migratory gulls (*L.audouinni, L.melanocephalus, L.fuscus* and *C.ridibundus*) that were admitted in the centre, with or without paresis, came from or upon release were seen in countries where there is a history of reporting outbreaks of ND or high virulent strains for poultry were detected in wild birds and *Columbidae* species (Germany, France, United kingdom, Spain, Belgium, The Netherlands, and Morocco) is preoccupying (Alexander 2011, RIAS 2011/2012a/2013, WAHID 2012). The measured correlation with poultry densities, although not significant, was stronger than other variables, and this finding is somewhat of concern if this disease is the causative agent of this syndrome. A concern that Glaser et al. (1999) was prompt to relate, when they were studying a series of epizootic outbreaks in *P.auritus* in North America, they referred that a threat of transmission was present because gulls are commonly found in the same habitats as the most sensitive wild species to ND and in poultry facilities, which is substantiated with the earlier identification by Wobeser et al. (1993) of APMV-1 virus with the same antigenic pattern of the viscerotropic velogenic typical of the 1970-74's panzootic in poultry in a gull and cormorants in outbreaks in 1990, in Canada. Moreover the recent study of Diel et al. (2012) is another example of such. This situation is further sustained by recent European attempts to assess the epidemiology status and screening NDV from wildlife, such are the cases of the studies of Camenisch, Bandli and Hoop (2008) in Switzerland, Lindh et al. (2012) in Finland and Snoeck et al. (2013) in Luxembourg. These efforts resulted of the need to understand the role that wildlife has in this disease, not only because of the proved exposure to these viruses, possible dissemination, the risk of exchange of virus between poultry and wild birds and a possible conversion to more virulent forms, but because, although still circumstantial, apparently there is a wild bird reservoir of a virulent NDV in Europe since the mid-90's, particularly in countries of the northern and western parts of the European Union (Alexander 2011).

In respect to **Salmonellosis**, in only one gull samples were collected for microbiology essays, in an approach that was similar to the one of the National Wildlife Centre in Wisconsin and described by Skerratt et al. (2005), where history and gross findings were the determinants that dictated which ancillary diagnostic should or not be used. However, these authors declare that this type of selection is more probable to induce failure when the detection of a pathogen is intended, when compared with a more systematic approach. In this study the selection of the ancillary exams chosen was based on the physical signs presented by these gulls in life, the necropsy findings attained and monetary constraints additionally influenced the number of procedures that could be performed. These factors explain why in another gull submitted to this institution, V0761/11/A, the presence of bacteria was noted in the necropsy examination, but no sample was collected for microbiology analysis for subsequent identification. Thus, the negativity in the detection of bacteria in one specimen, for which no cause of death could be determined, could indicate that probably the cause of death and disease was not due to a bacterial agent. Nevertheless, there are factors that can elicit a false negative and those could

have been present in this case: the concentration of the agent in the sample could have been minimal, the inflammatory process could have reduced the freely available bacteria, or the effects of freezing could also have been responsible for a low number of healthy pathogens available for isolation, even though a pre-enrichment medium was used in the case of *Salmonella* sp. detection (OIE 2010). The bird analysed was an immature *L.fuscus*, belonging to the age class for which a significant difference was determined as to the presence or not of syndrome upon admission, which is in accordance with the several studies published and reviewed associating cases of *Salmonella* sp. infection and disease in wild birds. In addition, considering the strong correlation of landfill density and proportion of paretic cases (despite the absence of statistical significance and the limitations already enumerated) and the potential for the development of bacterial infection and carriage of pathogen in these sites, circumstantial evidence that these Portuguese gulls may use this settings to search for food (at least one of the paretic gulls released was seen in a landfill, fig. 135 Ap.3.5.), is another strong point to consider when assessing the viability of this disease as the cause of this syndrome. However, bearing in mind the study of Negre, Bistuer and Millan (2008), a survey of diseases of *L.michahellis* feeding in a Spanish garbage dump from 2003-2007, where they used 900 gulls, none had lesions of *Salmonella sp.* but 64 had positive results in isolation, which led to the conclusion that gulls are asymptomatic carriers of this pathogen. Considering other problematic sites for bacterial contamination, Ferns and Mudge (2000) in their study of abundance, diet and salmonella contamination of gulls feeding at sewage outfalls and the works of Fenlon (1981/1983) on the same matter determined also higher rates of carriage in these sites and none indicated signs of disease. In this respect, although the correlation measured between the municipalities from where the Portuguese paretic gulls were retrieved with the distribution of wastewater treatment plants was negative, the role that outfalls may have in their life cycle and the dynamics of exposure and infection should not be overlooked in a future investigation. Similar findings of subclinical carriage were reported by several other studies, also conducted on the subject of relating salmonella and gulls, for instance the case of Butterfield et al. (1983) and Palmgreen et al. (2006). These authors also found positive gulls, but in a later re-sampling, cultures were negative for the presence of salmonella and/or the birds were found healthy and carried on their normal life cycle, as for instance, winter migration was not affected. So, given the existing data, it is still unknown if this pathogen could be a rightful cause for this disease in these animals.

On the subject of fungal infections that may affect wild birds, particularly **Aspergillosis**, none of the carcasses opened by the author or previous descriptions of the macroscopic findings in RIAS' database indicate the presence of grossly visible lesions that could clearly compromise the peripheral nerves, either directly or with involvement of organs in close proximity (e.g. kidneys) and subsequently elicit a paretic or paralytic presentation and/or signs of ataxia as stated by Joseph (2000). However, the importance of this pathogen cannot be neglected as it may be an important contributor to the death of gulls that were admitted in the centre already exhibiting opacity of the air sacs and milliar formations and larids that possibly were fully rehabilitated, but died with a disseminated granulomata presentation upon necropsy (as previously mentioned). Although no cytological examination of the respiratory lesions for the detection of specific traits of the genus *Aspergillus* was executed and no sample was collected for identification purposes (table 2), other diseases could provoke the same pathologic findings, as Atasever and Gumussoy (2004) state when considering Marek's disease, tuberculosis or neoplasias. However, respiratory lesion patterns are rare in these two infectious

conditions and the development of neoplasias is rarely documented (Threlfall 1967 and authors cited, Converse 2007b;Kaleta @Docherty 2007). The signs displayed in life by gulls assumed to be suffering from this disease were concordant with the clinical presentations reviewed of aspergillosis, the age classes of immature birds were the most representative (considering just the 4 with the grossly evident lesions and the one submitted to the faculty the relation was 4 to 1) and the yearly distribution of the cases are also in accordance with the literature. As Friend and Trainer (1969) refer, there are two main theories for the development of this disease and these can justify the lesions seen in these gulls. Either these birds were already carriers of the spores, but the paretic syndrome, malnutrition and dehydration due to the onset of paresis or the stress of captivity triggered the growth of them; or they inhaled these while in captivity and the same stressors could justify the occurrence of this disease. Taking into consideration the first model, it could explain the lesions observed in the air sacs of gulls that died within the first seven days upon admission, as the lesion pattern observed was preferentially located in this location and is typical of the initial stages of this disease, as Nardoni et al. (2006) state (inhaled spores can reach firstly the most caudal air sacs without contacting the lungs' epithelium). But this model could also explain the ones with signs of chronicity, where dimensioned nodules or flattened plaques were seen in the lungs and other organs outside the respiratory tract, some with blue-green, green to black velvety superficial texture owing to the growth of conidia, also described by Cacciuttolo et al. (2009) in *L.argentatus*, and are due to the presence of spores that remained in the gull and may originate dormant or chronic infections that extent to other tissues and organs (Nardoni et al. 2006;Converse 2007a). The presence of spores in this centre was not determined, but a study conducted by Nardoni et al. (2006) when confronted with cases of aspergillosis in their wildlife centre in captive free ranging gulls, detected large amounts of viable airborne *A.fumigatus*, ranging from 450 to 525 colony forming units per cubic metre, inside and outside the shelters. Therefore, the presence of this pathogen in RIAS cannot be disregarded owing to its ubiquitous and saprophytic nature and particularly the conditions in the recuperation chamber and flying pen (gravel in the first and vegetal matter in the second, where, in both, litter and vestigial quantities of uneaten food may be found). Also, in these two settings commingle of gulls with different causes of ingression and on different therapeutic protocols, some on antibiotics none on corticosteroids, could potentiate the contact with spores and inhalation (Nardoni et al. 2006). An interesting finding noticed while performing the necropsy of the 41 gulls and comparing specimens with traumatic lesions and entry causes of "Debilitation" or "Unknown" and that died in the centre, was that none of these (n=9), at least macroscopically, had the lesions considered more frequent of aspergillosis cases and one of them, a traumatism gull, remained in the centre for over a month (36 days). This could indicate that perchance paretic gulls may have a higher susceptibility to this pathogen owing to a more compromised immune system, which could also promote parasitic infection, virulence of bacterial and viral infection and explain the variety of findings on the necropsy due to the occurrence of possibly secondary diseases, but future research is needed before a statement pertaining to an immune supression/immune depression related to the possible etiologic agent of this syndrome can be made and the number of necropsies executed on other suspected causes of ingression should be higher for a comparable association.

In respect to **Sarcocystosis**, no gull had the typical lesions described in the pectoral muscle of waterfowl, nor in other less common tissues documented by Crawley et al. (1962), Box and Duszynski

(1977), Tuggle & Friend (1999), Spalding et al. (2002) and Wunschmann et al. (2010) or the ones of *L.argentatus* in Europe by Prakas et al. (2011). Then again, only gross lesions were searched for (or documented) and no sample was collected for microscopic observation of microcysts, which have been described in several species where the commonest presentation of the "rice breast disease" does not occur (Hoppe 1976;Munday et al. 1979). Studies of *Sarcocystis sp.* in muscles of wild birds are not available in Portugal, particularly when considering gulls. The signs here documented and the lesions found in this study are unspecific, but could be compatible with this disease, and gulls, on the word of Munday et al. (1979) were among the species with the highest prevalence, which included carnivorous, insectivorous and omnivorous avian species. As previously said, this condition is rarely the cause of death, normally is an adjunct that aids in the severity of another disease or promotes death by a decrease in the survival fitness, however it would be interesting to determine if this pathogen could be present in these species, as in accordance with Spalding et al. (2002) and Greiner (2007) the complete life cycle of this pathogen and also the list of definitive host is not fully known or complete, the pathogen exhibits intermediate host flexibility and wild birds are at risk of infection, especially when in close contact with other vertebrates and particularly, in certain cases, when in captivity.

Botulism is probably one of the most viable causes for the paretic disease displayed by these birds: the signs are similar to the description of Neimanis et al. (2007) in a specie of the same family of these birds and the pathologic lesions observed by them were also noted as the most frequent (distended gall bladder, absence of ingesta, cloacae replete with urates and faecal matter). Nonetheless, congestion of the intestines was not a finding documented in their study, but others have described it (chapter 2.2.5). However, these clinical and pathologic presentations aren´t specific of this condition and since it was impossible to pursue a definitive diagnosis of botulism by the golden standard of the mouse bioassay (table 2) there isn´t a guarantee that this syndrome could be attributable to these intoxications. Official outbreaks of botulism in Algarve don´t exist, however, in a waste water treatment facility in Faro, in 2010 and again in 2012, possible botulism eportinic events might have occurred, as the species affected, the high number of animals ill or deceased, the timing and the ecology of the place are strong circumstantial evidences that indicate that perchance botulism could be involved, though a correct diagnosis for these events was not yet attempted. Species of the families Anatidae and Rallidae were the most affected (*A.platyrhynchos*, *Anas strepera*, *Anas clypeata*, *Anas acuta*, *Anas crecca*, *Aythya farina, Tadorna tadorna*, *Netta rufina*, *Gallinula chloropus* and *Fulica atra*) and one individual of the Burnhinidae family, *Burhinus oedicnemus* were rescued in these two events. The rescued birds were displaying similar signs to the ones found in botulism cases, and in 2010 150 birds were received, moribund or already dead in the centre, between August and September; while in 2012, due to the active monitoring of Faro´s treatment plant, 344 were admitted from July to October 2012 (RIAS 2012b/2013). Minor events of the same nature have also been observed in 2011 in Faro and in another facility in Loulé, for which no equivalent data is available (RIAS 2012a;Água do Algarve written communication). Although, gulls are frequently seen in such places, as it is stated in the report made by RIAS as a result of the monitoring for which the centre is responsible (Rias 2012b) and personal observations when participating in this program, the Rho value was considered significant, but negative for the association (table 7, Ap.3.2.) which is an unexpected finding. Moreover, during these events no gull was retrieved from Faro or adjoining municipalities in 2010, but in 2012, 8 out of

the total sixteen were rescued from such places (data from 2012 could be biased towards more proximate counties due to collection constraints). However, considering all the complexity of these diseases and their usual course and as Poppenga and Tawde (2012) assert, the period of time between the manifestation of the first signs and death can be as short as a few hours, but in this case could be likely suitable for the dispersal of gulls to other municipalities where they were later rescued. Which is probably what happened in the study of *P.occidentalis* by Nol et al. (2004) for which no pattern could be determined between the sites where the possible source of the toxin was present and where the sick birds were found. Another notion to keep in mind is that these disease and mortality events are known because this structure is located in close proximity to RIAS and was later included in the aforementioned surveillance program, but factors that are known to predispose the development of this disease are easily found in this region. High temperatures, flood, geomorphologic alterations, the effects of human presence and drought in accordance to N-H-A.G and N-E-A.G (2012) are common in Algarve and could, in this case, provoke substantial alterations in the water levels and/or optimize the conditions for the development of a botulism outbreak. Drought, for instance, happens frequently and particularly in the eastern part of Algarve, between Ria Alvor and the hydrographical basin of Arade, where the highest retrievals of paretic gulls were made. These factors could also and possibly be allowing for other outbreaks to express in other more secluded habitats in the complex Algarvian wetlands of lagoons, marshes and others described in part 3.1. Although, it is still unknown the prevalence of spores of *C.botulinum* type C and E in the vast array of these different habitats in this region, in Europe surveys indicate a widely and variably distribution throughout the continent (Neimanis and Speck 2012). In accordance with Wobeser (1997) on the topic of marshes and on his personal opinion, cases of botulism in wild birds in these settings can be an endemic phenomena with the possibility to originate epizooties given that the most important drive, secondary intoxication, is enabled. He found, in a prone marsh and for a period of time of 20 years, individual birds affected by botulism in the summer, but also outside the commonly described typical outbreaks of botulism with high mortalities and sick birds in summer and early autumn. Sandler, Rocke, Samuel and Yuill (1993) additionally state that botulism type C spores are ubiquitous and may persist in sediments in marshes, either with or without the concomitant occurrence of outbreaks. Moreover Yule et al. (2006a) refer that epizootic episodes of mortality of fish by botulism E could occur frequently and may be rarely reported, owing to the settling of the carcasses in the bottom of freshwater/brackish habitats and apparent absence of dead or moribund birds, but these can be the cause of disease in birds that remain to be found. So the same, throughout Algarve and during the whole year, could be happening with these gulls that are at risk, especially when one considers their potential foraging behaviour and types of food ingested and habitats where they dwell.

Regarding **Algal toxicosis**, the only up to date information available of the presence of toxin producing organisms and levels of toxins produced is the result of the analysis of samples collected from indicator species of molluscs of lagoon/estuarine areas and offshore in view of the European regulation EC 853/2004 and 854/2004. A review of the information collected from 1986 to 2006 by Vale, Botelho, Rodrigues, Gomes and Sampayo (2006) indicated that the country's northwest coast is more prone to biotoxins events than other regions. In Algarve, toxins responsible for the paralytic shellfish poisoning in humans (PSP), particularly produced by *Gymnodinum cantenatum,* are not frequently detected in this region both inshore and offshore - only in 1994-1995 concentrations higher

than the regulatory limits (RL) were found and again only in 2006 were detected, but this time below the RL. Whereas, toxins that provoke the Diarrhetic Shellfish Poisoning (DSP), produced by *Dinophysis* species, are also found in the south, but are detected in higher levels than the previous and more frequently surpass the RL, especially in offshore molluscs than in organisms in lagoons or esturaries. Domoic acid, produced by *Pseudonitzschia autralis* and responsible for the Amnesic Shellfish Poisoning (ASP), is detected, but rarely reaches the RL in the south either offshore or in lagoons and estuaries. Frequently, when the concentration of the latter toxin peaks, it is a short-lived phenomenon (of normally one week) that usually doesn't repeat in subsequent weeks. Ictiotoxic and parasitic species (e.g. *Heterosigma akashiwo*, *Karlodinium micrum* and *Amyloodinium ocellatum*) have also been detected in Portugal and killed apparently only fish, in aquaculture infra-structures in Algarve (Moita, Palma & Vilarinho 2005). No traces of toxins of AZP (Azaspiracid Shelllfish Poisoning) were found and others like yessotoxins analogues were detected above the RL only in 2006 in Ria Formosa. The seasonal contamination by the first three groups of toxins coincides in part: DSP toxins are detected in May/June to November, the PSP's more in the summer or autumn and the ASP's may be quantified from spring to autumn. In the time frame of this study the general picture is not that much different from the previous data (fig.136 and table 11 in Ap.3.6). Considering the seasonality of these detections, the high range that this phenomena may have, the fact that although it is not known what was the normal diet of this birds but keeping in mind their omnivorous nature and the reviewed literature, these are all pointers that algal toxicosis might be a viable cause for the paretic presentation seen. However, when counting the number of cases that co-occurred in time and spatially with the bans of toxins known to cause paresis/paralysis, the values for each year are not very high to justify the numerous sick gulls found in these 4 years: 0/6 in 2009, 0/42 in 2010, 0/84 in 2011 and 3/16 in 2012, although in the first and last years there may be a bias in retrievals (table 11 Ap.3.6). The clinical and pathologic observations are again not conclusive, so if this disease is the rightful aetiological agent for the syndrome in study, at this stage, is still impossible to discern. This explanatory exposition of the likelihood for algal toxins to provoke paretic conditions was based on the accumulation and retained levels of such toxins in molluscs that led to restrictions to the collection and sale for human consumption as indicators of exposure. However, as Shumway et al. (2003) state, local shellfish toxicity is not the ideal measure for birds that are highly mobile and don´t feed exclusively on shellfish and in the banned sites, and doesn't translate clearly what is happening in more offshore distances. Shumway et al. (2003) also refer that, although these are good estimators of bloom formation, they aren´t a good indicator of the duration of the events and the toxic levels of other prey (e.g. fish). A notion that Nisbet (1983) affirmed, when defending that not all bird-HAB associated mortalities correlate with shellfish toxin levels. Therefore, and even though the diet of these gulls is unknown including the normal range of their movements or even the sites where they usually forage for food, the association of HAB events to this particular syndrome must not be overlooked, knowing additionally that time and spatial lag effects for exposure, passive dispersion of toxins by nature's elements (e.g. wind-driven flows) and time lag of the display of clinical signs or death owing to the pathogenesis of the intoxication or bioavailability of the compounds in the course of digestion, can be responsible for the temporal and spatial de-coupling of these occurrences and HABs (Kreuder et al. 2002;Sellner et al. 2003;Kvitek 2005;Landsberg et al. 2009). However, as previously reviewed some elements of the Laridae family have aversion behaviour towards the consumption of toxic prey, this

could be the case of these gulls in respect to mollusc species. Nevertheless, it is not known to what extent or even if migration pressures like the high energy/nutrients demands can surpass this survival strategy or if, possibly, a food shortage can circumvent it in more difficult times. Further investigation on this subject is still needed, and it would be interesting to further analyse the involvement of these kinds of biotoxins in the paretic syndrome displayed by these gulls, in Algarve, particularly by attempting to detect the presence of those in the tissues of co-occurring cases. Another point to consider is the possibility that toxin producing cyanobacteria in more restricted fresh or marine aquatic environments may be the cause of this disease, as agricultural, industrial, municipal wastes in conjunction with nutrient enrichment inputs by aquaculture practices may stimulate the formation of such blooms and are present in this region (for instance in southern dams cyanobacteria of the genera *Oscillatoria sp.* have been found dominating populations of such organism) (Carmichael & Li 2006;N-E-A.G 2012;N-H-A.G 2012). The role that emergent biotoxins, like the identified palytoxins, ciguatera toxins and tetrodotoxins in the Mediterranean (Vale 2011;Paredes, Rietjens, Vieities & Cabado 2011), could possibly take in this continental part of Portugal, if already present, is also something of concern. On the subject of heavy metal contamination, samples of two gulls were used for the detection of **Lead** and **Copper**, as they are usually the ones that can induce a paralytic or paretic presentation, and given the area in study and the species affected by this syndrome could be causes for this ailment. Hunting and fishing are activities practiced in this region and both metals were extracted, are used or can be emitted into the environment by the industrial sector due to activities such as ceramic, metallurgic or even by other sectors like wastewater treatment plants, agriculture, among others (Direcção-Geral de Energia e Geologia [DGEG] 2013;ICNF 2013;N-H-A.G 2012;N-E-A.G 2012). Taking into consideration the problematic of **lead** ammunition and its toxicity in wild birds, Portugal is a country of the European Union and by 2007 was member of several conventions (i.e. Convention on Biological Diversity, Bern, Bonn and Ramsar) and subscriber of several other agreements and declarations (African-Eurasian Waterbird Agreement [AEWA], Declaration on risk reduction for lead by the Organization of for Economic Co-operation and Development), and in doing so, obliged itself to adhere to the principals and resolutions of such, including, directly or indirectly, the ban on the use of lead shots (AEWA-Secretariat 2008, Mateo 2009). However, despite the definitive and growing body of knowledge pertaining to the toxic effects of the ingestion of these compounds, including national studies such as the one of Rodrigues, Figueiredo and Fabião (2001) in several lowlands of central Portugal between 1993-1995, which indicate that lead poisoning is killing wild birds, lead shots have only recently been banned and exclusively while waterfowl hunting or in wetlands included in classified areas (2nd article of ordinance nº288/2010).Thus, even though the ban is being progressively implemented, situations of lead intoxication can develop as a result of persistence of lead residues in aquatic ecosystem or in regions were the prohibition has yet to be applied completely, in contrast with countries like Denmark, Norway and The Netherlands where a complete prohibition was implemented (Fisher et al. 2006, Mateo 2009). Nevertheless, none of the gulls admitted had lead pellets, sinkers or any other lead material in their stomach, nor gun-shot wounds that could more likely place them in a environment where lead or shooting are frequent and could lead to exposure and intoxication (despite the possibility of regurgitation, defecation or digestion). Both gulls tested in this study for these metals were admitted in the same week and came from two of the counties with highest retrievals. They were of different species and age classes (Ap.2.5), but both displayed the same clinical signs: depressed

mental status, body score of 2, 7% of dehydration, green and pasty diarrhoea, flaccid cloacae, generalized moderate muscular weakness and posterior paresis of moderate severity. The necropsy examination for both was not conclusive as to the cause of paresis. The Rho value of the correlation rank was not significant for the association with variable "Lead mines" (table 7 Ap.3.2) and, although only one mine of rock salt is active in Algarve, the potential legacy effect of the others or even quarries in activity or abandoned (not assessed) is unknown when regarding these gulls. Nonetheless, in accordance with DGEG-Empresa de Desenvolvimento Mineiro (2011), numerous efforts for environmental recovery and prevention of future legacy points are underway especially in critical cases, but until 2009 none was carried out in Algarve (DGEG 2013), which could mean that perchance they are not so deleterious for the environment. The set of signs and findings in these 148 gulls could be compatible with saturnism, however the levels determined in these two birds were lower than the ones considered toxic or sub-clinical (table 2). And so probably this condition is not the etiologic agent of this disease. In respect to the other metal, **copper**, the case is altogether different. This is an essential element and despite varying with environmental contamination, is a reflection of physiological demands (Wenzel et al. 1996;Eisler 1988); so its detection was something expectable. Nonetheless, information of the toxic concentrations of this metal in tissues of wild birds, particularly aquatic/marine birds, has not been determined (at least to the same extent of other contaminants) and reports of toxicosis are rare (Degernes 2008).Copper industrial extraction was a common practice in Algarve, but nowadays all the mines are inactive (LNGE 2013, written communication). Several industries are known to use this metal (as well as lead), but data of their location and environmental impacts were not made available for this study. The correlation tested was not significant and the levels detected are difficult to interpret, as they may vary in accordance with the feeding ecology, intensity and timing of exposure in foraging zones and some physiological and biochemical characteristic of the pollutant (Savinov, Gabrielsen and Savinova 2003).Considering the investigation by Taggart et al. (2009) of metal levels in tissues of *Marmaronetta angustirostris* and *Oxyura leucocephala* and the authors cited, the values observed in this study are bellow what was found to be good estimators of a possible intoxication in several species. Nonetheless, the immature gull had a higher result. Eisler (1988) reviewed several data collected of several species and in his work is noted also this discrepancy, although not in the same order of magnitude. However, the present comparison is being made between two different species, so perhaps the juvenile *L.michahellis* had a higher value, as a result of duration of exposure due to its more sedentary nature; but local exposure, the type of food chosen or foraging inexperience leading to a possible increase in exposure or in protein catabolism with disruption of metal homeostasis, could also be reasonable explanations for this result (Debacker, Schiettecatte, Jauniaux & Bouquegneau 2001). High metabolic demands and growth could also justify this elevated value in the immature when compared with an older specimen, as was the case seen in nestlings of several days of *R.tridactyla* in the North sea described by Wenzel et al. (1996) or perchance nestling and immature *Pandion haliaetus* in the eastern United States between 1975 and 1982, described by Wiemeyer, Schmeling and Anderson (1987) when comparing with adults. Furthermore and given the scarcity of studies of toxicity in wild birds (specifically in gulls) and since none had the black discoloured livers as a result of a focal hepatic necrosis with cytoplasmatic pigmented granules of copper found in intoxication cases (Molnar 1983, Kobayashi, Shimada, Umemura & Nagai 1992), hardly one can discern with certainty if in fact copper intoxication is or not a

viable diagnosis. With respect to **Mercury** intoxication as another possible cause for this syndrome, no gull could and was tested in this study for its presence; however gulls admitted in RIAS were sampled for a study conducted by Santos, Monteiro, Soares and Loureiro (2011) on the subject of comparing the bioaccumulation of total mercury between the orders Pelecaniformes, Charadriiformes and Ciconiiformes in the continental portion of the country. Santos et al. (2011) found that the Order Pelecaniformes had significantly higher levels of mercury than the other two ($p<0,005$; Dun's test). A finding that authors like Burger and Gochfeld (1997), Burger (2002), Ochoa-acuña et al. (2002) and Goodale et al. (2008), also documented in other types of tissue samples and comparing several other species, including different species of gulls. These results are explained by all these authors in view of differences in the feeding behaviour or trophic positioning of the different species, therefore seagulls, that can have a more diversified diet and/or may not consume entirely high trophic fish, aren't perchance in such a critical state for mercury intoxication when compared with more strict fish-eating birds. Considering only the gulls' samples collected, these were submitted by RIAS since 2009 and the levels detected ranged from 0,38 to 5,72 ng/mg in dry weight. These values are lower than what is generally considered to be toxic by several authors reviewed by Ohlendorf (1993) and Wolfe et al. (1998). However, the specimens in analysis in this case belong to different diagnostic categories ("Traumatism", "Debilitation", "Disease" or "Unknown" cause of ingression) hence, they are not representative of paretic cases. In spite of these low values, seagulls dwell in habitats that can be more prone for accumulation than marine ecosystems as Frederick et al. (2002) and Evers et al. (2005) indicate, when comparing a variety of examples of freshwater and marine environments in respect to several wild bird species, and this could explain the range of these values. Gulls can also be exposed to more toxic-laden prey when frequenting fishery practices, as Arcos et al. (2002) state and which could be the case of these paretic gulls, as after their release they were frequently re-sighted in harbours (fig.134 Ap.3.5) and can regularly use this food source in these areas. Given that, definitive values of toxicity are absent and should be considered alongside with the evidences of pathologic problems, it would be interesting in the future to measure this contaminant in paretic gulls, bearing in mind: habitat comparisons and different geographic areas, different age classes, distinct species, moulting status or even the role of a possible demethylation reaction or the effects of selenium in the prevention of more deleterious effects. The investigation of mercury burdens in eggs, younger gulls or resident species could also be advantageous, as in agreement with Wenzel et al. (1996) and Burger and Gochfeld (1997) they are a good source of local data, given that: usually the migratory species reach breeding grounds before the laying period and so the levels in eggs can be a good estimative of the levels obtained by females in these sites, younger specimens depend fully on food collected locally and provided by the parents, and resident species normally are not so dispersive. The same could be equated in respect to other metals in order to understand their effects in wildlife dwelling in this region.

Considering **Pesticides** as a viable differential, one of the 8 gulls tested for their presence, specimen V0071/12/A (table 5 Ap.2.5), had a liver sample positive for an OP molecule. This gull, upon admission on the centre, was found extremely depressed and unaware of its surroundings (visual stimulation didn´t illicit a reaction), however with the sound of snapping fingers a mild, almost unnoticed, attempt to move the body or redirect the head towards the source of the stimuli was seen. This *L.fuscus* was practically completely immobile with the neck fully extended (fig.109). Dyspnoea,

flaccid cloacae and diarrhoea were additional signs found. It died in less than 48 hours and was frozen and sent to the faculty in May, where in July was examined. On the necropsy, procedure, as previously stated, some lesions/alterations could not be deem as consequences of a pathological process in course due to freezing and autolysis, however the lesion pattern seen in the lungs could be compatible with the respiratory insufficiency and signs noted in life and may be concordant with the pathological findings reported by Stone et al. (1984) and Glaser (1999) in cases of intoxication with these pesticides. This gull, of a migratory specie, was paralysed and considering the date of retrieval and on the word of Glaser (1999) the exposure to a pesticide in a bird while on migration and under high energy demands could result in more deleterious effects and this could justify the paralytic presentation of this gull. Also, all the remaining considered paretic had signs compatible with a possible involvement of pesticides, despite the short list of cues of dysfunction of the parasympathetic nervous system when compared with the reviewed cases in other species and in gulls by Whitney (2004). Only one gull tested positive, but only 8 out of 148 were analysed (5,4%), if in fact pesticide intoxication is the cause of this disease, the absence of other positive animals or the inability to identify the molecule detected or quantify its presence in order to compare with known or comparable LD_{50} or a limit of adverse effects, could have been due to: the fact that it is a qualitative/semiquantitative laboratory technique of low selectivity (when compared with others and in the absence of all the standards); the repeated freezing and thawing phenomena and the long time lapse between the moment of death and proper examination contributed to the breakdown of the compounds; liver breakdown could have originated compounds that interfered with the analysis as unwanted co-extractives; the detection level of the technique may have been too high or the concentration of the pesticide in the tissue could have been lower than this limit; the route of exposure could be other (e.g. dermal); the chronicity of this disease impeded the detection of residues or unknown anticholinesterase inhibitors were present (Smith, Thomas & Hulse 1995;Kwon et al. 2004). Therefore, the use of the colorimetric essay to measure the inhibition of brain cholinesterases (table 2) could have been useful in these cases, as brain activity may remain inhibited far longer than the persistence of the compounds in the viscera, even when decomposition is in progress (Prijono & Leighton 1991;Burn & Leighton 1996;Franson & Smith 1999). In spite of only one gull being positive for an OP pesticide, hypothetically, plenty of opportunities are present in Algarve for the exposure to these chemicals, given that at least 58 products (OP or CA) can be legally used in several of the crops already enumerated, and organic agriculture is nationally still incipient. For the year 2009, only 1% of Algarve's UAA was used for organic production, by only 0,2% of the region's holdings (INE 2011b; Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento do Território - Direcção Geral de Alimentação e Veterinária 2013). In fact, the rank correlation of Spearman was positive for the association of the geographic density of some vegetal holdings with proportion of paretic gulls, which strengthens the probability of pesticide involvement in this syndrome. The correlation was greater for the association with the crops that are less representative, when compared with fresh fruit holdings (INE2010a). Moreover, in a bird watching session attended in the training period in Faro, gulls were seen eating olives directly from the grooves and thus the use of farmland as a means to acquire food, if regularly displayed, could lead to an increase in the exposure to such toxics in these settings. However, as Franson and Smith (1999) declare a thorough history of each case is an important tool for the determination of the cause of death (or disease) alongside with the necropsy procedure and

appropriate ancillary laboratory tests. Even though data concerning age, species, description and course of the disease is available (like in this case), information of water quality, crop management, pesticide applications, that are quite useful, are unknown or not available to interpret in light of the findings gathered, and in this study the same occurred. Additionally, no bird mortality event at least in Olhão for the same time period of the positive *L.fuscus* has been described, or animals with the same presentation were submitted to RIAS. Nevertheless, individual incidents of pesticide poisoning instead of several die-offs, and die-offs of single species are possible as is clear in the number of examples referred by Blus and Henny (1997) and Fleischli et al. (2004). Therefore, given this data, pesticide exposure could be a viable cause for the disease herein documented, however a more systematic diagnostic approach and understanding the dynamics of gull populations in Algarve would be enlightening on this subject.

 As previously stated only one recent study is available concerning the effects of **Thiamine deficiency** on the presentation of paralysis in seagulls. When comparing gulls of RIAS with the ones of Balk et al. (2009), the clinical presentation overlaps in some of the signs, however a chronologic sequence so detailed as the one published by the latter was impossible to accomplish, given the passivity of retrieval of such birds (and possibly a bias towards cases of paresis, which potentially are more easily found and retrieved) and an absence of field observations. Additionally, the limited prospect to examine the full range of clinical signs in the centre, as well as possibly the altered behaviour in captivity, affected these descriptions and some signs could have been unnoticed or its right order may be misrepresentative. The other findings of this study cannot be compared with these gulls, because samples could be collected but the same tests could not be performed, neither in the centre nor in the faculty due to equipment and methodology constraints, so a tentative diagnosis of a thiamine deficiency or an involvement of this vitamin in the paretic syndrome is difficult to accomplish in the Portuguese case when compared with this northern European study. On the whole, the clinical signs and necropsy findings here compiled are similar to the ones previously described by these authors and the remaining aforementioned, although opisthotonus, a frequently described sign by them, was not documented in these gulls. Nonetheless and considering the results of the feeding trial of Swank (1940), if thiamine deficiency was chronic, opisthotonus didn´t develop, perchance the same occurred in these gulls. However, the absence of evidences on the course of the disease and the onset of signs in these gulls precludes the characterization of this disease as either acute or chronic. Additionally, the body scores and fat deposits are also not clear in this aspect: recent deaths had higher body scores, but the other categories of recent death in relation to date of admission were likely influenced by anthropogenic factors such as time of retrieval, immediate stress of captivity and development of opportunistic diseases. However, one *C.ridibundus (*V0584/11/A) in the course of the training period was found dead in a star gazing position. This adult was admitted in September 2011, exhibited the commonest set of signs and died over night, but if this is a post mortem artefact, or was due to the terminal agonic period or is a true sign, it's unknown. Moreover, very few had lesions similar to the ones of the cardiac failure documented in the chronic deficiency of thiamine in the study of Swank (1940) (hydropericardium, congestion and oedema of lungs, engorged liver, oedema in connective tissue, pectorals and thighs) and none exhibited grossly visibly evidences of cardiac necrosis. If the cause of this syndrome is a thiamine deficiency, the absence of opisthotonus in these gulls cannot be explained in this moment. Other sign that was not detected was the pigmentation changes resembling

the disorder *retinitis pigmentosa* described by Balk et al. (2009), no gull on the training period exhibited it and no similar description was found in the digital records. The absence of necropsy examinations in this Swedish study, unfortunately, further limits this comparison and is one of the aspects most criticized by other professionals like Rocke and Barker (2010). Accordingly, this differential is still nowadays subject of public and scientific debate, with critics, reviews of differentials, comments and replies being currently published like the ones of Balk et al. (2010); Sonne et al. (2012a/b) and Tillitt, Kraft and Honeyfield and Fitzsimons (2012), which are explicit of the need for more investigation, before the definitive acceptance of this differential can be acknowledged in the Baltic Sea. When considering the Portuguese panorama, the feeding habits and composition of the diet of these gulls for this roughly four year period is unknown and none, upon admittance in the centre, had or regurgitated ingesta with visible cues of what it ate previously. The places where they usually forage for food is another problem to consider, as information or works carried on that subject, specifically in Algarve, are not available. This lack of information is not something uncommon and has already been reported in studies like the one of Neimanis et al. (2007). When they were relating botulism as a proximate cause of paralysis in gulls, cues as to the possible source of botulinic toxins for the paralysis were absent and further investigation on the subject was considered extremely important. Furthermore, these gulls were practically found all over Algarve, so it is extremely challenging to understand this behaviour seeing that there isn´t an uniformity in the various municipalities, when bearing in mind the several habitats and ecosystems found (along with the diversity of food) and also the distribution and influence of human presence and activities, like was already mentioned on part 3. A conscientious analysis of the various food items present, that could conceivably constitute their normal diet and their nutritional values was not attempted. Research on specific nutrient deficiencies in minor species, comparable to the one of the aforesaid Riley et al. (2011), have also not been published to allow a possible extrapolation to more complex species. Algarve in terms of climate is probably one of the most favourable regions of Portugal for the yearly survival and in some cases wintering phase of the life cycle of several avian species, and given the resourcefulness presented by these birds and the multitude of types of food they are known to seek (Catry et al. 2010) plenty of alternatives could be available, even in the colder seasons for resident species and also for migratory ones that winter, pass or use Algarve as a stop-over. The absence of cues of death by starvation as the only cause, a possible indicator of shortage of food in potentially harsher seasons, is another pointer to consider in this last aspect, even though, in accordance to Wobeser and Kost (1992) it is something difficult to diagnose, especially by necropsy since emaciation is a terminal stage of several diseases. In this study, this sign was always accompanied by a series of abnormal findings that suggest other type of aetiology than simply emaciation by starvation and possibly hypovitaminosis. Therefore and considering all this, a deficit in a single nutrient, either by lack of food or a specific nutrient deficiency in it, through insufficient transfer between trophic levels, could hardly, alone, and in this case justify this syndrome, which was only found in gull species. However, Balk et al. (2009) suggested that increased needs for thiamine in these birds or an inability to use this vitamin are other possible explanations for their presentation, but in this study could not be further investigated. When regarding other nutrients particularly cases of negative human enrichment of the food-chain, it is true that some human activities as the ones indicated by Lemly (2004) are present in Algarve and these are also found in potentially the most problematic municipalities, however

information of its specific location/activity, process of exploitation, residue or by-products management and environmental impacts is not readily available to study the effects of this hypothetical enrichment of the ecosystem and its effects in avian populations.

Bearing in mind the different protocols and types of therapeutic approaches recommended for the diseases in consideration (table 2), in cases like metal intoxication, botulism among other diseases the use of vitamins, e.g. B complex and specifically B_1, is beneficial and recommended (Samour 2000). Thus protocol T used in this context to correct a possible thiamine deficiency, may have ameliorated other diseases that could be the cause of this syndrome. On the other hand, the dosage of 30 milligrams of thiamine per kg of body weight used, although in accordance with what is usually recommended for neurologic disorders or thiamine supplementation (Samour 2000), is different from the 50mg/kg used by Balk et al. (2009). The difference that 20 milligrams could have in the outcome of both conditions in this respect is not known, but valid for future investigations. In turn, the use of activated charcoal, the protocol used before the training period and proposed for toxic afflictions (table 2), could have been ineffective, as normally is administered early in the intoxication process, and at the time of admission there weren't present signs to assert that a toxic was present and was still available in the gastrointestinal tract for it to act upon and exert its effects. Altogether these approaches are not specific for the treatment of one disease and cannot identify the cause of these signs, and so the no association between outcome and the different protocols observed could have been due to these circumstances. In spite of the different protocols used and this result, between October 2009 and December 2012 sightings of ringed gulls, rehabilitees of this paretic syndrome, were reported and are indications of survival in nature of both resident and migratory species (Ap.3.5 fig.135 a, b, c, d and e). The data gathered indicates, as well, that their migration routes may have not been severely affected as is an example the *L.fuscus* of figure c. This gull was an adult that upon release in 2009, has been repeatedly seen in the same region for two consecutive years. In view of the fact that adult gulls may show a stronger philopatric instinct to their breeding sites than younger ones, which is probably what is seen in this case, apparently an impairment of this function doesn't occur, but more data is required to refine this hypothesis[3].

3.3.3. Methodology considerations, anthropogenic factors and the local and global importance of this study.

Because the submission of cases is spontaneous, the number of paretic gulls or mortalities should not be considered or confounded with prevalence or incidence of this disease in Algarve, as: it is difficult to determine the impact of this disease on seagulls' populations or the magnitude of ill cases or mortality (this information is not available); part of the admissions occurred in times where fidelity to sites can be less stronger, when compared with the breeding colonial formations (fig.102) and the low findings in warmer months probably corresponds to peaks of activity or ability to sense of predators and scavengers as Prosser, Nattrass and Prosser (2008) have stated. No systematic search for such carcasses or debilitated gulls was or is in course and probably predators and scavengers are active throughout the year, so the ones that were retrieved are an unknown proportion of those that are sick

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[3] As a result of a low number of re-sightings of RIAS' rehabilitated animals marked with a metallic ring, in December of 2010 began a specific project to study the success of rehabilitation, re-adaptation, movement, provenance and longevity of once ill *L.fuscus* and *L.michahellis*. Ever since they have been ringed with a black plastic ring with an alphanumerical code published in cr-birding www.cr-birding.be (RIAS2011).

or that actually died of this illness. In fact, this lack of knowledge of the number of gulls present in Algarve and their distribution is another factor that couldn't be assessed, but such information could be important, as it may be influencing some of the results here documented and could explain the significant differences assessed when comparing variable syndrome and variables "Species" and "Age class" or clinical signs "Body score" and "Weakness" and the same two variables. The significant differences assessed, probably a higher susceptibility of *L.fuscus* and sub-adult seagulls to this paretic syndrome, can be in fact influenced by the abundance of such animals in Algarve, or, in the case of age classes the temporal and spatial differential influx of these larids, habitat or foraging partition or even different food predilections, when considering the moment and place where the optimal conditions for the etiologic agent are gathered. This type of information is of difficult determination as is clear globally in the Wetlands International Waterbird Population Estimates website (http://wpe.wetlands.org/) where such data is limited both geographically and temporally and sometimes is evaluated as to its quality as only a best guess. Estimations of trends, in turn, are frequently unknown or of poor quality (WPE 2012/2013). Nationally, the projects are also limited in time and geographically, however recent efforts have been undertaken like is the case of Project Arenaria, a long term monitoring survey of the distribution and abundance of wild birds in the coast and Portuguese beaches of wintering populations that began in 2009 (Projecto Arenaria 2013). The results disclosed, until this moment, are of the years 2009 and 2010 and don´t differ greatly from previous surveys: the species most abundant nationally and in Algarve are still *L.fuscus* and *L.michahellis*. However, only non-estuarine regions were subjected to count and so other sites known to be chosen by species like *C.ridibundus* or with a more pelagic nature like *L.melanocephalus* were not considered, but were deemed of importance. Age classes were not sub-divided further than the two classes Adult and Immature. In the latter, the species were not identified which underestimates the number of birds that are *L.fuscus* or *L.michahellis*. Therefore and for this study this data makes it impossible to understand this part of the relationship between these variables.

As previously explained, several human factors may also be influencing the number of cases and their provenance. Human densities, tourism, summer holidays, the economic crisis and monetary constraints (the last ones explicit in Ap.3.7 fig.137) are probably the most significant for this region. Public awareness to the problematic of conservation and to the presence of RIAS in this region, as well as the acknowledgement of the activities developed by this centre, are other factors that may be affecting the rescues, specifically when international tourists are considered. Although RIAS develops frequently numerous educational and release sessions in several events and locations (Ap.2.2), it's connected to the new technologies and sometimes is the focus of attention by the national and international mass media (RIAS 2010/2011/2012a/2013); the impact that these actions/features have in the locals and tourists is something unknown and would be interesting to investigate. This aspect could be influencing this study and is relevant to future works, if the irregularities in retrievals continue and a possible bias could be developing given that these sessions tend to occur in counties in close proximity to RIAS.

Another point to take into consideration is the fact that only 148 gulls, out of the 780 that were admitted in RIAS, were regarded as having the presentation considered specific to the syndrome in study, which was based on the logged descriptions of the findings of the physical examinations. However, this conservative methodology excluded 27% of the 717 alive admissions (Ap.3.8 fig. 138),

including two *L.audouinii* gulls, that although classified as being ill due to a "Disease", "Debilitation" or "Unknown" cause (like the paretic gulls), had no written description of the physical examination to clarify if these birds were or not gulls suffering from this syndrome (Ap.3.9.fig.139). The absence of such records, in spite of the fact that the physical examination was performed and provided the basis for the classification on either one of these three categories, was probably due to the: arrival of a high number of animals, particularly young and juvenile on Spring and Summer in 2010, 2011 and 2012; the two outbreaks of disease in the wastewater treatment plant in 2010 and then again in 2012; the admittance in 2012 of 124 chicks of *Falco naumanni* of the European project LIFE07NAT/P/654; and admission of gulls with paresis (Ap.3.10/3.11,fig.140/141). These all required attention and care of a low number of volunteers and RIAS' permanent small team of 4 elements. The terms of the database, although correct, are vague and don´t improve this situation. The case of the category "Disease" is clear in this aspect. On the words of Wobeser (1997) cited by Wobeser (2006) "Disease is an impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factos (pp.5)" and clearly encompasses several different conditions, as what was seen in the database when analyzing gull admissions with logged information. Moreover Trocini, Pacioni, Warren, Butcher and Robertson (2008), when regarding databases of wildlife rescue centres, referred that they are usually more incident focused than diagnostically orientated and perhaps this is what happened in the case of paretic gulls. These cases of paresis were recorded as belonging to different categories, probably, because a definitive diagnosis was not reached to clarify the nature of this syndrome. This consistent use of different terms and the absence of the written descriptions of the signs or clinical history were what led to the exclusion of several seagulls, due to uncertainties as to what was found in the admission and if they were true paretic cases. These authors also indicate that recording errors are likely to happen due to the high number of different volunteers and this hindered the compilation of the data for this study and probably explains the case of gull, V0078/12/A. This bird upon admittance had a written description compatible with the signs regarded as specific of this syndrome (was classified as admitted due to suspicion of disease), but on the necropsy examination was found to have: a bloody wrist, hemorrhagic flank, three exposed fractured ribs with perforations of the adjoining air sacs, blood in the celomic cavity, hemorrhagic lungs, hypertrophied heart with dilated chambers (Ap.3.4 table10). These findings would likely render her immobile while alive, but were not concordant with the description of the clinical signs (a description of the presence of the fractures or the blood was absence) or the methodology used to delineate this study; hence the exclusion of these cases from the discussion of the necropsy findings. Therefore and taking this into consideration, even if only 148 out of 780 were paretic from 2009 to 2012, this value may be an underestimate of the true magnitude of the problem.

Other important factor that hindered this study was the impossibility to develop a more systematic diagnostic approach to determine the cause of this disease and the mortalities observed. As Trocini et al. (2008) state very few diagnostic exams and/or limited economic resources are available in rehabilitation centres, so these facilities are highly dependent on external institutions. In this particular case, only 9 animals could be submitted for further analysis. Lag time between moment of death of the birds and authorized submission of the carcasses and examination, including the subsequent disclosure of the results, methodologies used, samples tested and the total number of specimens that

could be analysed were additional factors that complicated this study (Ap.2.5 table 5). With more complex diagnostic procedures, it was expected that possibly the additional findings could enlighten some of the findings seen in the centre and alter the way these animals could be rehabilitated (e.g. use of alternative therapeutic protocols) or even influence other studies in progress in RIAS. But these delays, that lasted more than 6 months, prevented that from happening and conditioned the necropsy examinations performed by the author, that waiting for possible cues to keep in mind during the part of the necropsy examination *in situ* or an authorization to send more specimens, had to postpone the listing of gross pathologic findings in order to prevent a shortage of samples (given the problem of irregular retrievals) and couldn't collect beforehand the appropriate samples in fresh specimens and preserved them correctly to avoid problems with decomposition and autolysis. Furthermore, propositions for additional funding or partnerships with several institutions (including FMV-UL) were also attempted, but collaborations were refused, either because of limited resources by the latter or unfortunately lack of interest. This last one was probably influenced by monetary constraints imposed in these institutions during this economic crisis or maybe the apparent absence of a grand scale mortality or disease event affecting other types of birds, or more valuable species, was not alluring enough.

In spite of this, between October 2009 and December 2012 gulls of the species *L.fuscus*, *L.michahellis*, *C.ridibundus* and *L.melanocephalus* were the only admitted in RIAS displaying the described paretic syndrome. However, the absence of other seagull species or even other wild birds is not a proof that they are not affected by whatever it is causing this disease. Other gull species were admitted in RIAS and/or were present in the time-frame of the study in this part of the continent, including rare specimens of *L.glaucoides*, *L.hyperboreus* and some of the American group (Jara et al. 2010;Leitão & Cidraes-Vieira 2011;Aves de Portugal 2013).But as no systematic search for sick animals or deceased birds was or is in progress, more secluded areas may have been overlooked, the secretive behaviour of diseased birds may have hindered their finding or some of their phenotypic features (like small size) aided in their hiding, unnoticed decomposition or predation. Of the 148 paretic admissions, 66 were rescued from beaches, one of Algarve's settings more highly used by the locals and the transitory foreign population. Nonetheless, these birds can be found inhabiting other sites as the ones enumerated by Catry et al. (2010), amidst other species while roosting, foraging for food or in colonial formations where they can also prey on other birds or be predated. Consequently, for these reasons this syndrome can be an early warning of potential disease in other species that live in the same ecosystem and often share the same food items. This, in turn, could be problematic if one keeps in mind that a significant part of Algarve is included in the 2000 Natura Network, comprising nine Special Areas of Conservation and five Special Protection Areas in addition to five areas of the National Network of Protected Areas, one Biogenetic Reserve of Europe, four wetlands of the Ramsar convention and 10 Important Bird Areas (ICNF 2013b;SPEA 2013) and species like *S.hirundo* (nationally endangered), *Circus cyaneus*, *Ardeola ralloides* (already in a critical state) and *L.audouinii* (vulnerable but near threatened globally) are found in these settings and/or may be seen in close proximity to these animals (Cabral et al. 2005;N-H-A.G 2012).

Another aspect to keep in mind, an important factor that should be analysed in the future, is the one pertaining to the ability to migrate to more distant places that some of these larids have. It was assumed that the cause of this disease was present in this portion of the Portuguese continent,

however to what extent this natural phenomenon is influencing, or is responsible for this condition is something still not fully known. In accordance with Newton (2008) when considering true migratory species including gulls: several different individuals from several different breeding sites can gather in staging areas before the wintering migration; diet may change during this event and can hardly be assessed; all the dispersive movements, site fidelity to roost and foraging areas, overlapping of grounds and diets in different species, stop-over sites and temporal and geographical differential migration seen between distinct age classes (documented by Jorge et al. 2011) among several other aspects; are features regularly seen in these animals and are also factors of extreme importance when one tries to determine the cause of a disease, source and potentially the dispersion or dissemination of a pathogen or toxic. Moreover, the complexity of this aspect is enhanced when it is taken into consideration the African – European flyway, or even the presence of birds from other continents like America, or the incursion of European ones in such continents as noted by Hallgrimsson, Van Swelm, Gunnarsson, Johnson and Rutt (2011). Therefore, the cause of this paretic presentation may be present in another portion of the country or have its origin in other places, perchance in other countries, during other parts of the life cycle of true migrants, e.g. breeding grounds in northern latitudes. This hypothesis is still valid for the so called resident species, in this case *L.michahellis,* as they can be exposed through contact in Algarve or during their dispersive movements that can reach the 680km, as Arizaga et al. (2010) documented in a juvenile while studying the first five years of movements of a subspecies of *L.michahellis* in the bay of Biscay. Migration and other types of bird movements may also be influencing the expression of this syndrome, as some authors have already reported when considering infectious diseases and the immune system. Hubálek (2004) stated that the stress of migration may lower the resistance to infectious diseases and aid in the dissemination of this group of diseases and, in turn, Moñuz and De la Fuente (2003) have already demonstrated in *C.ridibundus* that winter and migration exert their pressures in the immune system by decreasing the circulating lymphocyte cell population. This could be another explanation for the significant associations between "Age classes", "Species" and the Paretic syndrome and possibly the relations between the same variables and the different clinical signs.

3.3.3. Other possible hypothesis for the paretic syndrome: one pathogenic agent/toxic, several agents acting together, several conditions in different birds or just weakness?

In this study only 11 aetiological causes for a paretic or paralytic presentation were considered, however in table 1 the list of differentials is vast and should be considered in future investigations as the clinical signs, necropsy findings and results of diagnostic tests don´t exclude completely some of the 11, but are also not characteristic of the other here dismissed. Nevertheless it was assumed from the beginning that one disease was the cause of this condition and could explain all these findings. However, this point of view can be somewhat reductive of the reality. If one considers that wildlife is subjected to multiple stressing pressures, it is likely that was herein reported could be due to not one pathogenic agent or toxic but several causes that acting together, in lower or non-pathogenic concentrations, could result in these unspecific and varied signs and findings as Pikula et al. (2010) documented in their study. These authors, when studying the pathologic effects of single and combined exposure to cyanobacteria toxins, lead and Newcastle virus in *Coturnix coturnix japonica*,

concluded that the necropsy findings and clinical signs found were concordant with an enhancement of avian toxicity, mortality and adverse effects seen in sub-lethal combinations. In this way, the low levels of lead, already considered a morbidity factor in at least *G.immer* by Stone and Okoniewski (2001), together with the absence of positive detections of pesticides due to possibily the presence of low undetectable concentrations in samples, debilities owing to migration or energy demands in colder seasons and other causes could explain the lack of uniformity of these results and be concordant with sub-clinical effects that interacting amongst them gain expression as a disease. Another viable hypothesis to be considered is related to the fact that it was presumed that all these signs belonged to the same syndrome (either mono or now multifactorial), however numerous manifestations of various diseases in different birds could be the reality, and this could easily explain the lengthy geographic distribution pattern and temporal occurrence of paretic gulls in Algarve. Other interesting point to consider is if in fact what was seen is a true paretic presentation, or perchance, weakness, caused by other condition that doesn´t exerts its effects exclusively in the neuromuscular system, is the rightful cause of the syndrome here described. All these are hypothesis that would be interesting to address in the future, given these results and all these possible scenarios.

4. Conclusions and further studies.

This preliminary study is the first to document and characterize a paretic syndrome that has been affecting several species of gulls in Algarve since 2009. The data compiled and the results obtained are not specific of a particular disease, but cannot exclude, in this moment, the different diseases studied or indicate the rightful cause of this syndrome. These results, their discussion and the suggestions made throughout this dissertation, including the reconnaissance of some of its limitations, provide baseline data for future research alternatives and underline the need for a more frequent and concerted effort between different institutions in a multidisciplinary approach in wildlife disease and surveillance both on field and in laboratory settings.

The aims of this investigation were generally achieved:

-18,97% of the gulls admitted were found to have this paretic syndrome, being the migrant species, *L. fuscus*, and age class sub-adults probably the most affected (p=0,02;p=0,00005). The most frequent signs documented were: depressed mental status without loss of conscience (58,8%); diarrhoea (43,9%); flaccid cloacae (70,3%); generalized muscular weakness (48,6%); moderate muscular weakness (46.6%); posterior paresis (69,6%) and moderate paresis (71,6%). This presentation probably aided in the detection of these birds and rescue, and may justify the low body scores and dehydration degrees presented by these gulls upon admission.

-Approximately half of the paretic gulls died in rehabilitation and the diferent protocols tested between September 2011 and March 2012 didn´t influence the outcome, at least significantly (p=0,7422). Nonetheless, in the case of protocol C, the administration of active charcoal was deleterious and contributed to the death of the more severely affected.

-Gross necropsy findings were unspecific, inconsistent and probably are due to the ongoing course of the disease, decomposition of the carcass, development of opportunistic diseases like aspergilosis or confounding features like the case of articular gout. Nonetheless, 45% (32 out of 71) of the examined carcasses had a thin-walled congested cloacae, distended with diarrhoea, and the intestines had

signs of oedema, vascular congestion and fluid faeces, findings concordant with the signs gathered in the physical examination.

-The results of more complex and specific diagnostic ancillary tests and the treatment trial were inconclusive as to the cause of this syndrome.

-When considering the geographic distribution of the paretic cases Loulé and Portimão were the two municipalities with the highest number of retrievals, however this finding is, in this moment, of difficult interpretation as these counties are heavily populated and one is in close proximity to RIAS (Loulé), features that aid in the rescue of sick birds. Problems with retrieval of sick birds, lack of information on gull population densities and their ethologic features are other factors that may be influence this aspect. Nonetheless, when considering the association of the proportion of sick birds per municipality and density of several activities, some were significant and when regarded with the positive detection of a pesticide in a deceased gull may indicate a potential source or possibly an exposure to a morbidity factor.

At this moment, it is impossible to ascertain a cause to this syndrome and doubts as to its nature (mono factorial, multi factorial,..) are still present. The shortcomings found are also factors that may be influencing some of the results obtained and are significant limitations when one tries to reach a conclusion as to the rightful cause or causes of this syndrome. The inconsistent use of ancillary diagnostic tests, limited funding, irregularities in the retrieval of sick birds, limited cooperation between institutions and exchange of data, limitations and restrictions usually felt while conducting scientific studies in wildlife centres, the scarcity of national studies in gulls and wildlife diseases and the negative public image of gulls were probably the main aspects that hindered this work and unfortunately are becoming more common nowadays. It is hoped that this data, the limitations acknowledged, the conclusions and suggestions presented be considered as a reference point for additional studies on the subject of paresis in wild birds, particularly with the intent to identify its rightful cause and the impacts that it could take in the species affected and, if needed, in other wild species and perchance domesticated animals and the human being.

Therefore, in future opportunities it would be advisable to proceed with a more comprehensive examination of fresh carcasses and sick birds, sampling a higher number of individuals with a more systematic use of ancillary diagnostic techniques. The data acquired should be combined with information on foraging and feeding habits of affected birds; characteristics of the habitats where they dwell; data of population dynamics, movements and ranges, provenance and roosting areas; among other biologic or ecology features. This requires the knowledge of these birds' natural histories and all their complex aspects, which is something still unknown when considering this region and the species affected, but is something of utmost importance and necessary to reach an accurate diagnosis and understand all the subsequent implications. New technologies of tracking, for instance the use of satellite transmitters, could be useful to fill the knowledge gaps seen in the traditional ringing methodologies and aid in this aspect. In order to prevent misinterpretations of new admissions in RIAS, small modifications in its database could be equated, particularly when considering the logged suspected cause of entry. Instead of the inconsistent use of the terms "Disease", "Intoxication", "Debilitation" and "Unknown" a new category, "Syndrome", could be added to differentiate these paretic gulls from other cases, while an accurate diagnosis is pending and limitations in human resources are still present and contributing to the lack of written reports of the physical examinations

and clinical histories. Additionally, if the problems with the retrieval of ill birds continue, reports of sightings of diseased gulls could be registered for future statistical analysis.

4.References.

Abou-Rawash, A.A., Yanai,T., Kano, R., Murakami, M., Masegi, T., Fukushi, H., Nagamine, T. & Hasegawa, A.(2008). Disseminated aspergillosis in a Whooper swan (*Cygnus Cygnus*).*Egypy J. Comp. Path & Clinic. Path*, 2(2), 161-174. Access Mar, 2012, available at: http://www.pathology-eg.com/publication2008_2/No.8_(161-174).pdf

Adams, D., Roblee, K. & Stone, W. (2009). Waterbird mortality as a result of type E botulism and nonnative invasive species, [poster]. In. *Odum Conf. 2009. New York Invasive Species Research Institute, Cornell University, Ithaca, NY*. Access Feb, 2012, available at: http://www.nyisri.org/odumposterB.aspx

Adrian, W.J., Spraker, T.R. & Davies, R.B.(1978). Epornitics of aspergillosis in Mallards (*Anas platyrhynchos*) in north central Colorado. *Journal of Wildlife Diseases*, 14, 212-217. Access Mar, 2012, available at: http://pubget.com/paper/650787/epornitics-of-aspergillosis-in-mallards-anas-platyrhynchos-in-north-central-colorado

Agreement on the conservation of African-Eurasian Migratory Waterbirds [AEWA] secretariat.(2008). *Update report on the use of non-toxic shot for hunting in wetlands*. AEWA technical series nº33 September 2008

Albers, P.H., Green, D.E. & Sanderson, C.J. (1996). Diagnostic criteria for selenium toxicosis in aquatic birds: dietary exposure, tissue concentrations, and macroscopic effects. *Journal of Wildlife Diseases*, 32(3), 468-485. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-32.3.468

Alexander, D.J.(2011). Newcastle disease in European Union 2000 to 2009. *Avian pathology*, 40(6), 547-558. Access May, 2013, available at: http://www.tandfonline.com/doi/pdf/10.1080/03079457.2011.618823

ALGAR (2012). *ALGAR: Valorização e tratamento de resíduos sólidos, S.A*. Access Oct, 2012, available at: http://www.algar.com.pt/pt/

Almansour, M.I. (2004). Using feathers as a biological indicator of lead environmental pollution. *Pakistan Journal of Biological Sciences,* 7(11), 1884-1887. Access Oct, 2012, available at: http://www.docsdrive.com/pdfs/ansinet/pjbs/2004/1884-1887.pdf

Anderson, D.M. (2009).Approach to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management*, 52, 342-347. Access Dec, 2011, available at www.whoi.edu/fileserver.do?id=58008&pt=2&p=28251

Anderson, D.M,, Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E. & Montresor, M. (2011). The globally distributed genus Alexandrium: multifaceted roles in marine ecosystems and impacts on human health. *Harmful algae,* 14, 10-35. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1568988311001405

Arcos, J.M., Oro, D. & Sol, D. (2001). Competition between the yellow-legged gull *Larus cachinnans* and audouin's gull *Larus audouinii* associated with commercial fishing vessels: the influence of season and fishing fleet. *Marine Biology,* 139(5), 807-816. Access Nov, 2012, available at: http://link.springer.com/article/10.1007%2Fs002270100651

Arcos, J.M., Ruiz, X., Bearhop, S. & Furness, R.W. (2002). Mercury levels in seabirds and their fish prey at the Ebro delta (NW mediterranean): the role of trawler discards as a source of contamination. *Marine ecology progress series*, 232, 281-290. Access Jan, 2013, available at: http://www.int-res.com/articles/meps2002/232/m232p281.pdf

Arizaga, J., Herrero, A., Galarza, A., Hidalgo, J., Aldalur, A., Cuadrado, J.F. & Ocio, G.(2010). First-year movements of yellow-legged gull (*L.michahellis lusitanius*) from the southeastern bay of Biscay. Waterbirds, 33(4), 444-450. Access Nov, 2011, available at: http://www.bioone.org/doi/full/10.1675/063.033.0403

ARKive (2012). *ARKive*. Access May, 2012, available at: http://www.arkive.org/

Atasever, A. & Gumussoy, K.S.(2004). Pathological, clinical and mycological findings in experimental aspergillosis infections of Starlings.*J. Vet.Med*, 51(1), 19-22. Access Mar, 2012, available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0442.2004.00598.x/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+BST+%2805%3A00-10%3A00+EDT%29+for+essential+maintenance

Augspurger, T., Smith, M.R., Meteyer, C.U. & Converse, K.A. (1996). Mortality of Passerines adjacent to a North Carolina corn field treated with granular carbofuran. *Journal of Wildlife Diseases* 32(1), 113-116. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-32.1.113

Australian Government – Department of the Environment and Heritage. (2006). *Hygiene protocols for the prevention and control of diseases (particularly beak and feather disease) in Australian birds - full necropsy protocol*. Australia: Commonwealth. Access Nov, 2011, available at: http://www.environment.gov.au/system/files/resources/9349e95b-85ec-4c40-a457-1a9fdcb76642/files/hygiene-protocols-all.pdf

Aves de Portugal. (2012). *Aves de Portugal*. Access Apr, 2012, available at: http://www.avesdeportugal.info/avesdeportugal.html

Aves de Portugal. (2013). *Aves de Portugal*. Access Apr, 2013, available at: http://www.avesdeportugal.info/avesdeportugal.html

Avibirds (2012). *Birds of Europe*. Acess Feb, 2012, available at: http://www.avibirds.com/euindex.html#.Up5F2tJdUgc

Bailey, T. A.(2000). Capture Paresia. In Samour, J. *Avian Medicine*. (2ªedição).(pp.266-268).Mosby Elsevier

Balk, L., Hagerroth, P-A., Akerman, G., Hanson, M., Tjarnlund, U., Hansson, T., Hallgrimsson, G.T., Zebuhr, Y., Broman, D., Morner, T. & Sundberg, H. (2009). Wild birds of declining European species are dying from thiamine deficiency syndrome. *PNAS,* 106(29), 12001-12006. Access Nov, 2011, available at: http://www.pnas.org/content/106/29/12001

Balk, L., Hagerroth, P.- A., Akerman, G., Hanson, M., Tjarnlund, U., Hansson, T., Hallgrimsson, G. T., Zebuhr, Y., Broman, D., Morner, T. & Sundberg, H.(2010). Reply to Rocke and Barker: the question is not whether birds are affected by thiamine deficiency or botulism, it is about the order of events.*PNAS*, 107(11). Access Nov, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2841946/pdf/pnas.200913140.pdf

Balseiro, A., Espí, A., Márquez, I., Pérez, V., Ferreras, M.C., García Marín, J.F. & Prieto, J.M. (2005). Pathological features in marine birds affected by the prestige´s oil spill in the North of Spain. *Journal of Wildlife Diseases*, 41(2), 371-378. Access Apr, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-41.2.371

Baron, R.L. & Johnson, H. (1964). Neurological disruption produced in hens by two organophosphate esters. *Brit.J.Pharmacol*. 23, 295-300. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1704093/pdf/bripharmchem00039-0081.pdf

Bates, F.Y., Barnes, D.M. & Higbee, J.M.(1968). Lead toxicosis in mallard ducks. *Bull.Wildlife Disease Association*, 4, 116-125.

Beaudette, F.R. (1945). Aspergillosis and parasitism in a gull. *Journal Series paper of the New Jersey Agricultural Experiment Station,* XVI, 99-101. Access Mar, 2012, available at: http://www.jstor.org/discover/10.2307/4509874?uid=3738880&uid=2&uid=4&sid=21103044950491

Beernaert, L.A., Pasmans, F., Waeyenberghe, L.V., Haesebrouck, F. & Martel, A. (2010). *Aspergillus* infections in birds: a review. *Avian pathology*, 39(5), 325-331. Access Apr, 2012, available at: http://www.tandfonline.com/doi/pdf/10.1080/03079457.2010.506210

Belas, A.; São Braz, B.; Moreira, A. & Carrapiço, B. (2011). Analytical detection of Metiocarbe in biologic samples. International Symposium for High-Performance Thin-Layer Chromatography-HPTLC In *Book of Abstracts, Basel, Switzerland 6 to 8 July 2011*, p.113

Bermejo, A., Carrera, E., De Juana A, E. & Teixeira, A. M. (1986). Primer censo general de gaviotas y charranes (laridae) invernantes in la Peninsula Iberica (Enero de 1984). *Ardeola,* 33 (1-2), 47-68. Access Mar, 2012, available at: http://www.ardeola.org/files/83.pdf

Berry, M.J. & Ralston, N.V.C.(2008). Mercury toxicity and the mitigating role of selenium. *Ecohealth,* 5(4), 456-459. Access Jan, 2012, available at: http://link.springer.com/article/10.1007%2Fs10393-008-0204-y

BirdLife International (2012). *IUCN red list for birds.* Access Apr, 2012, available at: http://www.birdlife.org

BirdLife International (2013). *IUCN red list for birds.* Access Apr, 2013, available at: http://www.birdlife.org

Blanco, J., Moroño, A., Franco, J. & Reyero, M.I. (1997). PSP detoxification kinetics in the mussel *Mytilus galloprovincialis*. one- and two- compartment models and the effect of some environmental variables. *Marine ecology progress series,* 158, 165-175. Access Nov, 2011, available at: http://www.int-res.com/articles/meps/158/m158p165.pdf

Blévin, P., Carravieri, A., Jaeger, A., Chastel, O., Bustamante, P. & Cherel, Y.(2013). Wide range of mercury contamination in chicks of southern ocean seabirds. *PLoS One*, 8, e54508. Access, May 2013, available at http://www.plosone.org/article/info:doi/10.1371/journal.pone.0054508

Blood, D.C.& Studdert, V.P. (1999). *Saunders comprehensive veterinary dictionary.* (2nd ed.). WB Saunders

Blus, L. J., Henny, C.J., Hoffman, D.J. & Grove, R.A. (1995). Accumulation in and effects of lead and cadminum on waterfowl and passerines in Northern Idaho. *Environmental Pollution*, 89(3), 311-318. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/026974919400069P

Blus, L. J. & Henny, C.J. (1997).Field studies and birds: unexpected and unique relations. *Ecological Applications* 7(4), 1125-1132. Access Mar, 2012, available at http://www.esajournals.org/doi/pdf/10.1890/1051-0761%281997%29007%5B1125%3AFSOPAB%5D2.0.CO%3B2

Boden, E. (2005). *Black´s veterinary dictionary.* (21st ed.). London: A&C Black Publishers limited

Boening, D.W. (2000). Ecological effects, transport, and fate of mercury: a general review. *Chemosphere,* 40, 1334-1351. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0045653599002830

Bond, A.L. & Diamond, A.W. (2009).Total and methyl mercury concentrations in seabird feathers and eggs. *Arch. Environ Contam Toxicol,* 56(2), 286-291. Access Jan, 2012, available at: http://link.springer.com/article/10.1007%2Fs00244-008-9185-7

Box, E.D. & Duszynski, D.W.(1977). Survey for *Sarcocystis* in the brown-headed cowbird (*Molothrus ater*): a comparison of macroscopic, microscopic and digestion techniques. *Journal of wildlife Diseases,* 13(4), 356-359. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-13.4.356

Box, E.D. & Duszynski, D.W.(1978). Experimental transmission of *Sarcocystis* from icterid birds to sparrows and canaries by sporocysts from the opossum. *Journal of Parasitology*, 64, 682-688. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/98622

Brand, C.J., Schmitt, S.M., Duncan, R.M. & Cooley, T. M. (1988a). An outbreak of type E botulism among common loon (*Gavia immer*) in michigan's upper peninsula. *Journal of wildlife diseases*, 24(3), 471-476. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-24.3.471

Brand, C.J., Windingstad, R.M., Siegfried, L.M., Duncan, R.M. & Cook, R.M.(1988b). Avian morbidity and mortality from botulism, aspergillosis, and salmonellosis at Jamaica bay wildlife refuge, New York, USA. *Colonial Waterbirds*, 11, 284-292. Access Apr, 2012, available at: http://www.nwhc.usgs.gov/publications/documents/88CJB.CW01.pdf

Brand, L.E., Campbell, L. & Bresnan, E. (2012). Karenia: the biology and ecology of a toxic genus. *Harmful algae*, 14, 156-178. Access Apr, 2013, available at: http://www.sciencedirect.com/science/article/pii/S156898831100148X

Brasso, R.L. & Cristol, D.A. (2008). Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolour*). *Ecotoxicology*, 17(2), 133-141. Access Jan, 2012, available http://link.springer.com/article/10.1007%2Fs10646-007-0163-z

Braune, B.M. & Gaskin, D.E. (1987). Mercury levels in bonaparte's gulls (*Larus philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. *Arch Environ. Contan. Toxicol,* 16, 539-549. Access Oct, 2011, available at: http://link.springer.com/article/10.1007%2FBF01055810#page-1

Brewer, L., Faribrother, A., Clark, J. & Amick, D. (2003). Acute toxicity of lead, steel, and iron-tungsten- nickel shot to mallard ducks (*Anas platyrhynchus*). *Journal of Wildlife Diseases,* 39(3), 638-648. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-39.3.638

Buckles, E.; Korich, J.; Ruiz, J.; Lucio-Martinez, B.; Torres, A.; Banda, A. & Mondal, S.(2012). *Avian necropsy examination*. [video]. Ithaca, New York: Cornell University College of Veterinary Medicine. Acess Feb, 2012, available at:http://www.partnersah.vet.cornell.edu/veterinarians/avian-necropsy-examination

Buekers, J., Steen Redeber, E. & Smolders, E. (2009). Lead toxicity to wildlife: derivation of a critical blood concentration for wildlife monitoring based on literature data- a review. *Science of total environment*, 407(11), 3431-3438. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0048969709000916

Burau, R.G. (1985). Environmental chemistry of selenium. *California Agriculture* 1985, 16-18. Access Jan, 2012, available at: http://ucce.ucdavis.edu/files/repositoryfiles/ca3907p16-62876.pdf

Burger,J. & Gochfeld, M.(1995a). Behaviour effects of lead exposure on different days for gull (*L. argentatus*) chicks. *Pharmacology Biochemistry and Behaviour*, 50(1), 97-105. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/009130579400290Y

Burger, J. & Gochfeld, M. (1995b).Effects of varying temporal exposure to lead on behaviour development in Herring gull (*L.argentatus*) chicks. *Pharmacology Biochemistry and Behavior*, 52(3), 601-608. . Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/009130579500147O

Burger, J. & Gochfeld, M. (1997). Risk, mercury levels, and birds: related adverse laboratory effects to field biomonitoring). *Environmental research*, 75, 160-172. Access Jan, 2012, available at http://www.sciencedirect.com/science/article/pii/S0013935197937786

Burger, J. (2002). Food chain differences affect heavy metals in bird eggs in Barnegat bay, New Jersey. *Environmental research section*, 90(1), 33-39. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0013935102943811

Burger, J. & Gochfeld, M. (2004). Effects of lead and exercise on endurance and learning in young herring gulls. *Ecotoxicology and Environmental Safety,* 57(2), 136-144. Access Out, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0147651303000356

Burger, J. & Gochfeld, M. (2005a). Effects of lead on learning in Herring gulls: an avian wildlife model for neurobehavioral deficits. *NeuroToxicology*, 26(4), 615-624. Access Oct, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0161813X05000082

Burger, J. & Gochfeld, M. (2005b). Lead and behavioural development: parental compensation for behaviourally impaired chicks. *Pharmacology, Biochemistry and Behavior*, 55(3), 339-349. Access Oct, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0091305796001037

Burger, J., Gochfeld, M., Jeitner, C., Burke, S., Stamm, T., Snigaroff, R., Snigaroff, D., Patrick, R. & Weston, J. (2007). Mercury levels and potential risk from subsistence foods from the aleutians. *Science of the total environment*, 384(1-3), 93-105. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0048969707005414

Burgess, N., Bond, A.L., Hebert, C.E., Neugebauer, E.& Champoux, L. (2013). Mercury trends in herring gull (*Larus argentatus*) eggs from atlantic canada 1972-2008: temporal change or dietary shift? *Environmental pollution*, 172, 216-222. Access May, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0269749112004022

Burn, J.D. & Leighton, F.A.(1996) .Further studies of brain cholinesterase: cholinergic receptor ratios in the diagnosis of acute lethal poisoning of birds by anticholinesterase pesticides . *Journal of Wildlife Diseases* 32(2), 216-224. Access Feb, 2013, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-32.2.216

Burr, E.W. (1981). Enzootic aspergillosis in wild Red Vented Cockatoos in the Philippines. *Mycopathologia*, 73, 21-23. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/7012630

Bushaw-Newton, K.L. & Sellner, K.G. (1999). Harmful Algal Blooms. In: NOAA's State of the Coast Report. *Silver Spring*, MD: National Oceanic and Atmospheric Administration. Access Feb, 2013, available at:http://state-of-coast.noaa.gov/bulletins/html/hab_14/hab.html

Buss, P.E. & Bengis, R.G.(2012). Cyanobacterial biointoxication in free-ranging wildlife. In Miller, R.E.& Fowler, M.E. (ed.). *Fowler´s zoo and wild animal medicine current therapy.* (pp.108-114).St. Louis Missouri: Elsevier Saunders

Butterfield, J., Coulson, J.C., Kearsey, S.V.; Monaghan, P.; McCoy, J.H. & Spain, G.E. (1983). The herring gull *Larus argentatus* as a carrier of *Salmonella. J.Hyg. Camb,* 91, 429-436. Access May, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129328/pdf/jhyg00021-0062.pdf

Cabral, M.J. (coord.); J. Almeida, P.R. Almeida, T. Delliger, N. Ferrand de Almeida, M.E. Oliveira, J.M. Palmeirim, A.I. Queirós, L. Rogado, M. Santos-Reis (eds.) (2005). *Livro Vermelho dos Vertebrados de Portugal*. Lisboa:Instituto da Conservação da Natureza.

Cacciuttolo, E., Rossi, G., Nardoni, S., Legrottaglie, R. & Mani, P. (2009). Anatomopathological aspects of avian aspergillosis. *Vet Res Commun*, 33, 521-527. Access Mar, 2012, available at: http://link.springer.com/article/10.1007%2Fs11259-008-9199-7

Cai, Z., Finnie, J.W. & Blumbergs, P.C. (2006). Avian riboflavin deficiency: an acquired tomaculous neuropathy. *Veterinary Pathology* 43(5): 780-781. Access Jan, 2012, available at: http://vet.sagepub.com/content/43/5/780.full.pdf+html

Camenisch, G., Bandli, R. & Hoop, R. (2008). Monitoring of wild birds for Newcastle disease virus in Switzerland using real time RT-PCR. Journal of wildlife diseases, 44(3), 772-776. Access, Nov, 2012, available at: http://www.zora.uzh.ch/13704/2/camenisch.pdf

Camphuysen, K., Van Dik, J., Witte, H. & Spaans, N. (2008). De voedselkeuze van kleine mantelmeeuwen en zilvermeeuwen en andere indicaties die aanwijzingen geven over het ruimtegebruik van deze vogelsoorten in de Noord-Hollandse kustwateren [Abstract]. *NIOZ Rapport 2008-12,* Koninklijk Nederlands Instituut voor Zeeonderzoek. Access Feb, 2012, available at: http://www.nioz.nl/files/afdelingen/Bibliotheek/NIOZ%20rapporten/nioz-report_2008-12.pdf

Carmichael, W.W. and Li, R.(2006). Cyanobacteria toxins in the Salton sea. *Saline Systems,* 2(5), Access Jan, 2012, available at: http://www.aquaticbiosystems.org/content/2/1/5

Carpenter, J.W., Pattee, O. H., Fritts, S.H., Rattner, B.A., Wiemeyer, S.N.; Royle, J.A. & Smith, M.R.(2003). Experimental lead poisoning in Turkey vultures (*Cathartes aura*). *Journal of Wildlife Diseases*, 39(1), 96-104. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-39.1.96

Carta Administrativa Oficial de Portugal [CAOP].(2013). *Carta administrativa oficial de Portugal*. Access Mar, 2013, available at: http://www.igeo.pt/produtos/cadastro/caop/inicial.htm

Catry, P., Costa, H., Elias, G. & Matias, R.(2010). *Aves de Portugal.ornitologia do território continental*. Lisboa:Assírio & Alvim.

Caudill, J.N. & Zimmerman, D.(2010). Botulism in wildlife. *Indiana wildlife disease news*, 5(2), 1-6. Access Jan, 2012, available at: http://digitalcommons.unl.edu/icwdmindiana/13/

Cawthorn, R.J. & Rainnie, D. (1981). Experimental transmission of *Sarcocystis* sp.(protozoa: sarcocystidae) between the shoveler (*Anas clypeata*) duck and the striped skunk (*Mephitis mephitis*). *Journal of wildlife diseases*, 17(3), 389-394. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-17.3.389

Chan, H.M., Scheuhammer, A.M., Ferran, A., Loupelle, C., Holloway & Weech, S.(2003). Impacts of mercury on freshwater fish-eating wildlife and humans. *Human and Ecological risk assessment,* 9(4), 867-883. Access Jan, 2012, available at: http://www.tandfonline.com/doi/abs/10.1080/713610013#.Up3SjtJdUgc

Chew, B.P. (1996). Importance of antioxidant vitamins in immunity and health in animals. *Animal Feed Science Technology* 59, 103-114. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/0377840195008918

Chou, S-J., Shieh, Y-C. & Yu, C-Y.(2008). Hematologic and biochemistry values of Black-faced spoonbills (*Platalea minor*) with recovering from botulism. *Journal of wildlife diseases,* 44(3), 781-784. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-44.3.781

Cívek, A., Dolejská, M., Karpískova, R., Dedicová, D. & Literák, I. (2007). Wild Black-headed gulls (*Larus ridibundus*) as an environment reservoir of *Salmonella* strains resistant to antimicrobial drugs. *European Journal Wildlife Research,* 53(1), 55-60. Access Jan, 2012, available at: http://link.springer.com/article/10.1007/s10344-006-0054-2

Clarkson, T.W.(1992). Mercury: major issues in environmental health. *Environmental health perspectives,* 100, 31-38. Access Jan, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1519577/pdf/envhper00370-0035.pdf

Clarkson, T.W. & Magos, L. (2006).The toxicology of Mercury and its chemical compounds.*Critical reviews in Toxicology,* 36(8), 609-662. Access Jan, 2012, available at: http://informahealthcare.com/doi/abs/10.1080/10408440600845619

Claro, M.A. & Pereira, M.A.(2009). *Alterações climáticas e turismo - uma questão em aberto*. Lisboa:Departamento de Prospectiva e Planeamento e Relações Internacionais - Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional.

Coffield, J.A. & Whelchel, D.D.(2012). Botulism neurotoxins. In Gupta, R.C. *Veterinary toxicology*. (Chapter 70). Elsevier inc

Coles, B. (2007). *Essentials of avian medicine and surg*ery. (3rd ed). Oxford:Blackwell Publishing Ltd.

Comissão de coordenação e desenvolvimento regional do Algarve. (2008). *Perfil e potencial económico-social do turismo náutico no Algarve*. Access Jan, 2013, available at: http://www.ccdr-alg.pt/site/modules.php?op=modload&name=CmodsDownload&file=index&req=getit&lid=728

Constanzo, G.R.(1990). *Sarcocystis* in american Black ducks wintering in New Jersey. *Journal of wildlife diseases,* 26(3), 387-389. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-26.3.387

Converse, K.A.(2007a). Aspergillosis. In chapter Thomas, N.C., Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 360-374). Blackwell Publishing.

Converse, K. (2007b) Avian tuberculosis In Thomas, N.C., Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 289-302). Blackwell Publishing

Coon, N.C. &Locke, L.N.(1967). Aspergillosis in a Bald eagle (*Haliaeetus leucocephalus*). *Bull. Wildlife Disease Assoc*, 4(2), 51. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/5689984

Cork, S.C., Alley, M.R., Johnstone, A.C. & Stockdale, P.H.G.(1999). Aspergillosis and other causes of mortality in the Stitchbird in New Zealand. *Journal of Wildlife Diseases*, 35(3), 481-486. Access Apr, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.3.481

Corson, M. S., Mora, M.A. & Grant, W.E. (1998). Simulating cholinesterase inhibition in birds caused by dietary insecticide exposure. *Ecological modelling*, 105, 299-323. Access Mar, 2012, available at: http://pubs.er.usgs.gov/publication/70021348

Coulson, J.C., Butterfield, J. & Thomas, C. (1983). The herring gull *Larus argentatus* as a likely transmitting agent of *Salmonella montevideo* to sheep and cattle. *J. Hyg.,* 91, 437-443. Access May, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129345/pdf/jhyg00021-0070.pdf

Cox, C. (1991). Pesticides and birds: from DDT to today's poisons. *Journal of Pesticide Reform* 11(4), 2-6. Access Dec, 2012, available at: http://www.wolf.sk/dok/pesticidy/birds.pdf

Crawley, R.R., Ernst, J.V. & Milton, J.L. (1962). *Sarcocystis* in a Bald eagle (*Haliaeetus leucocephalus*). *Journal of wildlife diseases,* 18(2), 253-255. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-18.2.253

Creekmore, L.H.(1999). Algal toxins In Friend, M. & Franson, J. C. (1999). *Field manual of wildlife diseases: general field procedures and diseases of birds.* (pp.263-266)Washington DC: U.S. Geological Survey

Crissey, S.D.; McGill, P. & Simeone, A-M. (1998). Influence of dietary vitamins A and E on serum alfa and gamma tocopherols, retinol, retinyl palmitate and carotenoid concentrations in Humboldt penguins (*Spheniscus Humboldti*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative physiology*, 121(4), 333-339. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1095643398101319

Cristol, D.A., Brasso, R.L., Condon, A.M., Fovargue, R.E., Friedman, S.L., Hallinger, K.K., Monroe, A.P. & White, A.E. (2008). The movement of aquatic mercury through terrestrial food webs. *SCIENCE,* 320. Access Jan, 2012, available http://www.sciencemag.org/content/320/5874/335.long

Crochet P.-A., Raty L., De Smet G., Anderson B., Barthel P.H., Collinson J.M., Dubois P.J., Helbig A.J., Jiguet F., Jirle E., Knox A.G., Le Maréchal P., Parkin D.T., Pons, J.-M., Roselaar C.S., Svensson L., van Loon A.J., Yésou P. (2010) *AERC TAC's taxonomic recommendations. (July 2010).* Access Mar, 2012, available at: http://www.aerc.eu/tac.html.

Custer, T.W.; Franson, J. C. & Pattee, O.H. (1984). Tissue lead distribution and hematologic effects in american kestrels (*Falco sparverius* L.) fed biologically incorporated lead. *Journal of Wildlife diseases,* 20(1), 39-43. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-20.1.39

Cuvin-Aralar, Ma.L.A. & Furness, R.W.(1991). Mercury and selenium interaction: a review. *Ecotoxicology and environmental safety,* 21(3), 348-364. Access Jan, 2012, available at http://www.ncbi.nlm.nih.gov/pubmed/1868791

Damkova, V., Paskova, V., Bandouchova, H., Hilscherova, K., Sedlackova, J., Novotny, L., Peckova, L., Vitula, F., Pohanka, M. & Pikula, J. (2011). Testicular toxicity of cyanobacterial biomass in Japanese quails. *Harmful algae*, 10(6), 612-618. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S156898831100045X

Daoust, P-Y. & Prescott, J.F.(2007) .Salmonellosis. In Thomas, N.J., Hunter, D. B. and Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 270-288)Blackwell Publishing

Daranas, A.H., Norte, M. & Fernández, J.J. (2001). Toxic marine microalgae review. *Toxicon*, 39, 1101-1132.

Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta tropica*, 78, 103-116. Access May, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0001706X00001790

Daury, R.W., Schwab, F.E. & Bateman, M.C. (1993). Blood lead concentrations of waterfowl from unhunted and heavily hunted marches of Nova Scotia and Prince Edward Island, Canada. *Journal of wildlife diseases,* 29, 577-581.

Davis, W.A. & McClung, L.S.(1940). Aspergillosis in wild Herring gulls. *Journal of bacteriology*, 321-323. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC374637/pdf/jbacter00744-0145.pdf

Davis, M.F. & Morishita, T.Y. (2001). *Poultry necropsy basics – extension factsheet.* Columbus:The Ohio State University Extension. Access Feb, 2012, available at: http://ohioline.osu.edu/vme-fact/0012.html

De Matos, R. (2008). Calcium metabolism in birds. *Veterinary Clinics: Exotic Animal Pratice* 11, 58-82. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1094919407000709

Debacker, V., Schiettecatte, L-S., Jauniaux, T. & Bouquegneau, J-M.(2001). Influence of age, sex and body condition on zinc, copper, cadmiun and metallothioneins in Common guillemots (*Uria aalge*) stranded at the Belgian coast. *Marine environmental research*, 52(2), 427-444. Acess Mar, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0141113601000964

Degernes, L.A. (1995). Toxicities in waterfowl. *Seminars in Avian and Exotic Pet medicine*, 4(1), 15-22. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055937X05800052

Degernes, L., Heilman, S., Trogdon, M., Jordan, M., Davison, M., Kraege, D., Correa, M. & Cowen, P.(2006).Epidemiologic investigation of lead poisoning in Trumpeter and Tundra Swan in Washington State, USA, 2000-2002. *Journal of wildlife diseases,* 42(2), 345-358. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-42.2.345

Degernes, L.A.(2008). Waterfowl toxicology: a review. *Vet clin Exot Anin*,11(2), 283-300. Access Mar, 2012, available at http://www.sciencedirect.com/science/article/pii/S1094919407000904

Delany, S. & Scott, D. (2006). Waterbird population estimates. *Wetlands International*, Wageningen, The Netherlands.

Diel, D.G., Miller, P.J., Wolf, P.C., Mickley, R.M., Musante, A.R., Emanueli, D.C., Shively, K.J., Pederson, K. & Afonso, C.L. (2012). Characterization of Newcastle disease viruses isolated from Cormorant and gull species in the United States in 2010. *Avian diseases,* 56, 128-133. Access Mar, 2013, available at: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=2120&context=icwdm_usdanwrc

Direcção geral da energia e geologia – Empresa de desenvolvimento mineiro [DGEG-EDM] (2011). *The legacy of abandoned mines – the context and action in Portugal.* Access Apr, 2013, available at: ec.europa.eu/environment/waste/mining/pdf/Appendix_III_to_Annex3.pdf

Direcção geral de energia e geologia [DGEG] (2013). *Minas com actividade produtiva declarada em 2010-2012.* Access Apr, 2013, available at: http://www.dgeg.pt/

DIVA-GIS. (2013). *Diva-gis.* Access Mar, 2013, available at: http://www.diva-gis.org/data/misc/countries_shp.zip

Docherty, D.E. & Friend, M. (1999) Newcastle disease. In Friend, M. Franson, J.C. *Field manual of wildlife diseases.* (p. 174-179). Washington DC.:U.S.Department of the Interior, U.S. Geological Survey

Dolezel, D., Koudela, B., Jirku, M., Hypsa, V., Oborník, M., Votýpka, J., Modrý, D., Slapeta, J.P. & Lukes, J. (1999). Phylogenetic analysis of *Sarcocystis* spp. of mammals and reptiles supports the coevolution of *Sarcocystis* spp. with their final hosts. *International Journal for Parasitology*, 29, 795-798. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0020751999000181

Duarte, E.L., Guerra, M.M. & Bernardo, F.M. (2002). *Salmonella* and *Listeria* spp. carriage by gulls (larids). *Revista portuguesa de Ciências Veterinárias*, 97(544), 181-187. Access Nov, 2011, available at: http://www.fmv.utl.pt/spcv/PDF/pdf12_2002/544_181_187.pdf

Dubey, J.P., Lindsay, D.S., Rezende, P.C.B. & Costa, A.J. (2000a). Characterization of an unidentified *Sarcocystis falcatula*- like parasite from the south American opossum, *Didelphis albiventris* from brazil.*J. Eukaryot Microbiology*, 47(6), 538-544. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/11128705

Dubey, J.P., Quist, C.F. & Fritz, D.L. (2000b). Systemic sarcocystosis in a wild turkey from Georgia. *Journal of wildlife diseases,* 36(4), 755-760. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-36.4.755

Dubey, J.P., Cawthorn, R.J., Speer, C.A. & Wobeser, G.A.(2003). Redescription of the *Sarcocysts* of *Sarcocystis rileyi* (apicomplexa: sarcocystidae). *J. Eukaryot microbial*, 50(6), 476-482. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/14733440

Dubey, J.P. & Morales, J.A.(2006). Morphologic characterization of *Sarcocystis sp. sarcocystis* from the Buffon´s macaw (*Ara ambigua*). *Acta parasitologica*, 51, 231-237. Access Mar, 2012, available at: http://link.springer.com/article/10.2478/s11686-006-0036-2

Duncan, R.M. & Jensen, W.I.(1976). A relationship between avian carcasses and living invertebrates in the epizootiology of avian botulism. *Journal of wildlife diseases*, 12, 116-126. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-12.1.116

Duncan, R.M., Stroud, R.K. & Locke, L.N. (1983). *Salmonella enteritidis* isolated from an Eared grebe (*Podiceps nigricollis*). *Journal of Wildlife Diseases*, 19(1), 63-64. Access Apr, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-19.1.63

Duszynski, D.W. & Box, E.D.(1978). The opossum (*Didelphis virginiana*) as a host for *Sarcocystis debonei* from Cowbirds (*Molothrus ater*) and Grackles (*Cassidix mexicanus*, *Quiscalus quiscula*). *J. Parasitology,* 64(2), 326-329. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/417165

Dwight-Jr., J. (1901). The sequence of moults and plumages of the laridae (gulls and terns). *The Auk*, 18(1), 49-63. Access Jun, 2012, available at: https://archive.org/details/jstor-4069675

Eagles-Smith, C.A., Ackerman, J.T., De La Cruz, S.E.W. & Takekawa, J. Y. (2009). Mercury bioaccumulation and risk to three waterbird foraging guilds is influenced by foraging ecology and breeding stage. *Environmental pollution*, 157, 1993-2002. Access Jan, 2012, available http://www.sciencedirect.com/science/article/pii/S0269749109001699

Eisler,R. (1980). Mercury hazards to fish, wildlife, and invertebrates: a synoptic review:biological report 85 April 1987 contaminant hazard reviews report n10. *U.S.Fish and wildlife service*, 10. Access Jan, 2012, available at: http://www.pwrc.usgs.gov/eisler/CHR_10_Mercury.pdf

Eisler, R. (1988). Lead hazards to fish, wildlife, and invertebrates: a synoptic review. *Contaminant hazard reviews report no 14 Biological report 85(1.14)* Access Feb, 2012, available at: http://www.pwrc.usgs.gov/eisler/CHR_14_Lead.pdf

Elliott, J.E, Langelier, K.M. & Wilson, L.K. (1996). Poisoning of bald eagles and Red-tailed hawks by carbofuran and fensulfothion in the Fraser delta of British Columbia, Canada. *Journal of Wildlife Diseases*, 32, 486-491. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-32.3.486

Equipa Atlas (2008) *Atlas das aves nidificantes em Portugal (1999-2005).* Lisboa:Instituto da Conservação da Natureza e da Biodiversidade/Assírio & Alvim

European Environment Agency (2013). *Corine land cover*. Access Mar, 2013, available at: http://www.eea.europa.eu/publications/COR0-landcover

Evelsizer, D.D., Clark, R.G. & Bollinger, T.K.(2010). Relationships between local carcass density and risk of mortality in molting mallards during avian botulism outbreaks. *Journal of Wildlife diseases*, 46(2), 507-513. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.2.507

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R. & Daigle, T. (2005). Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology*, 14(1-2), 193-221. Access Jan, 2012, available at: http://link.springer.com/article/10.1007%2Fs10646-004-6269-7

Falcon, M. (2004). Exotic Newcastle disease. *Seminars in Avian and Exotic Medicine*, 13(2), 79-85. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055937X04000052

Farley, J.M.; Romero, C.H.; Spalding, M.G.; Avery, M.L. & Forrester, D.J. (2001). Newcastle disease virus in Double-crested cormorants in Alabama, Florida and Mississippi. Journal of Wildlife Diseases, 37(4), 808-812.

Fenlon, D.R. (1981). Seagulls (*Larus* spp.) as vectors of *Salmonella*: an investigation into the range of serotypes and numbers of *Salmonella* in gull faeces. *J. Hyg. Camb.*, 86(2), 195-202. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2133874/pdf/jhyg00035-0057.pdf

Fenlon, D.R. (1983). A comparison of *Salmonella* serotypes found in the faeces of gulls feeding at sewage works with serotypes present in the sewage. *J. Hyg. Camb,* 91, 47-52. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129285/pdf/jhyg00019-0054.pdf

Ferns, P.N & Mudge, G.P. (2000). Abundance, diet and *Salmonella* contamination of gulls feeding at sewage outfalls. *Wat. Res*, 34(10), 2653-2660. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0043135499004273

Ferreyra, H., Romano, M. & Uhart, M. (2009). Recent and chronic exposure of wild ducks to lead in human-modified wetlands in Santa Fé Province, Argentina. *Journal of Wildlife Diseases,* 45(3), 823-827. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-45.3.823

Fisher, I.J., Pain, D.J. & Thomas, V.G. (2006). A review of lead poisoning from ammunition sources in terrestrial birds. *Biological conservation*, 131, 421-432. Access Jan, 2012, available at: http://www.ventanaws.org/pdf/species_condor_lead/LeadPoisoningStudyFisher_et_al2006.pdf

Fleischli, M.A., Franson, J.C., Thomas, N.J, Finley, D.L. & Riley.Jr, W.(2004). Avian mortality events in the United States caused by anticholisnesterase pesticides: a retrospective summary of national wildlife health center records from 1980 to 2000. *Environmental Contamination and Toxicology* 46, 542-550 Access Mar, 2012, available at http://www.ncbi.nlm.nih.gov/pubmed/15253053

Flickinger, E.L., Juenger, G., Roffe, T.J., Smith, M.R. & Irwin, R.J. (1991). Poisoning of Canada geese in Texas by parathion sprayed for control of Russian wheat aphid. *Journal of Wildlife Diseases*

27(2), 265-268. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-27.2.265

Forrester, D.J., Gaskin, J.M., White, F.H., Thompson, N.P., Quick Jr., J.A., Henderson, G.E., Woodard, J.C. & Robertson, W.D. (1977). An epizootic of waterfowl associated with a red tide episode in Florida. *Journal of wildlife diseases*, 13, 160-167. Access Dec, 2011, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-13.2.160

Forrester, D.J., Wenner, K.C., White, F.H., Greiner, E.C., Marion, W.R., Thul, J.E. & Berkhoff, G.A.(1980). An epizootic of avian botulism in a phosphate mine settling pond in Northern Florida. *Journal of Wildlife diseases*, 16(3), 323-327. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-16.3.323

Fowler, M. (2008). *Restraint and handling of wild and domestic animals.* (3rd ed). Iowa:Blackwell Publishing

Fowler, M.E. & Miller, R.E. (2008). *Zoo and wild animal medicine current therapy,* volume 6. St Louis Missouri: Saunders Elsevier

Franson, J.C.(1999). Mercury. In Friend, M. & Franson, J. C. *Field manual of wildlife diseases: general field procedures and diseases of birds.* (pp.337-340).Washington DC: U.S. Geological Survey

Franson, J. & Smith, M.R. (1999). Poisoning of wild birds from exposure to anticholinesterase compounds and lead: diagnostic methods and selected cases. *Seminars in Avian and Exotic Pet Medicine*, 8(1), 3-11. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055937X99800309

Franson, J.C., Petersen, M.R., Meteyer, C.U. & Smith, M.R. (1995).Lead poisoning of Spectacled eiders (*Somateria fischeri*) and of Common eiders (*Somateria mollissima*) in Alaska. *Journal of Wildlife diseases*, 31, 268-271. Access Feb, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-31.2.268

Frederick, P.C., Spalding, M.G. & Dusek, R. (2002). Wading birds as bioindicators of mercury contamination in florida, USA: annual and geographic variation. *Environmental toxicology and chemistry*, 21(1), 163-167. Access Jul, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/11804050

Frederick, P & Jayasena, N. (2010). Altered pairing behaviour and reproductive success in White ibises exposed to environmentally relevant concentrations of methylmercury. *Proceedings of the royal society,* 278, 1851-1857. Access Jan, 2012, available http://rspb.royalsocietypublishing.org/content/early/2010/11/24/rspb.2010.2189.full.pdf+html

Friend, M. & Trainer, D.O.(1969). Aspergillosis in captive herring gulls. *Bull.Wildlife diseases assoc*, 5(3), 271-275. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-5.3.271

Friend, M. (1999a). Aspergillosis. In In Friend, M. Franson, J.C. *Field manual of wildlife diseases.* (pp. 129-133). Washington DC.:U.S.Department of the Interior, U.S. Geological Survey

Friend, M. (1999b). Lead. In Friend, M. & Franson, J. C. *Field manual of wildlife diseases: general field procedures and diseases of birds*. (pp.317-334). Washington DC: U.S. Geological Survey

Friend, M. (1999c). Salmonellosis. In Friend, M. & Franson, J.C. *Field manual of wildlife diseases: general field procedures and diseases of birds*. (pp.99-109)   Washington DC: U.S.department of the interior U.S. Geological survey

Friend, M. & Franson, J. C. (1999). *Field manual of wildlife diseases: general field procedures and diseases of birds*. Washington DC: U.S. Geological Survey

Friend, M., Franson, J.C. & Anderson, W.L.(2009). Biological and societal dimensions of lead poisoning in birds in the USA. In Watson, R.T.; Fuller, M.; Pokras,M. & Hunt, W.G. (eds.) *Ingestion of lead from spent ammunition: Implications for wildlife and humans.* The Peregrine Fund, Boise, Idaho, USA. Access Oct, 2012, available at: http://www.peregrinefund.org/subsites/conference-lead/PDF/0104%20Friend.pdf

Gaffuri, A. & Holmes, J.P. (2012). *Salmonella* infections. In Widen-Gavier, D.; Duff, J.P. and Meredith, A. (2012). *Infectious diseases of wild mammals and birds in Europe.* (pp. 386-397) Blackwell publishing

Gangoso, L., Álvarez-Lloret, P., Rodríguez-Navarro, A.A.B., Mateo, R., Hiraldo, F. & Donázar, J.A. (2009). Long term effects of lead poisoning on bone mineralization in Vultures exposed to ammunition sources. *Environmental Pollution*, 157(2), 569-574. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0269749108004478

Garcia, M.E., Lanzarot, P., Rodas, V.L., Costas, E. & Blanco, J.L. (2007). Fungal flora in the trachea of birds from a wildlife rehabilitation centre in Spain. *Veterinarni Medicina,* 52, 464-470. Access Apr, 2012, available at: http://vri.cz/docs/vetmed/52-10-464.pdf

Garland, P.W. & Pritchard, S. (2009). *Other diseases, Poisons and Toxins: Nutritional disorders.*Elsevier

Getchell, R.G. & Bowser, P.R.(2006). Ecology of type E botulism within Dreissenid mussel beds. *The digest of Aquatic invaders. National aquatic nuisance species clearinghouse*, 17(2), 1-8. Access Dec, 2011, available at: http://www.glrc.us/documents/botulism/appendixD/ecology-mussel.pdf

Glaser, L.C. (1999) Organophosphorus and carbamate pesticides. In Friend, M. & Franson, J. C. (ed.). *Field manual of wildlife diseases: general field procedures and diseases of birds*.(pp.287-293) Washington DC: U.S. Geological Survey

Glaser, L.C., Barker, I.K., Weseloh, D.V.C., Ludwig, J., Windingstad, R.M., Key, D.W. & Bollinger, T.K. (1999). The 1992 epizootic of newcastle disease in Double-crested cormorants in North America. *Journal of wildlife disease*, 35(2), 319-330. Access Apr, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.2.319

Glibert, P.M., Anderson, D.M., Gentien, P., Granéli, E. & Sellner, K.G.(2005). Harmful algal blooms: The global complex phenomena of harmful algal blooms. *Oceanography,* 18(2), 136-147. Access Nov, 2011, available at: http://www.tos.org/oceanography/archive/18-2_glibert2.pdf

Gochfeld, M. & Burger, J. (1998). Apparent paralytic shellfish poisoning in captive herring gulls fed commercial scallops. *Toxicon,* 36(2), 411-415. Access Nov, 2011, available at: http://www.sciencedirect.com/science/article/pii/S0041010197001062

Goldstein, T., Zabka, T.S., DeLong, R.L., Wheeler, E.A., Ylitalo, G., Bargu, S., Silver, M., Leighfield, T., Van Dolah, F., Langlois, G., Sidor, I., Dunn, J.L. & Gulland, F.M.D.(2009). The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. *Journal of Wildlife Diseases*, 45(1), 91-108. Access Dec, 2011, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-45.1.91

Goodale, M.W., Evers, D.C., Mierzykowski, S.E., Bond, A.L., Burgess, N.M., Otorowski, C.I., Welch, L.J., Hall, C.S., Ellis, J.C., Allen, R.B., Diamond, A.W., Kress, S.W. & Taylor, R.J. (2008). Marine foraging birds as bioindicators of mercury in the gulf of Maine. *Ecohealth,* 5(4), 409-425. Access Jan, 2012, available at: http://link.springer.com/article/10.1007%2Fs10393-009-0211-7

Gordus, A.G. (1993). Lead concentrations in liver and kidneys of Snow geese during and avian cholera epizootic in California. *Journal of wildlife diseases,* 29, 582 – 586.

Goutner, V. & Furness, R.W.(1997). Mercury in feathers of Little egret *Egretta garzetta* and Night heron *Nycticorax nycticorax* chicks and in their prey in the Axios Delta, Greece. Archives *of environmental contamination and toxicology*, 32(2), 211-216. Access Mar, 2012, available at: http://link.springer.com/article/10.1007%2Fs002449900177

Greiner, E.C. (2007). Isospora, Atoxoplasma and Sarcocystis. In Thomas, N.J.; Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.(*pp. 108-119).Iowa: Blackwell Publishing

Gridwood, W.A., Fricker, C.R.; Munro, D.; Shedden, C.B. & Monaghan, P. (1985). The incidence and significance of *Salmonella* carriage by gulls (*Larus* spp.) in Scotland. *J.HYG. Comb*, 95(2), 229-241. Access May, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129538/pdf/jhyg00008-0020.pdf

Gries, C.L. & Scott, M.L. (1972). The pathology of thiamin, riboflavin, pantothenic acid and niacin deficiencies in the chick. *The Journal of Nutrition* 102(10), 1269-1286. Access Jan, 2012, available at: http://jn.nutrition.org/content/102/10/1269.full.pdf

Grue, C.E., Gibert, P.L. & Seeley, M.E. (1997). Neurophysiological and behavioral changes in non-target wildlife exposed to organophosphates and carbamate pesticides: thermoregulation, food consumption, and reproduction. *Amer.Zool*, 37, 369-388. Access Mar, 2012, available at: http://icb.oxfordjournals.org/content/37/4/369.full.pdf

Hall, A.J. & Saito, E.K. (2008). Avian wildlife mortality events due to salmonellosis in the United States, 1985-2004. *Journal of Wildlife Diseases*, 44(3), 585-593. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-44.3.585

Hallgrimsson, G.T., Van Swelm, N.D., Gunnarsson, H.V., Johnson, T.B. & Rutt, C.L.(2011).First two records of European-banded Lesser black-backed gulls, *Larus fuscus* in America. *Marine Ornithology*, 39,137-139. Access Apr, 2013, available at: http://www.marineornithology.org/PDF/39_1/39_1_137-139.pdf

Hamilton, S.J., Buhl, K.J. & Lamothe, P.J. (2002). Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern idaho near phosphate mining operations: September 2000. Columbia Environmental Research Center. SGS Western U.S. Phosphate Project. Acess Nov, 2012, available at: http://www.cerc.usgs.gov/Assets/UploadedFiles/ExternalDocs/91270.pdf

Hannett, G.E., Stone, W.B., Davis, S.W. & Wroblewski, D. (2011). Biodiversity of *Clostridium botulinum* type E associated with a large outbreak of botulism in wildlife from Lake Erie and Lake Ontario. *Appl. Environ.Microbiol,* 77(3), 1061-1068. Access Dec, 2011, available at http://aem.asm.org/content/77/3/1061.full.pdf+html

Harcourt-Brown, N.H. (2002). Orthopaedic conditions that affect the avian pelvic limb. *Veterinary clinics of North America : exotic animal practice*, 5(1), 49-81.

Harrison, G.J. & Ritchie, B.W. (1994). Making distinctions in the physical examination. In Ritchie, B.W., Harrison, G.J.& Harrison, L.R. (ed.). *Avian Medicine: principles and application.* (pp.144-175).Lake Worth Florida: Wingers Publishing

Harrison, G.J. & Lightfoot, T.L (2006). *Clinical avian medicine volume I e II*. Palm Beach, Florida: Spix Publishing Inc

Heinz, G.H. & Locke, L.N.(1976). Brain lesions in mallard ducklings from parents fed methylmercury. *Avian Dis*, 20(1), 9-17. Access Jun, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/1259664

Heinz, G.H. (1979) Methylmercury: reproductive and behavioural effects on three generations of Mallard ducks. *J. wild.Manage*, 43(2), 394-400. Access Jan, 2013, available at: http://www.jstor.org/discover/10.2307/3800348?uid=3738880&uid=2&uid=4&sid=2110305014183 1

Helander, B., Axelsson, J., Borg, H., Holm, K. & Bignert, A. (2009). Ingestion of lead from ammunition and lead concentration in White-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. *Science of the total environment*, 407(21), 5555-5563. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0048969709007049

Henny, C.J., Kolbe, E.J., Hill, E.F. & Blus, L.J. (1987).Case histories of Bald eagles and other raptors killed by organphosphorus insecticides topically applied to livestock. *Journal of Wildlife Diseases* 23(2), 292-295. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-23.2.292

Hifner, J.M., Hobson, K.A. & Elliott, J.E. (2011). Ecological factors differentially affect mercury levels in two species of sympatric marine birds of the North Pacific. *Science of total environment,* 409, 1328-1335. Access, May 2013, available at http://www.sciencedirect.com/science/article/pii/S0048969710013537

Hilbert, F., Smulders, F.J.M., Chopra-Dwasthaly, R. and Paulsen, P. (2012). *Salmonella* in the wildlife-human interface. *Food Research International*, 45(2), 603-608. Access Mar, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0963996911005096

Honour, S. M., Trudeau, S., Kennedy, S. & Wobeser, G. (1995a). Experimental vitamin A deficiency in mallards (*Anas platyrhynchos*): lesions and tissues vitamin A levels. *Journal of Wildlife Diseases* 31(3), 277-288. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-31.3.277

Honour, S., Kennedy, S., Trudeau, S. & Wobeser, G. (1995b). Vitamin A status of wild mallards (*Anas platyrhynchos*) wintering in saskatchewan. *Journal of Wildlife Diseases*, 31(3), 289-298. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-31.3.289

Hoppe, D.M.(1976). Prevalence of macroscopically detectable *Sarcocystis* in North Dakota ducks. *Journal of wildlife diseases,* 12(1), 27-29. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-12.1.27

Hubálek, Z., Skorpikova, V. & Horal, D.(2005). Avian botulism at a sugar beet processing plant in south moravia (czech republic).*Vet.Med.- Czech*, 50 (10), 443-445. Access Dec, 2011, available at: http://www.vri.cz/docs/vetmed/50-10-443.pdf

Hubálek, Z. (2004). An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of wildlife diseases*, 40(4), 639-659. Access Nov, 2013, available at: http://www.jwildlifedis.org/doi/abs/10.7589/0090-3558-40.4.639

Hunt, K.A., Hooper, M.J. & Littrell, E. (1995).Carbofuran poisoning in Herons: diagnosis using cholinesterase reactivation techniques. *Journal of Wildlife Diseases,* 31, 186-192. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-31.2.186

Iason, G.R., Duck, C.D. & Clutton-Brock, T:H. (1986). Grazing and reproductive success of Red deer: the effect of local enrichment by gull colonies. *Journal of Animal Ecology,* 55(2), 507-515. Access Apr, 2012, available at: http://www.jstor.org/discover/10.2307/4734?uid=3738880&uid=2&uid=4&sid=21103043824561

ICNF (2013) *Base de dados das zonas de caça.* Access Jan, 2013, available at: http://www.icnf.pt/portal/caca/zc/zonas-de-caca-em-actividade

ICNF (2013). *Natureza e areas classificadas.* Access Jan, 2013, available at: http://www.icnf.pt/portal/naturaclas

Instituto Nacional de Estatística [INE], I.P. (2010a). *Statistical yearbook of algarve region 2009.* Lisboa: Instituto Nacional de Estatística, I.P. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=102941307&PUBLICACOESmodo=2

INE, I.P. (2010b) *Estatísticas da pesca 2009.* Lisboa: Instituto Nacional de Estatística, I.P. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=89988890&PUBLICACOEStema=00&PUBLICACOESmodo=2

INE, I.P.(2010c) *Estatisticas demográficas 2009*. Lisboa: Instituto National de Estatística. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=89988890&PUBLICACOEStema=00&PUBLICACOESmodo=2

INE,I.P. (2011a).*Statistical yearbook of algarve region 2010*. Lisboa: Instituto Nacional de Estatística, I.P. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=130331854&PUBLICACOESmodo=2

INE, I.P. (2011b) Recenseamento agrícola 2009- análise dos principais resultados.Lisboa: Instituto Nacional de Estatística. Access Dec, 2012, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=119564579&PUBLICACOESmodo=2

INE, I.P.(2011c). *Indústria e energia em Portugal 2008-2009.* Lisboa: Instituto Nacional de Estatística. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=120933113&PUBLICACOESmodo=2

INE,I.P.(2012).*Statistical yearbook of algarve region 2011*. Lisboa: Instituto Nacional de Estatística, I.P. Access Jan, 2013, available at: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=150034457&PUBLICACOEStema=00&PUBLICACOESmodo=2

Insituto Português do Mar e Atmosfera [IPMA] (2011). Bivalves e biotoxinas. Access Nov, 2011, available at: https://www.ipma.pt/pt/pescas/bivalves/index.jsp

Instituto do Mar e da Atmosfera [IPMA]. (2012).Normais climatológicas. Access May, 2013, available at: https://www.ipma.pt/pt/oclima/normais.clima/

Irwin, J.C. (1972). The toxicity for ducks of disintegrated lead shot in a simulated-marsh environment. *Journal of wildlife diseases*, 8, 149-154. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-8.2.149

Jagoe, C.H., Bryan Jr., A.L., Brant, H.A., Murphy, T.M. & Brisbin Jr., I.L.(2002). Mercury in Bald eagle nestlings from South Carolina USA. *Journal of wildlife diseases,* 38(4), 706-712. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-38.4.706

Jakson, A.K., Evers, D.C., Folsom, S.B., Condon, A.M., Diener, J., Goodrick, L.F., McGann, A.J., Schmerfeld, J. & Cristol, D.A.(2011). Mercury exposure in terrestrial birds far downstream of an historical point source. *Environmental pollution*, 159, 3302-3308. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0269749111004799

Jansson, D.S. (2012) Aspergillosis. In Widen-Gavier, D.; Duff, J.P. and Meredith, A. *Infectious diseases of wild mammals and birds in Europe*. (pp. 455-461) Blackwell publishing

Jara, J., Alfrey, P., Costa, H., Matias R., Moore, C.C., Santos, J.L. & Tipper R. (2010) Aves de ocorrencia rara ou acidental em Portugal - relatório do comité português de raridades referente aos anos de 2008 e 2009. *Anuário ornitológico*, 7, 3-71. Access Nov, 2012, available at: http://www.spea.pt/fotos/editor2/spea_anuario_ornitologico_7_relatorio_cpr_p3_71.pdf

Jayasena, N., Frederick, P.C. & Larkin, I.L.V. (2011). Endocrine disruption in White ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury. *Aquatic toxicology*, 105(3-4), 321-327. Access Jan, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0166445X11001950

Jessup, D.A., Miller, M.A., Ryan, J.P., Nevins, H.M., Kerkering, H.A., Mekebri, A., Crane, D.B., Johnson, T.A. & Kudela, R.M.(2009). Mass stranding of marine birds caused by a surfactant-producing red tide. *PloSONE* 4(2). Access Dec, 2011, available at http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0004550

Johnson, W.D. & Storts, R.W. (1988). Peripheral neuropathy associated with dietary riboflavin deficiency in the chicken: light microscopic study. *Veterinary Pathology* 25(1), 9-16. Access Jan, 2012, available at: http://vet.sagepub.com/content/25/1/9.long

Jones, M.P. & Orosz, S.E. (1996). Overview of avian neurology and neurological diseases. *Seminars in Avian and Exotic Pet Medicine,* 5(3), 150-164. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055937X96800041

Jorge, P.E., Sowter, D. & Marques, P.A.M. (2011). Differential annual movement patterns in a migratory species: effects of experience and sexual maturation. *PLoS ONE,* 6(7):e22433.Access May, 2012, available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0022433

Joseph, V.(2000). Aspergillosis in raptors. *Seminars in avian and exotic pet medicine*, 9(2), 66-74. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055937X00800043

Julian, R.J. (2005). Production and growth related disorders and other metabolic diseases of poultry – a review. *The Veterinary Journal* 169(3), 350-369. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1090023304001121

Jung, K., Kim, Y., Lee, H. & Kim, J-T. (2009). *Aspergillus fumigatus* infection in two wild Eurasian black vultures (*Aegypius monachus* Linnaeus) with carbofuran insecticide poisoning: A case report. *The veterinary journal,* 179, 307-312. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1090023307003218

Kalas ,J.A., Steinnes, E. & Lierhagen, S. (2000). Lead exposure of small herbivorous vertebrate from atmospheric pollution. *Environmental Pollution*, 107(1), 21-29. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0269749199001554

Kaleta, E.F. & Docherty, D.E. (2007) Avian herpesviruses In Thomas, N.C., Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 63-86). Blackwell Publishing

Kaleta, E.F.(2012). Avian paramyxovirus infections In Widen-Gavier, D.; Duff, J.P. and Meredith, A. *Infectious diseases of wild mammals and birds in Europe*. (pp. 59-66) Blackwell publishing

Kapperud, G. & Rosef, O. (1983). Avian wildlife reservoir of *Campylobacter fetus* subsp.*jejuni*, *Yersinia spp.*, and *Salmonella* spp. in Norway. *Applied and Environmental Microbiology,* 45(2), 375-380. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC242295/pdf/aem00171-0033.pdf

Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Holt, R.D., Hudson, P., Jolles, A., Jones, K.E., Mitchell, C.E., Myers, S.S., Bogich, T. & Ostfeld, R.S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature,* 468, 647-652. Access May, 2013, available at: http://www.nature.com/nature/journal/v468/n7324/full/nature09575.html

Kendall, R.J., Brewer, L.W. & Hitchcock, R.R. (1993).Response of Canada geese to a turf application of diazinon AG500. *Journal of Wildlife Diseases,* 29(3), 458-464. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-29.3.458

Kilpi, M. & Ost, M. (1998). Reduced availability of refuse and breeding output in a herring gull (*Larus argentatus*) colony. *Ann. Zool. Fennici*, 35, 37-62. Access Dec, 2012, available at: http://www.sekj.org/PDF/anzf35/anzf35-037p.pdf

Kim, E.Y., Saeki, K., Tanabe, S., Tanaka, H. & Tatsukawa, R.(1996). Specific accumulation of mercury and selenium in seabirds. *Environmental Pollution,* 94(3), 261-265. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0269749196001108

Kim, L.M., King, D.J. , Curry, P.E., Suarez, D.L., Swayne, D.E., Stallkneht, D.E., Slemons, R.D., Pederson, J.C. & Senne, D.A. (2007). Phylogenetic diversity among low-virulence Newcastle disease viruses from waterfowl and shorebirds and comparison of genotype distributions to those of poultry-origin isolates. *Journal of Virology*, 81(22), 12641-12653. Access Mar, 2012, available at: http://jvi.asm.org/content/81/22/12641.long

Kirkpatrick, C.E. (1986). Isolation of *Salmonella* spp. from a colony of wading birds. *Journal of wildlife diseases,* 22(2), 262-264. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-22.2.262

Klasing, K.C. (2008). Nutriotional diseases. In Saif, Y.M. (eds). *Diseases of Poultry* (12ed)(pp.1121-1149) Blackwell Publishing

Kobayashi, Y., Shimada, A., Umemura, T. & Nagai, T.(1992). An outbreak of copper poisoning in Mute swans (*Cygnus olor*). *J VET MED SCI*, 54(2), 229-33. Access Apr, 2013, available at: http://www.ncbi.nlm.nih.gov/pubmed/1606252

Kojadinovic, J., Bustamante, P., Churlaud, C., Cosson, R.P. & Le Corre, M. (2007). Mercury in seabird feather: insight on dietary habits and evidence for exposure levels in the western Indian Ocean. *Science of total environment,* 384(1-3), 194-204. Access Jan, 2012, available at: http://archimer.ifremer.fr/doc/2007/publication-3416.pdf

Koster, M.D., Ryckman, D.P., Weseloh, D.V.C. & Struger, J. (1996). Mercury levels in Great lakes herring gull (*Larus argentatus*) eggs, 1972-1992. *Environmental Pollution,* 93(3), 261-270. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0269749196000437

Kreuder, C., Mazet, J.A.K., Bossart, G.D., Carpenter, T.E., Holyoak, M., Elie, M.S. & Wright, S.D. (2002). Clinicopathologic features of suspected brevetoxicosis in Double-crested cormorants (*Phalacrocorax auritus*) along the Florida Gulf coast. *Journal of Zoo and Wildlife Medicine,* 33(1), 8-15. Access Nov, 2011, available at: http://www.ncbi.nlm.nih.gov/pubmed/12216798

Kuiken, T., Leighton, F.A., Wobeser, G., Danesik, K.L., Riva, J. & Heckert, R. A. (1998). An epidemic of Newcastle disease in Double-crested cormorants from Saskatchewan. *Journal of wildlife diseases*, 34(3), 457-471. Access Apr, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-34.3.457

Kuiken, T., Wobeser, G., Leighton, F. A., Haines, D.M., Chelack, B., Bogdan, J., Hassard, L., Heckert, R.A. & Riva, J. (1999). Pathology of Newcastle disease in Double-crested cormorants from Saskatchewan, with comparison of diagnostic methods. *Journal of wildlife diseases*, 35(1), 8-23. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.1.8

Kvitek, R.G.(1991). Sequestered paralytic shellfish poisoning toxins mediate Glaucous-winged gull predation on bivalve prey. *The Auk,* 108(2), 381-392. Access Nov, 2011, available at: http://www.jstor.org/discover/10.2307/4087964?uid=3738880&uid=2&uid=4&sid=21103050141831

Kvitek, R. & Bretz, C. (2005). Shorebird foraging behaviour, diet, and abundance vary with harmful algal bloom toxin concentrations in invertebrate prey. *Marine ecology progress series,* 293, 303-309. Access Nov, 2011, available at: http://www.int-res.com/articles/meps2005/293/m293p303.pdf

Kwon, Y-K., Wee, S-H. & Kim, J-H. (2004).Pesticide poisoning in wild birds in korea from 1998 to 2002. short communications .*Journal of Wildlife Diseases*, 40(4), 737-740. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-40.4.737

Lagerquist, J.E., Davison, M. & Foreyt, W.J. (1994). Lead poisoning and other causes of mortality Trumpeter (*Cygnus buccinator*) and Tundra swans (*C. columbianus*) in western Washington. *Journal of wildlife diseases*, 30(1), 60-64. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-30.1.60

Lambert, K.F., Evers, D.C., Warner, K.A., King, S.L. & Selin, N.E. (2012). Integrating mercury science and policy in the marine context: challenges and opportunities. *Environmental research*, 119, 132-142. Access Jan, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0013935112001855

Landsberg, J. H., Vargo, G.A., Flewelling, L.J. & Wiley, F.E.(2007). Algal Biotoxins. In Thomas, N.J.; Hunter, D.B.& Atkinson, C.T.(2007). *Infectious diseases of wild birds*. (pp.431-456) Iowa: Blackwell Publishing

Landsberg, J.H., Flewelling, L.J. & Naar, J.(2009). *Karenia brevis* red tides, brevetoxins in the food web, and impacts on natural resources: decadal advancements. *Harmful algae,* 8(4), 598-607. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1568988308001571

Latif, B., Vellayan, S., Omar, E., Abdullah, S. & Desa, N.M. (2010). *Sarcocystis* among wild captive and zoo animals in Malaysia. *Korean J. Parasitol*, 48(3), 213-217. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2945795/pdf/kjp-48-213.pdf

Lawson, B., MacDonald, S., Howard, T., Macgregor, S.K. & Cunningham, A.A.(2006). Exposure of garden birds to aflatoxins in Britain. *Science of the Total Environment*, 361(1-3), 124-131. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0048969705007163

Lefebvre, K.A., Noren, D.P., Schultz, I.R., Bogard, S.M., Wilson, J. & Eberhart, B-T. L. (2007). Uptake, tissue distribution and excretion of domoic acid after oral exposure in Coho salmon (*Oncorhynchus kisutch*). *Aquatic toxicology,* 81(3), 266-274. Access Oct, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0166445X06004450

Lehel, J., Laczay, P., Déri, J., Darin, E.G. & Budai, P. (2010). Model study on the clinical signs and residue concentrations of sublethal cabofuran poisoning in birds. *Journal of wildlife diseases* 46(4), 1274-1278. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.4.1274

Leighton, F.A. & Heckert, R.A. (2007). Newcastle disease and related avian paramyxoviruses. In Thomas, N.C., Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 3-16). Blackwell Publishing

Leitão, A.H. & Cidraes-Vieira, N.(2011). Noticiário ornitológico, 2009-2010. *Anuário ornitológico*, 8, 53-93.

Lemly, A.D. (2004). Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicology and Environmental Safety*, 59(1), 44–56. Access Jan, 2012, available at: http://linkinghub.elsevier.com/retrieve/pii/S0147651303000952

Lévesque, B., Brousseau, P., Simard, P., Dewailly, E., Meisels, M., Ramsay, D. & Joly, J. (1993). Impact of the Ring-billed gull (*Larus delawarensis*) on the microbiological quality of recreational water. *Applied and Environmental Microbiology*, 59(4), 1228-1230. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC202265/pdf/aem00033-0298.pdf

Lewis, L. & Schweitzer, S.H.(2000). Lead poisoning in a Northern bobwhite in Georgia. *Journal of Wildlife Diseases*, 36, 180-183. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-36.1.180

Lindberg, A., Skarin, H., Knutsson, R., Blomqvist, G. & Baverud, V.(2010). Real-time PCR for *Clostridium botulinum* type C neurotoxin (BoNTC) gene, also covering a chimeric C/D sequence – application on outbreaks of botulism in poultry. *Veterinary Microbiology,* 146, 118-123. Access Dec, 2011, available at: http://www.sciencedirect.com/science/article/pii/S0378113510002312

Lindh, E., Ek-Kommonen, C., Vaananen, V-M., Alasaari, J., Vaheri, A., Vapalahti, O. & Huovilainen, A.(2012). Molecular epidemiology of outbreak-associated and wild-waterfowl-derived Newcastle disease virus strains in Finland, including novel class I genotype. *Journal of clinical microbiology* ,50(1), 3664-3673. Access Oct, 2012, available at: http://jcm.asm.org/content/50/11/3664.long

Lindsay, R.C. & Dimmick, R.W.(1983).Mercury residues in Wood ducks and Wood ducks foods in Eastern Tennese. *Journal of Wildlife diseases,* 19(2), 114-117. Access, Apr 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-19.2.114

Litaker, R.W.; Vandersea, M.W.; Faust, M.A.; Kibler, S.R.; Nau, A.W.; Holland, W.C.; Chinain, M.; Holmes, M.J. & Tester, P.A. (2010). Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus. Toxicon* 56(5), 711-730. Access Nov, 2011, available at: http://www.sciencedirect.com/science/article/pii/S004101011000214X

Literák, I., Cizek, A. & Honza, M. (1992). Using examinations of young Black-headed gulls (*Larus ridibundus*) for the detection of *Salmonella* in the environment. *Acta vet Brno*, 61, 141-146. Access Apr, 2012, available at: http://actavet.vfu.cz/pdf/199261020141.pdf

Locke, L.N., Bagley, G.E. & Young, L.T. (1967). The ineffectiveness of acid-fast inclusions in diagnosis of lead poisoning in Canada geese. *Bull. Wildlife Diseases Association*, 3(4), 176. Access Jan, 2012, available at: http://pubs.er.usgs.gov/publication/5221274

Locke, L.N. & Friend, M. N.(1989). 13.2.4 Avian Botulism: geographic expansion of a historic disease. In Waterfowl management handbook. US Fish & Wildlife Service. *Waterfowl Management Handbook*. University of Nebraska, Lincoln. Access Feb, 2012, available at: http://www.nwrc.usgs.gov/wdb/pub/wmh/13_2_4.pdf

Lowenstine, L.J. (2008) Algal bloom toxicity in marine animals. In Miller, R.E.& Fowler, M.E. (eds). *Fowler´s zoo and wild animal medicine current therapy.* (pp.341-348) St. Louis Missouri: Elsevier Saunders

Magalhães, N.S.; Gonçalves, A.I.R.; Afonso-Roque, M.M.; Madeira de Carvalho, L.M.(1998). Contribution for the study of the hemilthofauna of birds of prey from rehabilitation centres in Portugal. [Communication]. In *4th annual reunion of the Portuguese society of Parasitology*, UTAD, Vila Real, November 1998

March, G.L., John, T.M., McKeown, B.A., Sileo, L. & George, J.C. (1976). The effects of lead poisoning on various plasma constituents in the Canada Goose. *Journal of wildlife diseases,* 12(1), 14- 19. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-12.1.14

Marques, P.A.M., Costa, A.M., Rock, P. & Jorge, P.E. (2009). Age-related migration patterns in *Larus fuscus* spp. *Acta ethologica,* 12(2), 87-92. Access Apr, 2012, available at: http://link.springer.com/article/10.1007%2Fs10211-009-0060-y

Martinez, R. & Wobeser, G. (1999). Immunization of ducks for type C botulism. *Journal of Wildlife Diseases*, 35(4), 710-715. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.4.710

Martínez-Haro, M., Vinuela, J. & Mateo, R. (2007a). Exposure of birds to cholinesterase-inhibiting pesticides following a forest application for tick control. *Environmental Toxicology and Pharmacology* 23(3), 347-349. Access Mar, 2012, available at http://www.sciencedirect.com/science/article/pii/S1382668906001761

Martínez-Haro, M.; Mateo, R.; Guitart, R.; Soler-Rodríguez, F.; Pérez-López, M.; María-Mojica, P. García-Fernández, A.J. et al. (2007b). Relationship of the toxicity of pesticides formulations and their commercial restrictions with the frequency of animal poisonings. *Ecotoxicology and Environmental Safety* 69(3), 396-402. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0147651307001017

Marvin, C., Painter, S. & Rossmann, R. (2004). Spatial and temporal patterns in mercury contamination in sediments of the Laurentian Great Lakes. *Environmental Research,* 95, 351-362. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S001393510300210X

Massango, M.G., Myburgh, J.G., Labuschagne, L., Govender, D., Bengis, R.G. & Naicker, D. (2010). Assessment of *Microcystis* bloom toxicity associated with wildlife mortality in the Kruger National Park South Africa. *Journal of Wildlife Diseases*, 46(1), 95-102. Access Dec, 2011, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.1.95

Mateo, R., Dolz, J.C., Aguillar Serrano, J.M., Bellirure, J. & Guitart, R. (1997). An epizootic of lead poisoning in Greater flamingos (*Phoenicopterus ruber roseus*) in Spain. *Journal of Wildlife diseases,* 33(1), 131-134. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-33.1.131

Mateo, R. (2009). Lead poisoning in wild birds in Europe and regulations adopted by different countries. In Watson, R.T.; Fuller, M.; Pokras, M. &Hunt, W.G. (eds). *Ingestion of lead from spent ammunition: Implications for wildlife and humans*. Boise:The Peregrine Fund. Access Oct, 2012, available at: http://www.peregrinefund.org/subsites/conference-lead/PDF/0107%20Mateo.pdf

McLelland, J.M., Reid, C., McInnes, K., Roe, W.D. & Gartrell, B.D.(2010). Evidence of lead exposure in a free-ranging population of Kea (*Nestor notabilis*). *Journal of Wildlife Diseases*, 46(2), 532-540. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.2.532

Mereb, G., Rosetti, F., Castelli, E., Ganuza, R., Ottavioni, L., Cavallero, D. & Calderón, A.(1999). Description of a botulism type C outbreak among waterbirds in the providence of la Pampa, Argentina. *Anaerobe,* 5, 191-193. Access Dec, 2011, available at: http://pubget.com/paper/pgtmp_540a9afcca3f8db1ea82011880a99e95/description-of-a-botulism-type-c-outbreak-among-waterbirds-in-the-province-of-la-pampa-argentina

Michigan Sea Grant College Program (2007). Type E Botulism confirmed in waterfowl deaths. *Sea Grant Michigan*, Nov, 2007.

Mihaylov, G., Petrov, V., Marutsov, P., Simeonov, R. & Simeonova, G.(2008).A case of aspergillosis in a Bearded vulture. *Trakia Journal of Sciences*, 6(1), 144-146. Access Mar, 2012, available at: http://tru.uni-sz.bg/tsj/Vol6N01_2008_supplement/G.Mihaylov2.pdf

Mikaelian, I., Daaignault, D., Duval, M-C. & Martineau, D. (1997). *Salmonella* infection in wild birds from Quebec. *Canadian Veterinary Journal,* 38(6), 385. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1576894/pdf/canvetj00091-0067a.pdf

Miller, P.L., Kim, L.M., Ip, H.S. & Afonso, C.L. (2009). Evolutionary dynamics of Newcastle disease virus. *Virology,* 391(1), 64-72. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0042682209003237

Miller, R.E.& Fowler, M.E. (2012). *Fowler´s zoo and wild animal medicine current therapy.* St. Louis Missouri: Elsevier Saunders

Mineau, P. and Tucker, K.R.(2002). Improving detection of pesticide poisoning in birds. *J. Wildlife Rehab*, 25(2), 4-13. Access Jun, 2012, available at: http://nctc.fws.gov/csp/resources/pesticides/Terrestrial%20Effects/ID%20Pesticide%20Incidents%20Part%20I.pdf

Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento do Território-Direcção-Geral de Alimentação e Veterinária (2013). *Guia dos produtos fitofarmacêuticos:lista dos produtos com venda autorizada*. Lisboa:Direcção-Geral de Alimentação e Veterinária. Access Apr, 2013, available at: http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=4046540&cboui=4046540

Mitchell, M.A. & Tully-Jr., T.N. (2009). *Manual of exotic pet practice.*St. Louis Missouri: Saunders Elsevier

Mohapatra, S.P., Nikolova, I. & Mitchell, A. (2007). Managing mercury in the Great lakes: an analytical review of abatement policies. *Journal of Environmental Management,* 83(1), 80-92. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0301479706000648

Moita, M.T.; Palma, A.S. & Vilarinho, M.da G.(2005).Blooms de fitoplâncton na costa portuguesa. *IPIMAR divulgação*, 31, 1-4. Access Mar, 2012, available at: http://www.cienciaviva.pt/img/upload/BLOOMS%20DE%20FITOPL%C3%82NCTON%20NA%20COSTA%20PORTUGUESA.pdf

Molina, B. (2009). Gaviota reidor, sombría y patiamarilla en España. Población en 2007-2009 y método de censo. *SEO/BirdLife*Madrid Access May, 2013, available at: http://www.seo.org/wp-content/uploads/2012/04/27_gaviotascomunes.pdf

Molnar, J.J.(1983). Copper storage in the liver of the Wild mute swan (*Cygnus olor*). Its possible relation to pollution of harbor waters by antifouling paints. *Arch Pathol Lab Med*, 107(12), 629-632. Access, May, 2013, available at http://www.ncbi.nlm.nih.gov/pubmed/6357141

Monfort, P., Piclet, G. & Plusquellec, A. (2000).*Listeria innocua* and *Salmonella panama* in estuarie water and seawater: a comparative study.*Water Research,* 34(3), 983-989*.* Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0043135499001980

Monteiro, L.R., Granadeiro, J.P. & Furness, R.W.(1998).Relationship between mercury levels and diet in Azores seabirds. *Marine ecology progress series*, 166, 259-265. Access Jan, 2012, available at: http://www.int-res.com/articles/meps/166/m166p259.pdf

Moorman, T.E., Baldassarre, G.A. & Richard, D.M.(1991). The frequency of *Sarcocystis* spp. and its effect on winter carcass composition of Mottled ducks. *Journal of Wildlife Diseases*, 27(3), 491-493. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-27.3.491

Munday, B.L.(1977). A species of *Sarcocystis* using owls as definitive hosts. *Journal of Wildlife Diseases,* 13, 205-207. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-13.2.205

Munday, B.L., Hartley, W.J., Harrigan, K.E. & Obendorf, D.L.(1979). *Sarcocystis* and related organisms in Australian wildlife: II. survey findings in birds, reptiles, amphibians and fish. *Journal of wildlife diseases,* 15, 57-73. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-15.1.57

Munday, B.L. (1983). An isospora parasite of masked owls producing *Sarcocystis* in rats. *Journal of Wildlife Diseases,* 19(2), 146-147. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-19.2.146

Munir, S., Sharma, J.M. & Kapur, V. (2005). Transcriptional response of avian cells to infection with Newcastle disease virus. *Virus research*, 107, 103-108. Access Apr, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0168170204002837

Muñoz, F.J. and De la Fuente, M.D.(2003). Lymphoid distribution in the migratory gull *Larus ridibundus*. *Comparative biochemistry and Phsysiology Part A: Molecular & Integrative Physiology*, 136, 749-756 http://www.sciencedirect.com/science/article/pii/S1095643303002435

Munoz, R.V., Hacker, C.S. & Gesell, T.F.(1976). Environmentally acquired lead in the Laughing gull, *Larus atricilla*. *Journal of Wildlife Diseases*, 12(2), 139-142. Access Jan, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/933304

Munson, L. (2000).Necropsy procedures for wild animals. *University of California Davis: Wildlife Conservation Society.* Access, Feb, 2012, available at *www.wcs.org/~/media/Files/pdfs/necropsy2.ashx*

Naar, J.P., Flewelling, L.J., Lenzi, A., Abbott, J.P., Granholm, A., Jacocks, H.M., Gannon, D., Henry, M., Pierce, R., Baden, D.G., Wolny, J. & Landsberg, J.H.(2007). Brevetoxins, like ciguatoxins, are potent ichthyotoxic neurotoxins that accumulate in fish. *Toxicon*, 50(5), 707-723. Access Nov, 2011, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2652748/pdf/nihms68724.pdf

Nakade, T., Tomura, Y., Jin, K., Taniyama, H., Yamamoto, M., Kikkawa, A., Miyagi, K., Uchida, E., Asakawa, M., Mukai, T., Shirasawa, M. & Yamaguchi, M.(2005). Lead poisoning in whooper and tundra swans. *Journal of Wildlife Diseases,* 41(1), 253-256. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-41.1.253

Nardoni, S., Ceccherelli, R., Rossi, G. & Mancianti, F.(2006). Aspergillosis in *Larus cachinnans michahellis*: survey of eight cases. *Mycopathologia,* 161, 317-321.

Nayak, B., Militino Dias, F., Kumar, S., Paldurai, A., Collins, P.L., Samal, S.K.(2012). Avian paramyxovirus serotypes 2-9 (APMV-2-9) vary in the ability to induce protective immunity in chickens against challenge with virulent Newcastle disease virus (APMV-1). V*accine*, 30(12) 2220-2227. Access May, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0264410X11020391

Negre, N., Bistuer, A. and Millan, J.(2008). A survey for diseases in Yellow-legged gulls (*L.michahellis*) feeding at Majorca garbage dump, Spain. In *Book of abstracts EWDA 8th conference of European wildlife diseases assotiation Croatia Rovinj 2-5 october* pp.79

Neimanis, A., Gavier-Widén,D., Leighton, F., Bollinger, T., Rocke, T. & Morner, T.(2007). An outbreak of type C botulism in herring gulls (*Larus argentatus*) in Southeastern Sweden. *Journal of wildlife diseases*, 43(3), 327-336. Access Dec, 2011, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-43.3.327

Neimanis, A. & Speck, S. (2012). Clostridium species and Botulism. In Widen-Gavier, D.; Duff, J.P. and Meredith, A. *Infectious diseases of wild mammals and birds in Europe*. (pp. 417-423) Blackwell publishing

NEMUS-ECOSSISTEMA-AGRO.GES [N-E-A.G]. (2012). *Plano de gestão das bacias hidrográficas integradas nas regiões hidrográficas 6 e 7.*Agência Portuguesa do Ambiente. Access Feb, 2013, available at: http://www.apambiente.pt/?ref=16&subref=7&sub2ref=9&sub3ref=834.

NEMUS-HIDROMOD-AGRO.GES.[N-H-A.G] (2012). *Plano de gestão das bacias hidrográficas que integram a região hidrográfica das ribeiras do algarve (RH8).* Agência Portuguesa do Ambiente, I.P. *Access Feb, 2013,* available at: http://www.apambiente.pt/?ref=16&subref=7&sub2ref=9&sub3ref=834.

Newton, I. (2008). *The migration ecology of birds*. San Diego:Academic Press

Nisbet, I.C.T.(1983). Paralytic shellfish poisoning: effects on breeding terns. *Condor,* 85(3), 338-345. Access Nov, 2011, available at: http://www.jstor.org/discover/10.2307/1367071?uid=3738880&uid=2&uid=4&sid=21103050141831

Nol,P., Rocke, T.E., Gross, K. & Yuill, T.M.(2004). Prevalence of neurotoxin clostridium botulinum type C in the gastrointestinal tracts of tilapia (*Oreochromis mossambicus*) in the Salton sea. *Journal of wildlife diseases,* 40(3), 414-419. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-40.3.414

Ochiai, K., Hoshiko, J.K., Tsuzuki, T. & Itakura, C. (1993). A survey of lead poisoning in wild waterfowl in Japan. *Journal of Wildlife Diseases*, 29(2), 349-352. Access Oct, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-29.2.349

Ochiai, K., Kimura, T., Uematsu, K., Umemura, T. & Itakura, C.(1999). Lead poisoning in wild waterfowl in Japan. *Journal of Wildlife Diseases,* 35, 766-769. Access Jan, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.4.766

Ochoa-acuña, H., Sepúlveda, M.S. & Gross, T.S. (2002). Mercury in feathers from chilean birds: influence of location feeding strategy, and taxonomic affiliation. *Marine Pollution Bulletin*, 44, 340-349. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0025326X01002806

Ohlendorf,H.M.(1993). Marine birds and trace elements in the temperate North Pacific. *The status, ecology and conservation of marine birds of the north pacific*. Access Sep, 2012, available at:http://pubs.er.usgs.gov/publication/5210652

Olias, P., Gruber, A.D., Heydorn, A.O., Kohls, A., Mehlhorn, H., Hafez, H.M. & Lierz, M.(2009). A novel sarcocystis-associated encephalitis and myositis in racing pigeons. *Avian Pathology*, 38(2), 121-128. Access Mar, 2012, available at: http://www.tandfonline.com/doi/pdf/10.1080/03079450902737847

Olias, P.; Gruber, A.D.; Kohls, A.; Hafez, H.M.; Heydorn, A.O.; Mehlhorn, H. & Lierz, M.(2010). Sarcocystis species lethal for domestic pigeons. *Emerging infectious diseases,* 16(3), 497-499.

Olias, P., Gruber, A.D., Hafez, H.M., Lierz, M., Slesiona, S., Brock, M. & Jacobsen, I.D.(2011). Molecular epidemiology and virulence assessment of *Aspergillus fumigatus* isolates from White stork chicks and their environment.*Veterinary Microbiology*, 148, 348-355. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0378113510004165

Olsen, K.M. & Larsson, H. (2004) *Gulls of north america, europe, and asia.(*2nd edition). Princenton University Press

Onodera, H., Oshima, Y., Henriksen, P. & Yasumoto, T. (1997). Confirmation of anatoxin-a(s), in the cyanobacterium *Anabaena Lemmermannii*, as the cause of bird kills in Danish lakes. *Toxicon,* 35(11), 1645-1648. Access Dec, 2011, available at http://www.sciencedirect.com/science/article/pii/S004101019700038X

Ordinance nº288/2010, of 27th of May. Diário da República nº 10 – Ist series. Ministério da Agricultura, Pescas e Alimentação, da Saúde e do Ambiente e Recursos Naturais. Lisboa pp. 1832-1833

Oro, D. & Martínez-Abraín, A.(2007). Deconstructing myths on large gulls and their impact in threatened sympatric waterbirds. *Animal Conservation,* 10(1), 117-126. Access May, 2013, available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-1795.2006.00082.x/abstract

Ortiz, N.E. & Smith, G.R.(1994). Landfill sites, botulism and gulls.*Epidemiol. Infect.*, 112, 385-391. Access Dec, 2011, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2271455/pdf/epidinfect00044-0149.pdf

Oslon, E.J., Wusnchmann, A. & Dubey, J.P.(2007). *Sarcocystis* sp.- associated meningoencephalitis in a Bald eagle (*Haliaeetus leucocephalus*). *Journal of vet diagn invest*, 19, 564-568. Access Mar, 2012, available at: http://vdi.sagepub.com/content/19/5/564.long

Pain, D.J.; Fisher, I.J. & Thomas, V.G.(2009). A global update of lead poisoning in terrestrial birds from ammunition sources. In Watson, R.T.; Fuller, M.; Pokras, M. & Hunt, W.G. (eds.). *Ingestion of lead from spent ammunition: implications for wildlife and humans*. Boise:The Peregrine Fund Access Nov, 2012, available at: http://www.peregrinefund.org/subsites/conference-lead/PDF/0108%20Pain.pdf

Palmgren, H., Aspán, A., Broman, T., Bengtsson, K., Blomquist, L., Bergstrom, S., Wollin, R. & Olsen, B. (2006). *Salmonella* in Black-headed gulls (*Larus ridibundus*); prevalence, genotypes and influence on *Salmonella* epidemiology*. Epidemiol. Infect.*, 134(3), 635-644. Access Mar, 2012, available at: http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=431958

Paredes, I., Rietjens, I.M.C.M., Vieites, J.M. & Cabado, A.G. (2011). Update of risk assessments of main marine biotoxins in the European Union. *Toxicon*, 58, 336-354. Access Fev, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0041010111002091

Pattee, O.H., Wiemeyer, S.N., Mulhern, B.M., Sileo, L. & Carpenter, J.W. (1981). Experimental lead-shot poisoning in Bald eagles. *The Journal of Wildlife management,* 45(3), 806-810. Access Mar, 2011, available at: http://www.jstor.org/discover/10.2307/3808728?uid=3738880&uid=2&uid=4&sid=21103048872751

Pattee, O.H., Carpenter, J.W., Fritts, S.H., Rattner, B.A., Wiemeyer, S.N., Royle, J.A. & Smith, M.R. (2006). Lead poisoning in captive Andean condors (*Vultur gryphus*). *Journal of wildlife diseases*, 42, 772-779. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-42.4.772

Pearson, G.L.(1969).Aspergillosis in wintering mallards. *Bull. Wildlife Disease Assoc,* 5, 404-405. Access Mar, 2012, available at: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1107&context=usgsnpwrc

Peel, M.C.; Finlayson, B.L. and McMahon, T.A.(2007). Updated world map of the koppen-geiger climate classification. *Hydrology and earth system sciences*,11, 1633-1644. Access Jan, 2013, available at: http://www.hydrol-earth-syst-sci.net/11/1633/2007/hess-11-1633-2007.html

Perrot, J.K. & Armstrong, D.P.(2011).*Aspergillus fumigatus* densities in relation to forest succession and edge effects: implications for wildlife health in modified environment. *EcoHealth, 8*(3)*, 290-300.* Access Mar, 2012, available at: http://link.springer.com/article/10.1007%2Fs10393-011-0716-8

Pikula, J., Bandouchova, H., Hilscherova, K., Paskova, V., Sedlackova, J., Adamovsky, O., Knotkova, Z., Lany, P., Machat, J., Marsalek, B., Novotny, L., Pohanka,M., Vitula, F.(2010).Combined exposure to cyanobacterial biomass, lead and the Newcastle vírus enhances avian toxicity. *Science of the total environment,* 408, 4984-4992. Access Apr, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0048969710007448

Pikula, J., Hajkova, P., Bandouchova, H., Bednarova, I, Adam, V., Bemklova, M., Kral, J., Ondracek, K., Osickova, J., Pohanka, M., Sedlackova, J., Skochova, H., Sobotka, J., Treml, F. & Kizek, R.(2013). Lead toxicosis of captive vultures: case description and responses to chelation therapy. *BMC Veterinary Research*, 9(11), 1-10.Access May, 2013, available at: http://www.biomedcentral.com/content/pdf/1746-6148-9-11.pdf

Pisani, J.M., Grant, W.E. & Mora, M.A. (2008). Simulating the impact of cholinesterase-inhibiting pesticides on non-target wildlife in irrigated crops. *Ecological Modelling,* 210(1-2), 179-192. Access Mar, 2012, available at http://www.sciencedirect.com/science/article/pii/S0304380007003742

Poissant, L., Zhang, H.H., Canário, J., Constant, P. (2008).Critical review of mercury fates and contamination in the arctic tundra ecosystem. *Science of the total environment,* 400(1-3), 173-211. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0048969708007031

Pollock, C.(2006) Diagnosis and treatment of avian renal disease. *Veterinary Clinics of North America: Exotic Animal Practice,* 9(1), 107–128. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S109491940500068X

Pons, J.-M., Hassanin, A. & Crochet, P.-A. (2005). Phylogenetic relationships within the *Laridae* (Charadriiformes: *Aves*) inferred from mitochondrial markers. *Molecular Phylogenetics and Evolution* 37(3), 686-699. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1055790305001776

Poot, M.(2003). Offshore foraging of Mediterranean gulls *Larus melanocephalus* in Portugal during the winter*. Atlantic seabirds*, 5(1), 1-12.

Poppenga, R. H. & Oehme, F. W. (2010). Pesticide use and associated morbidity and mortality in veterinary medicine. In Krieger, R. (eds).*Hayes's handbook of pesticide toxicology*.(pp. 285-300).

Poppenga, R.H. & Tawde, S.(2012). Avian Toxicology. In Gupta, R.C. Veterinary toxicology. Elsevier

Prakas, P. & Butkauskas, D.(2012). Protozoan parasites from genus *Sarcocystis* and their investigations in Lithuania. *Ekologija Lietuvos mokslu akademija*, 58(1), 45-58. Access Mar, 2012, available at: http://www.lmaleidykla.lt/ojs/index.php/ekologija/article/view/2349

Prakas, P., Kutkiené, L., Sruoga, A. & Butkauskas, D.(2011). *Sarcocystis* sp.from the herring gull (*Larus argentatus*) identitied to be *Sarcocystis wobeseri* based on cyst morphology and DNA results. *Parasite Research*, 109(6), 1603-1608. [Abstract]. Access Mar, 2013, available at: http://link.springer.com/article/10.1007%2Fs00436-011-2421-5

Prijono, W.B. & Leighton, F.A.(1991). Parallel measurement of brain acetylcholinesterase and the muscarinic cholinergic receptor in the diagnosis of acute, lethal poisoning by anti-cholinesterase pesticides. *Journal of wildlife disease*, 27, 110-115. Access Dec, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-27.1.110

Projecto Arenaria (2013). *Projecto arenaria: monitorização da distribuição e abundância de aves nas praias e costas de Portugal*. Access May, 2013, available at: https://sites.google.com/site/projectoarenaria/Home

Prosser, P., Nattrass, C. and Prosser, C.(2008). Rate of removal of bird carcasses in arable farmland by predators and scavengers. *Ecotoxicology and environmental safety*, 71, 601-608. Access Apr, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0147651307002539

Puschner, B. and Roegner, A.(2012). Cyanobacterial (blue-green algae) toxins. In Gupta, R.C. *Veterinary toxicology* (pp 953-965). Elsevier

Quessy, S. & Messier, S. (1992). Prevalence of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. in Ring-billed gulls (*Larus delawarensis*). *Journal of wildlife diseases*, 28(4), 526-531. Access May, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-28.4.526

Quinn, P.J.; Carter, M.E.; Makey, B. & Carter, G.R.(1994).*Clinical veterinary microbiology*. Spain: Mosby eLSEvier Limited

R Core Team (2013). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Access Mar, 2013, available at: *http://www.R-project.org/*

Ramos, R., Cerdà-Cuéllar, M., Ramírez, F., Jover, L. & Ruiz, X. (2010). Influence of refuse sites on the prevalence of *Campylobacter* spp. and *Salmonella* serovars in seagulls. *Applied and Environmental Microbiology*, 76(9), 3052-3056. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2863452/pdf/2524-09.pdf

Rattner, B.A. & McGowan, P.C. (2007). Potential hazards of environmental contaminants to avifauna residing in the chesapeake bay estuary. *Waterbirds*, 30, 63-81. Access Mar, 2012, available at: http://pubs.er.usgs.gov/publication/70031211

Redig, P.T., Fuller, M.R. & Evans, D.L. (1980). Prevalence of *Aspergillus fumigatus* in free-living Goshawks (*Accipiter gentilis atricapillus*). *Journal of Wildlife Diseases*, 16(2), 169-174. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-16.2.169

Reed, K.D., Meece, J.K., Henkel, J.S. & Shukla, S.K. (2003). Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, Influenza A and enteropathogens. *Clinical medicine & research*, 1(1), 5-12. Access May, 2013, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1069015/

RIAS (2010).Relatório de actividades de 2009.

RIAS (2011).Relatório de actividades de 2010.

RIAS (2012a) Relatório de actividades de 2011.

RIAS (2012b) Relatório de Monitorização de avifauna na etar de faro-nascente 2011.

RIAS (2013).Relatório de actividades de 2012

Riley, S., Rinchard, J., Honeyfield, D.C., Evans, A.N. & Begnoche, L. (2011). Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low Alewife abundance. *North American Journal of Fisheries Management* 31(6), 1052-1064. Access Jan, 2012, available at http://www.glsc.usgs.gov/publications/2011/1638

Ritchie, B.W.; Harrison, G.J.& Harrison, L.R. (1994). *Avian Medicine: principles and application.* Lake Worth Florida: Wingers Publishing

Rock, P. (2005). Urban gulls: problems and solutions. *British birds*, 98, 338-355. Access Nov, 2011, available at: www.mouettesenville.be/_files/rock-urban-gulls.pd

Rocke, T.E. & Samuel, M.D. (1991). Effects of lead shot ingestion on selected cells of the Mallard immune system. *Journal of wildlife diseases*, 27(1), 1-9. Access Jan, 2012, available at: http://www.nwhc.usgs.gov/disease_information/lead_poisoning/publications/91TER.JWD01.pdf

Rocke, T.E. & Brand, C.J.(1994).Use of sentinel mallards for epizootiologic studies of avian botulism. *Journal of wildlife diseases*, 30(4), 514-522. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-30.4.514

Rocke, T.E & Friend, M. (1999). Avian Botulism. In Friend, M. & Franson, J. C. (1999). *Field manual of wildlife diseases: general field procedures and diseases of birds.* (pp.271-286).Washington DC: U.S. Geological Survey

Rocke, T.E.(2006). The global importance of avian botulism.*Waterbirds around the world*, Eds. G.C.Boere, C.A. Gallbraith & D.A. Stroud. The Stationery office, 422-426. Access Feb 2012, available at http://jncc.defra.gov.uk/PDF/pub07_waterbirds_part4.2.3.pdf

Rocke, T. E. & Bollinger, T. K. (2007). Avian botulism. In Thomas, N.C., Hunter, D.B. & Atkinson, C.T. *Infectious diseases of wild birds.* (pp. 377-416). Blackwell Publishing

Rocke, T.E. & Barker, I. (2010).Proposed link between paralytic syndrome and thiamine deficiency in Swedish gulls not substantiated. *PNAS*, 107(4). Access Mar, 2012, available at: http://www.pnas.org/content/107/4/E14.full.pdf+html

Rodrigues, D., Figueiredo, M. & Fabião, A. (2001). Mallard *Anas platyrhynchos* lead poisoning risk in Central Portugal. *Wildfowl*, 52, 169-174. Access Apr, 2013, available at: wildfowl.wwt.org.uk/index.php/wildfowl/article/view/1104/1104

Rodrigues, A.L.; Correia, M.I.; Belas, A.; São Braz, B.; Carrapiço, B.; Moreira, A. & Silva, A.J.(2005). Screening analytical methods for pesticides detection in animal tissues. *Toxicology Letters,* 158 (suppl.1), 142-143.

Rodrigues, A.L.; Moreira, A.S.; São Braz, B.; Carrapiço, B. & Silva, A.J. (2006). Screening and confirmatory methods for strychnine determination in biological matrices. *Journal of Veterinay Pharmacology and Therapeutics*, 29 (1), 345-346.

Rodríguez, J.J., Oliveira, P.A., Fidalgo, L.E., Ginja, M.M., Silvestre, A.M., Ordoñez, C., Serantes, A.E.; Gonzalo-Orden, J.M. & Orden, M.A. (2010). Lead toxicity in captive and wild mallards (*Anas platyrhynchos*) in Spain. *Journal of Wildlife diseases*, 46(3), 854-863.Access Nov, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.3.854

Rose, K.; Newman, S.; Uhart, M. & Lubroth, J. (2006). *FAO animal production and health manual: Wild bird highly pathogenic avian influenza surveillance - sample collection from healthy, sick and dead brids.* Roma: Food and Agriculture Organization of the United Nations FAO. Access Dec, 2011, available at: http://www.fao.org/docrep/010/a0960e/a0960e00.HTM

Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D., Skåre, J.U., Gabrielsen, G.W. (2009) Persistent organic pollutants and mercury in dead and dying Glaucous gulls (*Larus hyperboreus)* at Bjørnøya (Svalbard). *Science of the Total Environment*, 407(23), 6009-6016. Access Jan, 2013, available at: http://www.sciencedirect.com/science/article/pii/S004896970900761X

Samour, J.(2000). *Avian Medicine.* (2ªedição).Mosby Elsevier

Samuel,W.M., Pybus, M.J. & Kocan, A.A. (2001). *Parasitic diseases of wild Mammals.* (2nd ed). Iowa State University Press/Ames

Sanchez, C.R. & Murray, S.Z. (2005).Diagnosis and successful treatment of a presumptive case of aspergillosis in a Micronesian kingfisher (*Halcyon cinnamomina cinnamomina*). *Avian diseases*, 49, 309-312. Access Apr, 2012, available at: http://www.jstor.org/discover/10.2307/1593586?uid=3738880&uid=2&uid=4&sid=21103044455201

Sanderson, G.C., Anderson, W.L., Foley, G.L., Havera, S.P., Skowron, L.M., Brawn, J.W., Taylor, G.D. & Seets, J.W. (1998). Effects of lead, iron, and bismuth alloy shot embedded in the breast muscles of game-farm mallards. *Journal of Wildlife disease*, 34(4), 688-697. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-34.4.688

Sandler,R., Rocke, T.E., Samuel, M.D. and Yuill, T.M.(1993).Seasonal prevalence of *Clostridium botulinum* type C in sediments of a Northern California wetland.*Journals of wildlife diseases* 29(4), 533-539 Access Jun, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-29.4.533

Santos, C.S.A., Monteiro, M.S., Soares, A.M.V.V. & Loureiro, S. (2011). Bioaccumulation of mercury in birds: a comparative study in ciconiformes, charadriiformes and pelecaniformes. *In 1st Europe meeting SETAC annual metting, 15-19 May, Milan, Italy*

Savinov, V.M., Gabrielsen, G. and Savinova, T.N. (2003). Cadmium, zinc, copper, arsenic, selenium and mercury in seabirds from the Barents sea: levels, inter-specific and geographical differences. *The science of the total environment*, 306(1-3), 133-158. Access Mar, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0048969702004898

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B. & Murray, M.W.(2007).Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio*, 36(1), 12-18. Access Jan, 2012, available at http://www.ncbi.nlm.nih.gov/pubmed/17408187

Schmidt, R.E. (2006). Types of renal disease in avian species. *Veterinary Clinics of North America: Exotic Animal Practice,* 9(1), 97–106. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S1094919405000642

Schreiber, E.A. & Burger, J. (2001*). Biology of marine birds.* Florida: CRC Marine biology series. CRC Press

Seewagen, C.L. (2010).Threats of environmental mercury to birds: knowledge gaps and priorities for future research. *Bird Conservation International,* 20(2), 112-123. Access Dec, 2012, available at: http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7820478

Sekercioglu, C.H., Daily, G.C. & Ehrlich, P.R. (2004). Ecosystem consequences of bird declines. *PNAS,* 52, 18042-18047. Access May, 2013, available at: http://www.pnas.org_cgi_doi_10.1073_pnas.0408049101

Sellner, K.G., Doucette, G.J. & Kirkpatrick, G.J.(2003). Harmful algal blooms: causes, impacts and detection. *Journal of Industrial Microbiology Biotechnology*, 30, 383-406. Access Dec, 2011, available at http://link.springer.com/article/10.1007%2Fs10295-003-0074-9

Shearn-Bochsler, V. (2008). The impact of harmful algal blooms on wildlife. In *Symposium on diagnostic pathology of diseases of aerial, terrestrial and aquatic wildlife 2008.*USGS National Wildlife Health Center

Shin, N-R., Byun, S.H., Chun, J.H., Shin, J.H., Kim, Y.J., Kim, J-H., Rhie, G-e., Chung, H. M., Mo, I-P. & Yoo, C-K. (2010). An outbreak of type C botulism in waterbirds: Incheon, Korea. *Journal of Wildlife Diseases*, 46(3), 912-917. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.3.912

Shumway, S.E., Allen, S.M. & Dee Boersma, P.(2003). Marine birds and harmful algal blooms: sporadic victims or under-reported events? *Harmful Algae*, 2, 1-17. Access Dec, 2011, available at http://www.sciencedirect.com/science/article/pii/S1568988303000027

Sidor, I.F., Pokras, M.A., Major, A.R., Poppenga, R.H., Taylor, K.M. & Miconi, R.M.(2003). Mortality of Common loons in New England, 1987 to 2000. *Journal of Wildlife Diseases*, 39(2), 306-315. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-39.2.306

Sileo, L. & Fefer, S.I. (1987). Paint chip poisoning of Laysan albatross at Midway Atoll. *Journal of wildlife diseases*, 23(3), 432-437. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-23.3.432

Simões. R.R., Poirel, L., Da Costa, P.M. & Nordmann, P. (2010). Seagulls and beaches as reservoirs for multidrug- resistant *Escherichia coli. Emerging infectious diseases,*16(1), 110-112. Access Feb, 2012, available at: http://wwwnc.cdc.gov/eid/article/16/1/09-0896_article.htm

Skarin, H., Lindberg, A., Blomqvist, G., Aspán, A. & Baverud, V.(2010). Molecular characterization and comparison of *Clostridium botulinum* type C avian strains. *Avian Pathology*, 39(6), 511-518. Access Dec, 2011, available at: http://www.tandfonline.com/doi/pdf/10.1080/03079457.2010.526923

Skerratt, L.F.; Franson, J.C.; Meteyer, C.U. & Hollmén, T.E.(2005). Causes of mortality in Sea ducks (*Mergini*) necropsied at the USGS-National Wildlife Health Center. *Waterbirds,* 28(2), 193-207. Access Mar, 2013, available at: http://www.nwhc.usgs.gov/publications/documents/05LFS.W01.pdf

Skocovska, B., Hilscherova, K., Babica, P., Adamovsky, O., Bandouchova, H., Horakova, J., Knotkova, Z., Marsalek, B., Paskova, V. & Pikula, J.(2007). Effects of cyanobacterial biomass on the Japanese quail. *Toxicon*, 49(6), 793-803. Access Dec, 2011, available at http://www.sciencedirect.com/science/article/pii/S0041010106004612

Smith, G.R., Turner, A. & Till, D.(1987).Factors affecting the toxicity of rotting carcasses containing *Clostridium botulinum* type E. *Epidem. Inf,* 100, 399-405. Access Dec, 2011, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2235363/

Smith, M.R., Thomas, N.J. and Hulse, C.(1995). Application of brain cholinesterase reactivation to differentiate between organophosphorus and carbamate pesticide exposure in wild birds. *Journal of wildlife diseases* 31(2), 263-267. Access Jan, 2013, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-31.2.263

Smith, E.A., Grant, F., Ferguson, C.M.J. & Gallacher, S. (2001). Biotransformations of paralytic shellfish toxins by bacteria isolated from bivalve molluscs. *Applied and environmental microbiology*, 67(5), 2345-2353. Access Dec, 2011, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC92876/pdf/am002345.pdf

Snoeck, C.J.; Marinelli, M.; Charpentier, E.; Sausy, A.; Conzemius, T.; Losch, S. and Muller, C.P.(2013). Characterization of Newcastle disease viruses in wild and domestic birds in Luxembourg from 2006-2008. *Applied and environmental microbiology*, 79(2), 639-645.

Sonne, C.; Alstrup, A.K.O.; Therkildsen, O.R.(2012a). A review of the factors causing paralysis in wild birds: implications for the paralytic syndrome observed in the Baltic sea. *Science of the Total Environment,* 416, 32-39. Access Jan, 2013, available at http://www.sciencedirect.com/science/article/pii/S0048969711014318

Sonne, C., Alstrup, A.K.O., Therkildsen, O.R.(2012b). Reply to Tillitt et al. 2012: Thiamine deficiency: a viable hypothesis for paralytic syndrome in Baltic birds. *Science of the Total environment*, 433, 563-564. Access May, 2013, available at: http://pure.au.dk/portal/en/publications/reply-to-tillit-et-al-2012-thiamine-deficiency-a-viable-hypothesis-for-paralytic-syndrome-in-baltic-birds(cc965d69-0c8e-4c28-8850-324d9410e011).html

Souza, M.J. & Degernes, L.A.(2005). Mortality due to aspergillosis in wild swans in Northwest Washington State, 2000-02. *Journal of Avian Medicine and Surgery*, 19, 98-106. Access Apr, 2012, available at: http://www.bioone.org/doi/abs/10.1647/2004-001?journalCode=avms

Spalding, M.G., Akinson, C.T. &Carleton, R.E. (1994). *Sarcocystis* sp. in wading birds (ciconiiformes) from Florida. *Journal of Wildlife Diseases,* 30, 29-35. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-30.1.29

Spalding, M.G., Frederick, P.C., McGill, H.C., Bouton, S.N. & McDowell, L.R. (2000a). Methylmercury accumulation in tissues and its effects on growth and appetite in captive Great egrets. *Journal of wildlife diseases*, 36, 411-422. Access, Mar 2012, available at http://www.wec.ufl.edu/faculty/frederickp/publications/Spalding%20et%20al%2000%20Methylmercur%20accumulation%20in%20tissues%20and%20its%20effects%20on%20growth%20and%20appetite.pdf

Spalding, M.G., Frederik, P.C., McGill, H.C., Bouton, S.N., Richey, L.J., Schumacher, I.M., Blackmore, C.G.M. & Harrison, J.(2000b). Histologic, neurologic, and immunologic effects of methylmercury in captive Great egrets. *Journal of wildlife diseases*, 36, 423-435. Access, Mar 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-36.3.423

Spalding, M.G., Yowell, C.A., Lindsay, D.S., Greiner, E.C. & Dame, J.B. (2002). *Sarcocystis meningoencephalitis* in a northern gannet (*Morus bassanus*). *Journal of Wildlife Diseases*, 38(2), 432-437. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-38.2.432

SPEA (2013). *IBAs.* Access Jan, 2013, available at: http://www.spea.pt/en/study-and-conservation/ibas/

Stocker, L. (2005). *Practical wildlife care.* (2nd ed). Blackwell Publishing

Stone, W.B., Overmann, S.R. & Okoniewski, J.C.(1984).Intentional poisoning of birds with parathion.*The Condor,* 86,(3) 333-336. Access Mar, 2012, available at http://www.jstor.org/discover/10.2307/1367004?uid=3738880&uid=2&uid=4&sid=21103050813661

Stone, W.B. & Okoniewski, J.C. (2001). Necropsy findings and environmental contaminants in Common loons from New York. *Journal of wildlife diseases*, 37(1), 178-184. Access May, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-37.1.178

Swank, R. (1940). Avian thiamin deficiency: a correlation of the pathology and clinical behavior. *The Journal of Experimental Medicine*, 71(5), 683-702. Access Jan, 2012, available at: http://jem.rupress.org/content/71/5/683.long

Taggart, M.A., Green, A.J., Mateo, R., Svanberg, F., Hillstrom, L., Meharg, A.A. (2009).Metal levels in the bones and livers of globally threatened Marbled teal and White-headed duck from El Hondo, Spain. *Ecotoxicology and Environmental safety,* 72, 1-9. Access Dec, 2011, available at: http://www.sciencedirect.com/science/article/pii/S0147651308002121

Tarello, W.(2011). Etiologic agents and diseases found associated with clinical aspergillosis in falcons. *International Journal of Microbiology*, 2011, 1-6. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3132614/pdf/IJMB2011-176963.pdf

Tennakoon, S., Perera, B. & Haturusinghe, L. (2009). Intentional poisoning cases of animals with anticholinesterase pesticide-carbofuran in Sri Lanka. *Legal Medicine,* 11, 500-502. Access Mar, 2012, available at: http://www.legalmedicinejournal.com/article/S1344-6223(09)00037-6/abstract

Thomas, N.J.; Hunter, D.B.& Atkinson, C.T.(2007). *Infectious diseases of wild birds.*Iowa: Blackwell Publishing

Thompson, H.M.; Langton, S.D. & Hart, A.D.M. (1995). Prediction of inter-species differences in the toxicity of organophosphorus pesticides to wildlife – a biochemical approach. *Comp. Biochem. Physiol Part C: Pharmacology, Toxicology and Endocrinology*, 111(1), 1-12. Access Mar, 2012, available at: http://www.sciencedirect.com/science/article/pii/074284139500040E

Threlfall, W.(1967). Diseases and Pathological conditions of the herring gull (*L.argentatus* Pontopp.) excluding helminth infestations. *Bulletin wildlife diseases association,* 3, 62-67. Access Out, 2011, available at: http://www.avibirds.com/pdf/Z/Zilvermeeuw4.pdf

Tiffany, M.A., Wolny, J., Garret, M., Steidinger, K. & Hurlbert, S.H.(2008). Dramatic blooms of *Prymnesium sp.* (Prymnesiophyceae) and *Alexandrium margalefi* (Dinophyceae) in the Salton sea, California. *Proceedings of SPIE*, 23(5), 620-629. Access Nov, 2012, available at: http://www.tandfonline.com/doi/abs/10.1080/07438140709354041#.Up392dJdUgc

Tillitt, D.E., Kraft, C.E., Honeyfield, D.C. and Fitzsimons, J.D.(2012). Thiamine deficiency: a viable hypothesis for paralytic syndrome in Baltic birds. Commentary on Sonne et al.., 2012. A review of the factors causing paralysis in wild birds:implications for the paralytic syndrome observed in the Baltic Sea. *Science of the Total Environment* 43. Acess May, 2013, available at: http://www.sciencedirect.com/science/article/pii/S0048969712009138

Tizard, I. (2004). Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine*, 13(2), 50-66. Access Apr, 2012, available at: http://vetmed.tamu.edu/common/docs/public/schubot/SalmonellosisinWildBirds.pdf

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W. P., Adams, N.G. & Trick, C.G. (2012). *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health, *Harmful algae* 14, 271-300. Access Jan, 2013, available at: http://www.sciencedirect.com/science/article/pii/S1568988311001533

Trocini,S., Pacioni, C., Warren, K., Butcher, J. & Robertson, I.(2008). Wildlife disease passive surveillance: the potential role of wildlife rehabilitation centres. 6th National Wildlife Rehabilitation Conference. Camberra. Access Feb, 2013, available at: http://www.awrc.org.au/uploads/5/8/6/6/5866843/trocini_surveillance.pdf

Tsipoura, N., Burger, J., Newhouse, M., Jeitner, C., Gochfeld, M. & Mizrahi, D. (2011). Lead, mercury, cadmium, chromium, and arsenic levels in eggs, feathers, and tissues of Canada geese of the New Jersey meadowlands. *Environmental research*, 111(6), 775-784. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0013935111001423

Tuggle, B.N. & Friend, M.(1999). Sarcocystis. In Friend, M. & Franson, J. C. *Field manual of wildlife diseases: general field procedures and diseases of birds.*(pp.219-222)Washington DC: U.S. Geological Survey

Tully Jr.,T.N., Dorrestein, G.M. & Jones, A. K. (2000). *Handbook of avian medicine*. (2nd ed.) Oxford:Reed Educational and Professional Publishing Ltd

Turismo do Algarve.(2012a) *A guide to nature tourism in the algarve*. Faro: Turismo do Algarve. Access Feb, 2013, available at: http://www.iltm.net/ExhibitorLibrary/3521/Nature_Tourism_Guide_6.pdf

Turismo do Algarve.(2012b) Guia de observação de aves no Algarve. Access Feb, 2013, available at: http://www.visitalgarve.pt/pressroom.file.php?fileID=123&file=guia_observacao_aves_baixa_resolucao_pt.pdf

Vale, P., Botelho, M.J., Rodrigues, S.M., Gomes, S.S. & Sampayo, M.A.de M.(2006). Two decades of marine biotoxin monitoring in bivalves from Portugal (1986-2006): a review of exposure assessment. *Harmful algae*, 7, 11-25. Access Dec, 2011, available at: http://www.afbini.gov.uk/portugal_monitoring_review_1986-2006.pdf

Vale, P.(2011). Biotoxinas emergentes en aguas europeias e novos riscos para a saude publica. *Revista Portuguesa de Saúde Pública*, 29(1), 77-87. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0870902511700104

Van Deventer, M.(2007). *Brevetoxins in marine birds: evidence of trophic transfer and the role of prey fish as toxin vector.* Florida:Master thesis University of South Florida Graduate School Access Nov, 2012, available at: http://scholarcommons.usf.edu/cgi/viewcontent.cgi?article=3391&context=etd

Wada, H., Cristol, D.A., McNabb, F.M.A. & Hopkins, W.A. (2009). Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. *Environ. Sci. Technol,* 43(15), 6031-6038. Access Jan, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/19731714

Wakamatsu, N., King, D.J., Seal, B.S., Kamal, S.K. & Brown, C.C. (2006). The pathogenesis of Newcastle disease: a comparison of selected Newcastle disease virus wild-type strains and their infectious clones. *Virology*, 353, 333-343. Access Nov, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0042682206004090

Waldenstedt, L. (2006). Nutritional factors of importance for optimal leg health in broilers: a review. *Animal and feed science and technology,* 126(3), 291-307. Access Jan, 2012, available at: http://www.journals.elsevierhealth.com/periodicals/anifee/article/PIIS0377840105003238/abstract

Ward, F.P., Fairchild, D.G. & Vuicich, J.V. (1970). Pulmonary aspergillosis in prairie falcon nest mates. *Journal of Wildlife Diseases,* 6, 80-83. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pubmed/16509138

Wenzel, C., Adelung, D. & Theede, H. (1996) Distribution and age-related changes of trace elements in kittiwake *Rissa tridactyla* nestling from an isolated colony in the german bight, north sea. *The science of the total environment,* 193(1), 13-26. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S004896979605320X

Wheeler, W.E. & Gates, R.J.(1999). Spatial and temporal variation in lead levels related to body condition in the Mississippi valley population of Canada geese. *Journal of Wildlife Disease,* 35(2), 78-186. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-35.2.178

White, D.H.; Hayes, L.E. & Bush, P.B. (1989). Case histories of wild birds killed intentionally with famphur in georgia and west virginia. *Journal of Wildlife Diseases*, 25(2), 184-188. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-25.2.184

White, M. (2011). *Consultant: a diagnostic support system for veterinary medicine*. Ithaca, New Yorke: Cornell University College of Veterinary Medicine. Acess Dec, 2011, available at: http://www.vet.cornell.edu/consultant/consult.asp

Whitney, H. (2004). Wildlife diseases factsheet: Pesticide poisoning of seagulls. *Government of Newfoundland and Labrador- Department of Natural Resources*, AP051, July 2004. Access Jan, 2012, available at: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1023&context=zoonoticspub

Wickware, A.B. (1944) Sarcosporidiosis in a Black duck. *Canadian Journal of Comparative Medicine,* 8, 15-17. Access Mar, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1660773/pdf/vetsci00314-0017.pdf

Wiemeyer, S.N., Schmeling, S.K. & Anderson, A.(1987). Enviromental pollution and necropsy data for Osprey from the Eastern United States, 1975-1982. *Journal of wildlife diseases*, 23(2), 279-291. Access Nov, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-23.2.279

Wildcove, D.S. & Wikelski, M. (2008). Going, going, gone: is animal migration disappearing? *PLoS Biology,* 6(7), 1361-1364. Access May, 2013, available at: http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.0060188

Wilson, H.M., Oyen, J.L. & Sileo, L.(2004). Lead shot poisoning of a Pacific loon in Alaska. *Journal of Wildlife Diseases*, 40(3), 600-602. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-40.3.600

Windingstad, R.M. & Hinds III, L.S. (1987). Lead poisoning in Canada geese on Plum island, Massachusetts. *Journal of Wildlife Diseases,* 23(3), 438-442. Access Jan, 2012, available at: http://www.nwhc.usgs.gov/disease_information/lead_poisoning/publications/87RMW.JWD02.pdf

Wobeser, G. (1969).Apparent favourable response of lead poisoning in a duck to treatment with a chelating agent. *Bull. Wildlife Disease Assoc*, 5, 120. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-5.2.120

Wobeser, G., Raininnie, D.J., Smith-Windsor, T.B. & Bogdan, G.(1983). Avian botulism during late autumn and early spring in Saskatchewan. *Journal of wildlife diseases,* 19(2), 90-94. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-19.2.90

Wobeser, G. & Kost, W. (1992).Starvation, staphylococcosis, and vitamin A deficiency among mallards overwintering in Saskatchewan. *Journal of Wildlife Diseases* 28(2), 215-222. Access Jan, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-28.2.215

Wobeser, G., Leighton, F.A., Norman, R., Myers, D.J., Onderka, D., Pybus, M.J., Neufeld, J.L., Fox, G.A. & Alexander, D.J. (1993). Newcastle disease in wild water birds in western Canada, 1990. *Canadian Veterinary Journal*, 34(6), 353-359. Access Apr, 2012, available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1686568/

Wobeser, G. (1997). Avian botulism – another perspective. *Journal of Wildlife Diseases,* 33(2), 181-186. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-33.2.181

Wobeser, G., Bollinger, T., Leighton, F.A., Blakley, B. & Mineau, P.(2004). Secondary poisoning of eagles following intentional poisoning of coyotes with anticholinesterase pesticides in western Canada. *Journal of Wildlife Diseases*, 40(2), 163-172. Access Mar, 2012, available at http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-40.2.163

Wobeser, G.A. (2006). *Disease in wildlife: investigation and management*. (2nd ed.) Springer

Wobeser, G.A. (2007) Parasitism: costs an effects. In Samuel,W.M., Pybus, M.J. & Kocan, A.A. (2001). *Parasitic diseases of wild Mammals*. (2nd ed) (pp.3-9). Iowa State University Press/Ames

Wolfe, M. F., Schwarzbach, S. & Sulaiman, R.A. (1998).Effects of mercury on wildlife: a comprehensive review. *Environmental Toxicology and Chemistry*, 17(2), 146-160. Access Jan, 2012, available at: http://onlinelibrary.wiley.com/doi/10.1002/etc.5620170203/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+BST+%2805%3A00-10%3A00+EDT%29+for+essential+maintenance

Woo, G-H., Kim, H-Y., Bae, Y-C., Jean, Y.H., Yoon, S-S., Bak, J.; Hwang, E.K. & Joo, Y-S.(2010). Outbreak of botulism (clostridium type C) in wild waterfowl: Seoul, Korea, *Journal of wildlife diseases,*46(3), 951-955. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.3.951

Work, T. (2000). *Avian necropsy manual for biologists in remote refuges*. U.S. Geological Survey National Wildlife Health Center Hawaii Field Station. Access Feb, 2012, available at: http://www.nwhc.usgs.gov/hfs/Globals/Products/Sea%20bird%20manual%20english.pdf

Work, T. M., Klavitter, J. L., Reynolds, M.H. & Blehert, D.(2010). Avian Botulism: a case study in translocated endangeres Laysan ducks (*Anas laysanensis*) on Midway Atoll. *Journal of Wildlife Diseases,* 46(2), 499-506. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-46.2.499

Work, T.M. & Smith, M.R.(1996).Lead exposure in Laysan albatross adults and chicks in Hawaii: prevalence, risk factors , and biochemical effects. *Arch. Environ. Contam. Toxicol*, 31, 115-119. Access Jan, 2012, available at: http://www.nwhc.usgs.gov/hfs/Globals/Products/Lead%20exposure%20in%20Laysan%20albatross.pdf

World Organization of Animal Health [OIE] (2009) *Newcastle disease*. Terrestrial Animal Health Code. OIE, Paris: OIE. Access Jan, 2012, available at: http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/NEWCASTLE_DISEASE__FINAL.pdf

World Organization of Animal Health [OIE] (2009) *Salmonellosis*. Terrestrial Animal Health Code. OIE, Paris: OIE.

World animal health information Database [WAHID]. (2012). *World animal health information database interface. Access Feb,* 2013, available at: http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home

Waterbird population estimates [WPE] (2012). *Waterbirds populations estimates online database.* Access Apr, 2012, available at: http://wpe.wetlands.org/

WPE (2013). *Waterbirds populations estimates online database.* Access Apr, 2013, available at: http://wpe.wetlands.org/

Wunschmann, A., Rejmanek, D., Cruz- Martinez, L. & Barr, B.C.(2009). *Sarcocystis falcatula*-associated encephalitis in a free-ranging Great horned owl (*Bubo Virginianus*). *Journal of Veterinary Investigation, 2*1, 283-287. Access Mar, 2012, available at: http://vdi.sagepub.com/content/21/2/283.long

Wunschmann, A., Rejmanek, D., Conrad, P.A., Hall, N., Cruz-Marinez, L., Vaughn, S.B. & Barr, B.C. (2010). Natural fatal *Sarcocystis falcatula* infections in free-ranging eagles in North America. *Journal of veterinary diagnostic investigation, 22,* 282-289. Access Mar, 2012, available at: http://vdi.sagepub.com/content/22/2/282.long

Xavier, M.O., Soares, M.P., Cabana, A.L., Da Silva-Filho, R.P., Ruoppolo, V., Meireles, M.C.A. & Severo, L.C. (2011). Clinical and pathological findings of aspergillosis in Magellanic penguins (*Spheniscus magellanicus*). *Ci.Anim.Bras.*, 12(3), 520-524. Access Apr, 2012, available at: *www.revistas.ufg.br/index.php/vet/article/view/4014/9706*

Young, E.A., Cornish, T.E. & Little, S.E. (1998). Concomitant mycotic and verminous pneumonia in a Blue jay from Georgia. *Journal of Wildlife Diseases*, 34(3), 625-628. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-34.3.625

Yule, A.M., Barker, I.K., Austin, J.W. & Moccia, R.D.(2006a). Toxicity of *Clostridium botulinum* type E neurotoxin to Great lakes fish: implications for avian botulism. *Journal of wildlife diseases,* 42(3), 479-493. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-42.3.479

Yule, A.M., LePage, V., Austin, J.W., Barker, I.K. & Moccia, R.D. (2006b). Repeated low-level exposure of the Round goby (*Neogobius Melanostomas*) to *Clostridium botulinum* type E neurotoxin. *Journal of Wildlife diseases*, 42(3), 494-500. Access Dec, 2011, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-42.3.494

Zinkl, J.G. & Hyland, J.M.(1977). Aspergillosis in Common crows in Nebraska, 1974. *Journal of Wildlife Diseases*, 13, 191-193. Access Apr, 2012, available at: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1041&context=nebgamestaff

Zinkl, J.G., Jessuo, D.A., Bischoff, A.I., Lew, T.E. & Wheeldon, E.B. (1981).Fenthion poisoning of wading birds. *Journal of Wildlife Diseases* 17(1), 117-119. Access Mar, 2012, available at: http://www.jwildlifedis.org/doi/pdf/10.7589/0090-3558-17.1.117

Zolfaghari, G., Esmaili-Sari, A., Ghasempouri, S.M. & Kiabi, B. H. (2007). Examination of mercury concentration in the feathers of 18 species of birds in Southwest Iran. *Environmental research,* 104(2), 258-265. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S001393510600260X

Zolfaghari, G., Esmaili-Sari, A., Ghasempouri, S.M., Baydokhti, R.R. & Kiabi, B.H. (2009).A multispecies-monitoring study about bioaccumulation of mercury in Iranian birds (Khuzestan to Persian gulf): effects of taxonomic affiliation and trophic level. *Environmental research,* 109, 830-836. Access Jan, 2012, available at: http://www.sciencedirect.com/science/article/pii/S0013935109001273

5. Appendices.

Appendix 1.

1.1. List of all the species mentioned throughout this paper, their scientific names and corresponding English and Portuguese common ones.

Scientific name	English common name	Portuguese common name
Aegypius monachus	Cinereous vulture	Abutre-preto
Aix sponsa	Wood duck	Pato-carolino
Alca torda	Razorbill	Torda-mergulheira
Alosa pseudoharengus	Alewife	Alosa
Anas acuta	Northern pintail	Arrabio
Anas clypeata	Northern shoveler	Pato-trombeteiro
Anas crecca	Euroasian teal	Marrequinha-comum
Anas laysanensis	Laysan duck	Pato-de-Laysan
Anas platyrhynchos	Mallard	Pato-real
Anas strepera	Gadwall	Frisada
Apteryx australis	Southern brown kiwi	Kiwi-castanho
Aquila chrysaetos	Golden eagle	Águia-real
Ardea albus	Great egret	Garça-branca-grande
Ardea herodias	Great blue heron	Garça-azul-grande
Ardeola ralloides	Squacco heron	Papa-ratos
Asio otus	Long-eared owl	Bufo-pequeno
Athene noctua	Little owl	Mocho galego
Aythya affinis	Lesser scaup	Zarro-americano
Aythya ferina	Common pochard	Zarro-comum
Branta Canadensis	Canada goose	Ganso-do-Canadá
Bubo bubo	Euroasian eagle-owl	Bufo-real
Bubo virginianus	Great horned owl	Bufo-da-virgínia
Bufo calamita	Natterjack toad	Sapo-corredor
Burhinus oedicnemus	Euroasian stone-curlew	Alcaravão
Cassidix mexicanus	Boat-tailed grackle	Irauna-mexicano
Chamaeleo chamaeleon	Mediterranean chameleon	Camaleão-comum
Chroicocephalus ridibundus	Black-headed gull	Guincho-comum
Circus cyaneus	Hen harrier	Tartaranhão-azulado
Clangula hyemalis	Long-tailed duck	Pato-rabilongo
Clupea spp.	Herring	Arenque
Columba livia	Rock pigeon	Pombo-das-rochas
Corvus brachyrhynchos	American crow	Corvo-americano
Corvus corax	Common raven	Corvo
Coturnix coturnix japonica	Japanese quail	Codorniz-japonesa
Cygnus buccinator	Trumpeter swan	Cisne-trombeteiro
Cygnus columbianus columbianus	Tundra swan	Cisne-pequeno
Didelphis virginiana	Virginia opossum	Gambá
Discoglossus galganoi	Iberian painted frog	Rã-de-focinho-pontiagudo
Emys orbicularis	European pond turtle	Cágado-de-carapaça-estriada
Erinaceus europaeus	European hedgehog	Ouriço-cacheiro
Falco naumanni	Lesser kestrel	Peneireiro-das-torres
Fulica atra	Eurasian coot	Galeirão-comum
Gallinula chloropus	Common moorhen	Galinha-d'água
Gavia immer	Great northern loon	Mobelha-grande
Gymnogyps californianus	California condor	Condor-da-califórnia
Gyps fulvus	Griffon vulture	Grifo
Haliaeetus leucocephalus	Bald eagle	Águia-de-cabeça-branca
Larus argentatus	European herring gull	Gaivota prateada
Larus atricilla	Laughing gull	Gaivota-risonha
Larus audouinii	Audouin´s gull	Gaivota-de-Audouin
Larus cachinnans	Caspian gull	Gaivota do Cáspio
Larus californicus	California gull	Gaivota californiana
Larus canus	Common gull	Gaivota-parda
Larus delawarensis	Ring-billed gull	Gaivota-de-bico-riscado
Larus fuscus	Lesser Black-backed gull	Gaivota-d'asa-escura
Larus genei	Slender-billed gull	Gaivota-de-bico-fino

Larus glaucoides	Iceland gull	Gaivota-polar
Larus glaucescens	Glaucous-winged gull	Gaivota-de-Bering
Larus hyperboreus	Glaucous gull	Gaivota-hiperbórea
Larus marinus	Great black-backed gull	Gaivotão-real
Larus melanocephalus	Mediterranean gull	Gaivota-de-cabeça-preta
Larus michahellis	Yellow-legged gull	Gaivota-argêntea
Larus minutus	Little gull	Gaivota-pequena
Larus novaehollandiae	Silver gull	Gaivota-prata
Larus pacificus	Pacific gull	Gaivota do Pacífico
Larus philadelphia	Bonaparte´s gull	Gaivota de Bonaparte
Larus pipixcan	Franklin´s gull	Gaivota de Franklin
Larus smithsonianus	American herring gull	Gaivota-prateada-americana
Marmaronetta angustirostris	Marbled teal	Pardilheira
Mauremys leprosa	Spanish pond turtle	Cágado-mediterrânico
Mephitis mephitis	Striped skunk	Mofeta
Molothrus ater	Brown-headed cowbird	Tordo-de-cabeça-castanha
Morus bassanus	Northern gannet	Ganso-patola
Natrix natrix	Grass snake	Cobra-de-água-de-colar
Neogobius melanostomus	Round goby	Caboz
Netta rufina	Red-crested pochard	Pato-de-bico-vermelho
Nycticorax nycticorax	Black-crowned night heron	Goraz
Octopus vulgaris	Common octopus	Polvo comum
Opisthonema oglinum	Thread herring	Sardinha-bandeira
Oreochromis mossambicus	Mozambique tilapia	Tilapia moçambicana
Oxyura leucocephala	White-headed duck	Pato-de-rabo-alçado
Pandion haliaetus	Osprey	Águia-pesqueira
Pelecanus erythrorhynchos	American white pelican	Pelicano-branco-americano
Pelecanus occidentalis	Brown pelican	Pelicano-pardo
Phalacrocorax aristotelis	European shag	Corvo-marinho-de-crista
Phalacrocorax auritus	Double-crested cormorant	Corvo-marinho-de-orelhas
Phalacrocorax carbo	Great cormorant	Corvo-marinho-de-faces-brancas
Phalacrocorax penicillatus	Brandt´s cormorant	Corvo marinho-de-Brandt
Pipistrellus pygmaeus	Soprano pipistrelle	Morcego-pigmeu
Podiceps grisegena	Red-necked grebe	Mergulhão-de-pescoço-vermelho
Quiscalus quiscula	Common grackle	Irauna-comum
Rissa tridactyla	Black-legged kittiwake	Gaivota-tridáctila
Salmo spp.	Salmon	Salmão
Salvelinus namaycush	Lake trout	Salvelino-lacustre
Sardina pilchardus	Sardine	Sardinha
Scomber japonicus	Chub mackerel	Cavalinha
Somateria fischeri	Spectacled eider	Eider-de-lunetas
Somateria mollissima	Common eider	Eider
Spheniscus humboldti	Humboldt penguin	Pinguim-de-Humboldt
Sprattus spp.	Sprat	Espadilha
Sterna dougallii	Roseate tern	Andorinha-do-mar-rósea
Sterna hirundo	Common tern	Andorinha-do-mar-comum
Sterna paradisaea	Artic tern	Andorinha-do-mar-árctica
Sturnus vulgaris	Common starling	Estorninho-malhado
Ruditapes decussatus	Grooved carpet shell	Amêijoa-boa
Tadorna tadorna	Common shelduck	Pato-branco
Tarentola mauritanica	Moorish secko	Osga-moura
Thalasseus maximus	Royal tern	Garajau-real
Thalasseus sandvicensis	Sandwich tern	Garajau-comum europeu
Tyto alba	Barn owl	Coruja-das-torres
Tyto novaehollandiae	Australian masked owl	Coruja-das-torres australiana
Xema sabini	Sabine's gull	Gaivota de Sabine

1.2. Different Species and Life cycles.

Figure 1 – *Larus atricilla:* Adult with chicks, Doug Wechlser. Adapted from: ARKive (2012).
Figure 2 – Immature feeding on a prawn, Erica Olsen. Adapted from: ARKive (2012).
Figure 3 – Juveniles with first winter plumage, Robin Chittenden. Adapted from: ARKive (2012).
Figure 4 – *L. atricilla* with winter plumage, Robin Chittenden. Adapted from: ARKive (2012).
Figure 5 – Species range. Adapted from: BirdLife International (2013).

Figure 6 – *Larus philadelphia:* Adult prenuptial plumage, Melvin Grey. Adapted from: ARKive (2012).
Figure 7 – Young swimming, Ralph Reinhold. Adapted from: ARKive (2012).
Figure 8 – First season, David Beadle. Adapted from: ARKive (2012)
Figure 9 – Non-breeding plumage, Glenn Bartley. Adapted from: ARKive (2012).
Figure 10 – Species range. Adapted from: BirdLife International (2013).

Color scheme for the maps:

Resident   Non Breeding   Breeding
Non Breeding (Probable)   Breeding (Probable)

Figure 11 – Species range of *Larus pipixcan*. Adapted from: BirdLife International (2013).
Figure 12 – Species range of *Larus delawarensis*. Adapted from: BirdLife International (2013).

Figure 13 – Species range of *Larus glaucoides*. Adapted from: BirdLife International (2013).
Figure 14 – Species range of *Larus hyperboreus*. Adapted from: BirdLife International (2013).
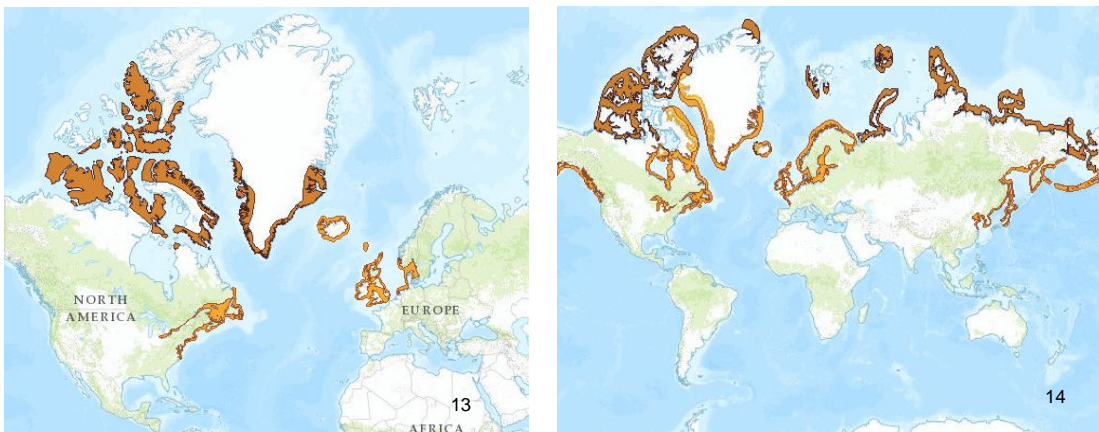
Figure 15 – *Larus cachinans:* Pair of gulls, Jordi Bas Casas. Adapted from: ARKive (2012).
Figure 16 – Juvenile, Mike Read. Adapted from: ARKive (2012).
Figure 17 – Adult and Juvenile, Mike Read. Adapted from**:** ARKive (2012).
Figure 18 – Adult, Fabrice Cahez. Adapted from: ARKive (2012).
Figure 19 – Species range. Adapted from: BirdLife International (2013).

Figure 20 – Species range of *Xema sabini*. Adapted from: BirdLife International (2013).
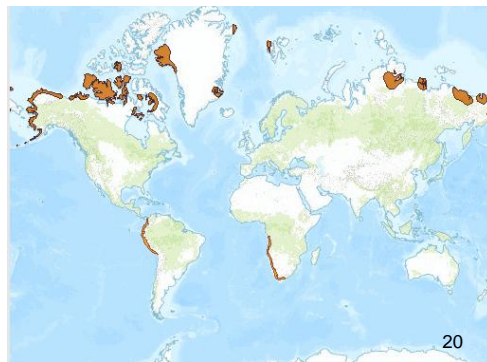
Figure 21 – *Larus genei:* Courtship display, Hellio & Van Ingen. Adapted from: ARKive (2012).
Figure 22 – Juvenile first winter plumage, Richard Brooks. Adapted from: ARKive (2012).
Figure 23 – Immature, Yossi Eshbol. Adapted from: ARKive (2012).
Figure 24 – Winter plumage, Richard Brooks. Adapted from: ARKive (2012).
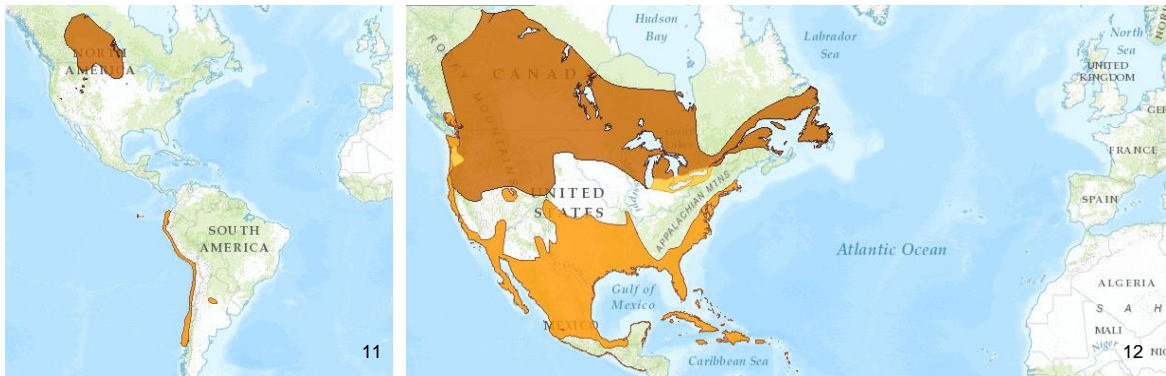**Figure 25** – Species range. Adapted from: BirdLife International (2013).
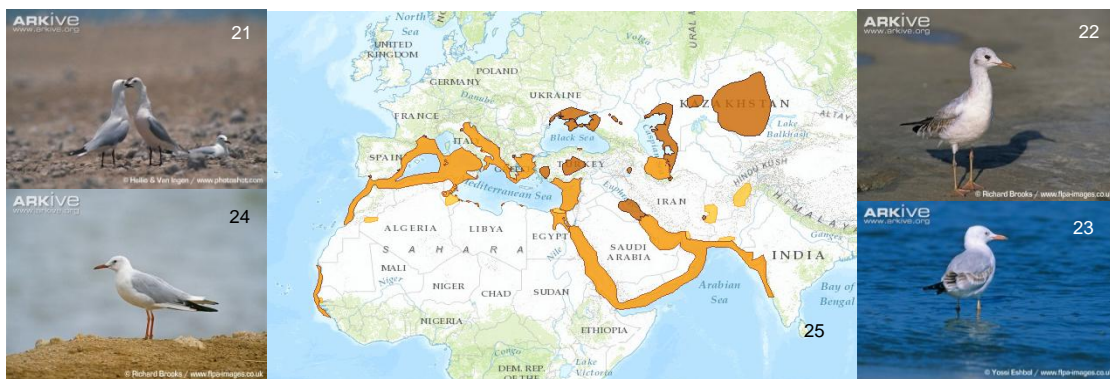
Figure 26 – *Larus audouinii:* Adult at water edge, Jose B. Ruiz. Adapted from: ARKive (2012).
Figure 27 – At nest in a colony, Jose B. Ruiz. Adapted from: ARKive (2012).
Figure 28 – Adult with chicks, Juan Manuel Borrero. Adapted from: ARKive (2012).
Figure 29 – Gulls on posts, Mike Read. Adapted from: ARKive (2012).
Figure 30 – Species range. Adapted from: BirdLife International (2013).

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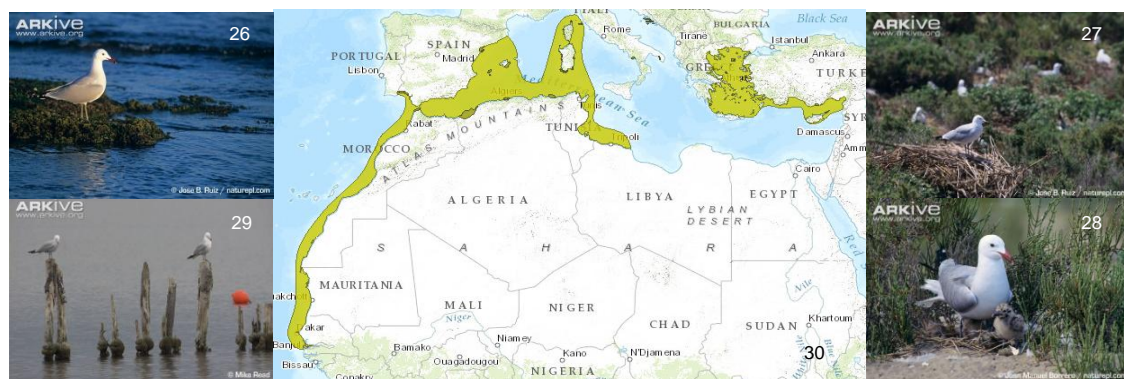
Figure 31 – *Larus argentatus:* Adult with chicks, Doug Wechsler. Adapted from: ARKive (2012).
Figure 32 – First year gull, David Cottridge. Adapted from: ARKive (2012).
Figure 33 – Attempting to steal from *Ardea herodias*, Kerstin Hinze. Adapted from: ARKive (2012).
Figure 34 – Adult, Chris O'Reilly. Adapted from: ARKive (2012).
Figure 35 – Species range. Adapted from: BirdLife International (2013).
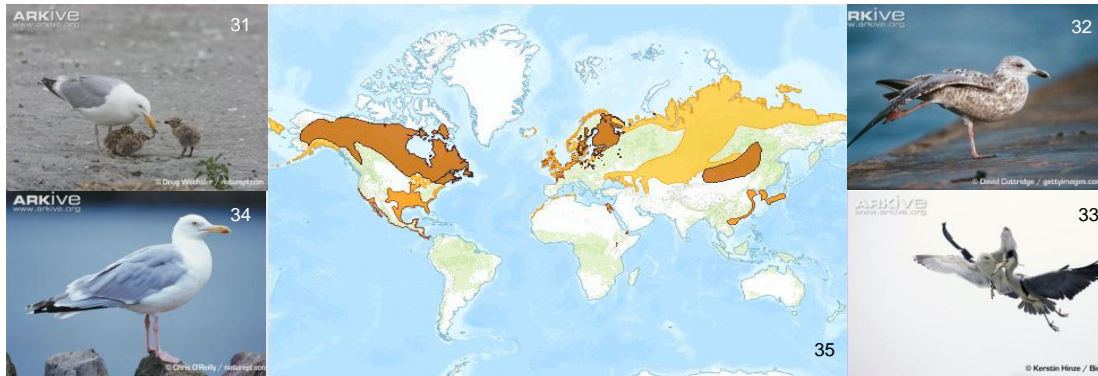
Figure 36 – *Larus canus:* Chick with adult, Mark Newman. Adapted from: ARKive (2012).
Figure 37 – Juvenile, Steve Young. Adapted from: ARKive (2012).
Figure 38 – Species range. Adapted from: BirdLife International (2013).
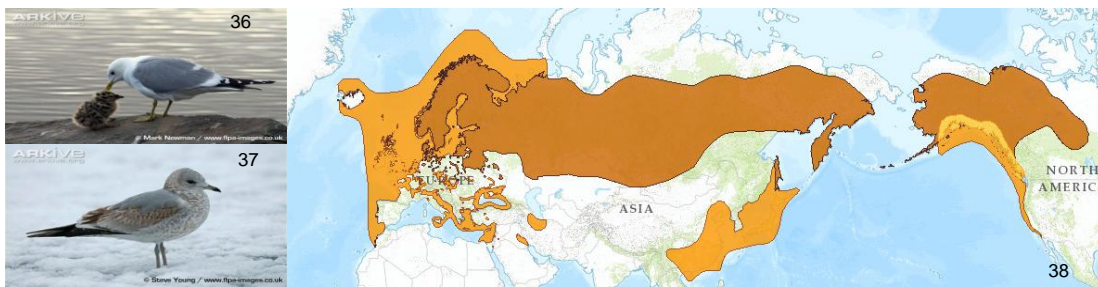
Figure 39 – *Larus minutus:* Non-breeding plumage paddling in water, Frank and Joyce Burack. Adapted from: ARKive (2012).
Figure 40 – Breeding plumage, Markus Varesvuo. Adapted from: ARKive (2012).
Figure 41 – Immature standing in water, Markus Varesvuo. Adapted from: ARKive (2012).
Figure 42 – Juvenile flock, Markus Varesvuo. Adapted from: ARKive (2012).
Figure 43 – Species range. Adapted from: BirdLife International (2013).
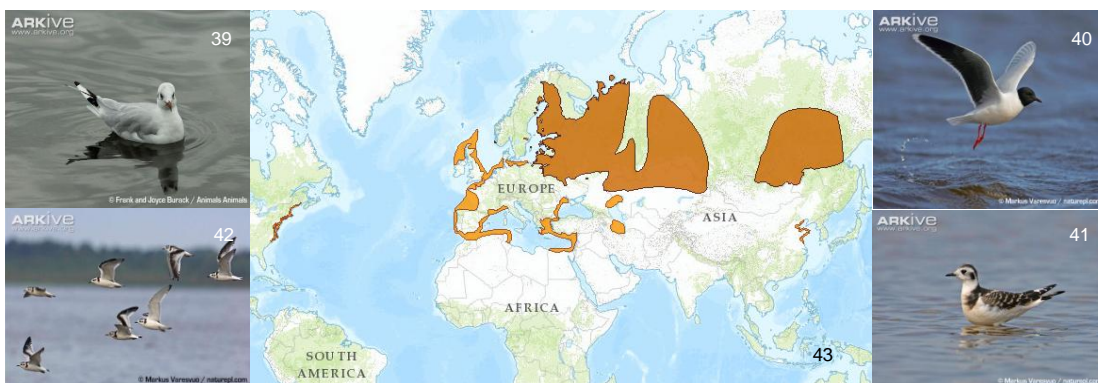
Figure 44 – *Larus marinus:* Breeding plumage feeding young, G. K. Brown. Adapted from: ARKive (2012).
Figure 45 – Swallowing *Alca torda* chick, Paul Hobson. Adapted from: ARKive (2012).
Figure 46 – Juvenile feeding on fish, John **Cancalosi. Adapted from: ARKive** (2012).
Figure 47 – Taking off, Graham Eaton. Adapted from: ARKive (2012).
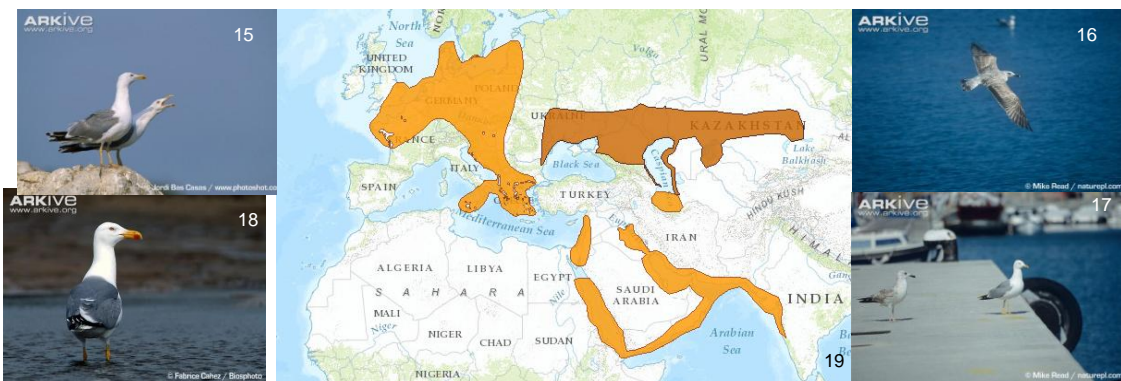**Figure 48** – Species range. Adapted from: BirdLife International (2013).

Figure 49 – *Rissa tridactyla:* Defending territory,Kenneth Day. Adapted from: ARKive (2012).
Figure 50 – Adult and chicks, Pete Oxford. Adapted from: ARKive (2012).
Figure 51 – Adult and fledglings, Doug Allan. Adapted from: ARKive (2012).
Figure 52 – Roosting on a iceburg, Tui de Roy. Adapted from: ARKive (2012).
Figure 53 – Species range. Adapted from: BirdLife International (2013).

Figure 54 –*Larus melanocephalus:* Courtship (male feeding female), Johan de Meester. Adapted from: ARKive (2012).
Figure 55 – Feeding of the chicks, Thomas Roger. Adapted from: ARKive (2012).
Figure 56 – Juvenile, Roger and Liz Charlwood. Adapted from: ARKive (2012).
Figure 57 – Winter plumage, Neil Bowman. Adapted from: ARKive (2012).
**Figure 58** – Species range. Adapted from: BirdLife International (2013). Adapted from: ARKive (2012).

Figure 59 – *Chroicocephalus ridibundus:* Winter plumage, Chris Gomersall. Adapted from: ARKive (2012).
Figure 60 – Flock following plough in winter, Chris Gomersall. Adapted from: ARKive (2012).
Figure 61 – Summer plumage, Laurie Campbell. Adapted from: ARKive (2012).
Figure 62 – Carrying nesting material, Alan Williaws. Adapted from: ARKive (2012).
Figure 63 – Feeding from water waste from a fish processing plant, Kenneth W. Fink. Adapted from: ARKive (2012).
Figure 64 – Species range. Adapted from: BirdLife International (2013).
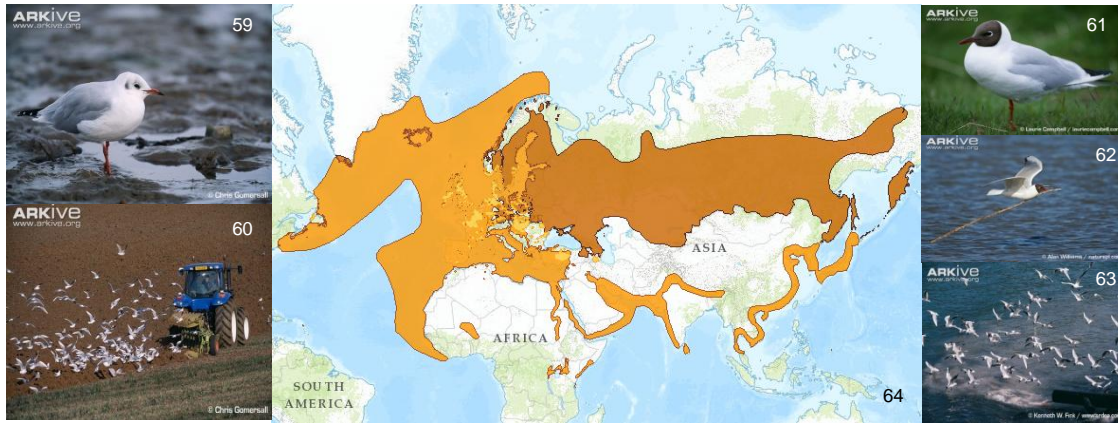
Figure 65 –*Larus fuscus:* Feeding chicks, David & Katie Urry. Adapted from: ARKive (2012).
Figure 66 – Juvenile, Roy Waller. Adapted from: ARKive (2012).
Figure 67 – Hunting and feeding on a rabbit, Brian Hewitt. Adapted from: ARKive (2012).
Figure 68 – Adult, Wild Wonders of Europe. Adapted from: ARKive (2012).
Figure 69 – Species range. Adapted from: BirdLife International (2013).
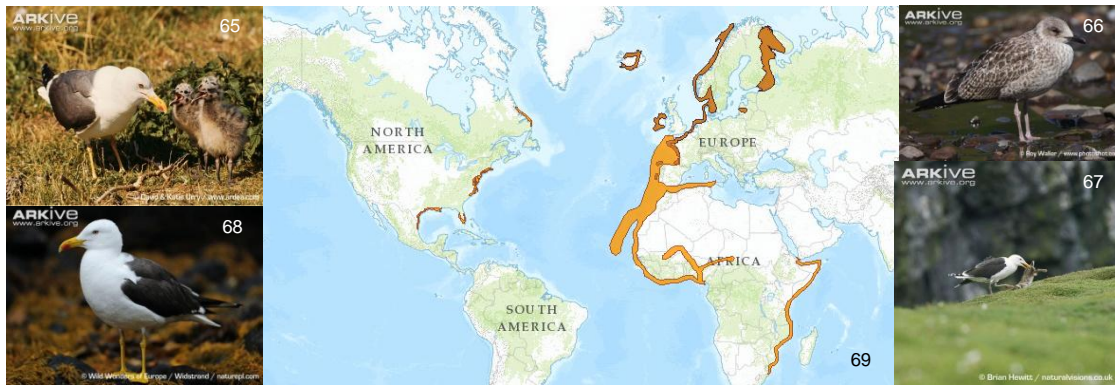
Figure 70 – Species range of *Larus michahellis*. Adapted from: BirdLife International (2013).
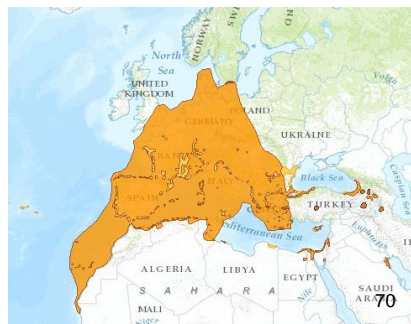
Appendix 2 – Methods.

2.1. Description of the area in study.

Figure 83 – A, Europe EPSG4326:WGS 84, source: Google satellite ®; B, Main regions of continental Portugal EPSG:3763-ETRS89/Portugal TM06, source: CAOP(2013); C, Figure in detail with the Municipalities and Civil parishes of Algarve and the location of RIAS; D, Land use EPSG3035:ETRS89/ETRS-LAEA, source: Corine Land cover 2006 by European Environment Agency (2013).
Note: S.B.Alportel and V.R.S.A. stand for São Brás de Alportel and Vila Real de Santo António.
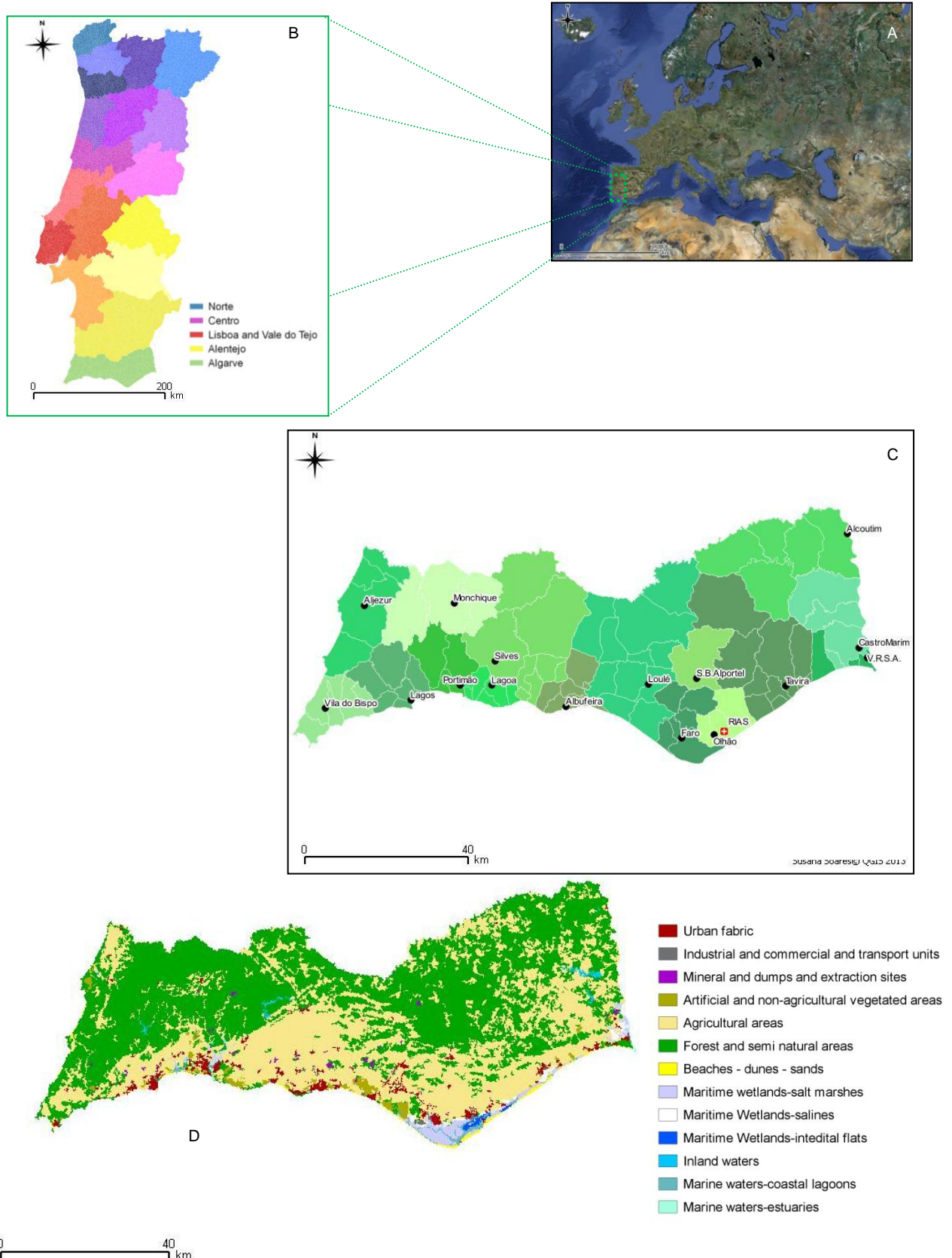
2.2. RIAS and report of the author´s training period.

In the last year of her Integrated Masters in Veterinary Medicine, the author took a training period of six months (between September fifth 2011 and March third of 2012) in the Wildlife Rehabilitation and Investigation Centre of Ria Formosa, RIAS (Centro de Recuperação e Investigação de Animais Selvagens). This centre is one of the ten Portuguese wildlife rehabilitation centres still in activity and the only one serving the south portion of Portugal. It belongs to the Rede Nacional de Centros de Recuperção para a Fauna (National Network of Rehabilitation centres), coordinated by the Institute of Conservation of Nature and Forests (ICNF). Although the physical spaces belongs to the ICNF and Ria Formosa's National Park (PNRF), its management is, since 2009, of the responsibility of a non-governmental association, ALDEIA (http://www.aldeia.org/), in partnership with ICNF and fundings of ANA (Airports of Portugal) by means of a Business & Biodiversity initiative, among other partners. This centre is located in the municipality of Olhão, civil parish of Quelfes, with geographical coordinates 37 2.057'N 7 48.797'O (EPSG 4326:WGS 84). As is explicit in its denomination, the main purposes of this centre are: rehabilitation of native wildlife (and its subsequent release) and investigation, by contributing with data and samples for several studies, thus enabling an increase in the national knowledge of this kind of fauna but also of the risk factors that threaten their conservational status. This institution partakes directly in the reduction or mitigation of such factors, by its participation on diverse projects like the Programa Antídoto (Antidote Program), Projects Life Trachemys (LIFE09NAT/ES/000529) and Life Estepárias (LIFE07NAT/P/654). Additionally, the ringing/marking of all the rehabilitees in the centre and specific ringing projects of mainly anatids, larids and vultures are other important sources for scientific information that are granted by this centre. Another great objective of this centre is linked with the effort that is employed in the education of the common citizen towards the environment, giving primacy to concepts like biodiversity, sustainability, conservation among others related. In this way, this wildlife centre holds several activities of environmental education to raise awareness to the problematic and need for conservation of Biodiversity and Nature in its facilities, but also in a number of external events and facilities like schools, hotels, fairs and other public venues. It allows volunteering work in its premises, participation in the release of the rehabilitated animals, financial adoption of injured animals and the application to several courses and workshops regarding Conservation, Rehabilitation and Identification of Wildlife (all accessible to anyone in its website http://rias-aldeia.blogspot.pt/ and social networks like facebook). In addition to this, RIAS, allows students of the most varied backgrounds and courses, but principally veterinary and biology, to gain theoretical knowledge and practical experience in the rehabilitation of native wildlife, specifically in areas such as: nutrition, food management, clinic examination, surgery, ancillary diagnostic tests, necropsy diagnosis, besides acquisition of other skills through the participation in events/courses/workshops organized by it and also external projects of which the centre is a partner (RIAS 2011, 2012a;2013).

The author´s training period was supervised by Dr. Hugo Lopes, clinic director of RIAS, and co-supervised by Professor Doctor Luís Manuel Madeira de Carvalho of the Faculty of Veterinary Medicine of the University of Lisbon (FVM-UL). The author followed the daily activities of this centre, five days a week (from Monday to Thursday and Sundays) almost 10 hours per day with the exception of Sundays and holidays when the centre was only open for 3 hours. This timetable and the number of days of training per week could be increased and was during this period, particularly when it was

needed, either because of the arrival of debilitated animals outside the normal labouring hours, the need and interest to follow up problematic cases or the high number of treatments to perform in times of high influx of ill fauna.

During this period 260 debilitated animals were admitted in this centre, additionally 39 entered already dead, and 46 were by this time in the rehabilitation process, comprising a total of 345 animals. From the beginning of the training period till completion the class of Aves was the most representative (fig.93) with 266 specimens of 48 different species of several groups (raptors, seagulls, among others).This is probably due to the fact that when diseased, birds are more easily discovered and retrieved. The 3 species with the highest absolute frequencies of admission were *L. fuscus* (f=67), *L.michahellis* (f=38) and *Athene noctua* (f=20). The second classe with the highest number of individuals was the Reptilia (12 *Chamaeleo chamaeleon*; 16 *Mauremys leprosa*; three *Emys orbicularis;* one *Natrix natrix* and one *Tarentola mauritanica*), a finding that was expectable, given the fact that Algarve is one of the Portuguese regions famous for the presence of these chameleons and because the centre is one of the institutions responsible for the Portuguese part of the European Project life + Trachemys. Mammalia and Amphibia classes were the less represented: 4 *Erinaceus europaeus* and 1 *Pipistrellus pygmaeus* in the first; and 2 *Bufo calamita* and 1 *Discoglossus galganoi* in the second, probably owing to, in a certain degree, the typical secretive habits of such animals when ill and possibly to a lesser proneness or increased difficulty in the collection of these animals, particularly in the case of Amphibia.

Figure 93 – Distribution of the relative frequencies of the animals in rehabilitation in the centre by class during the training period (n=306).

Considering the causes or suspected ailments of the retrieved animals, either alive or death admittances in RIAS, upon collection of the medical history and physical examination, traumatism and disease cases were the most usual in these 6 months (fig.94).

Figure 94 – Causes of admission of alive and dead animals between September and early March, during the 6 months, n=345.
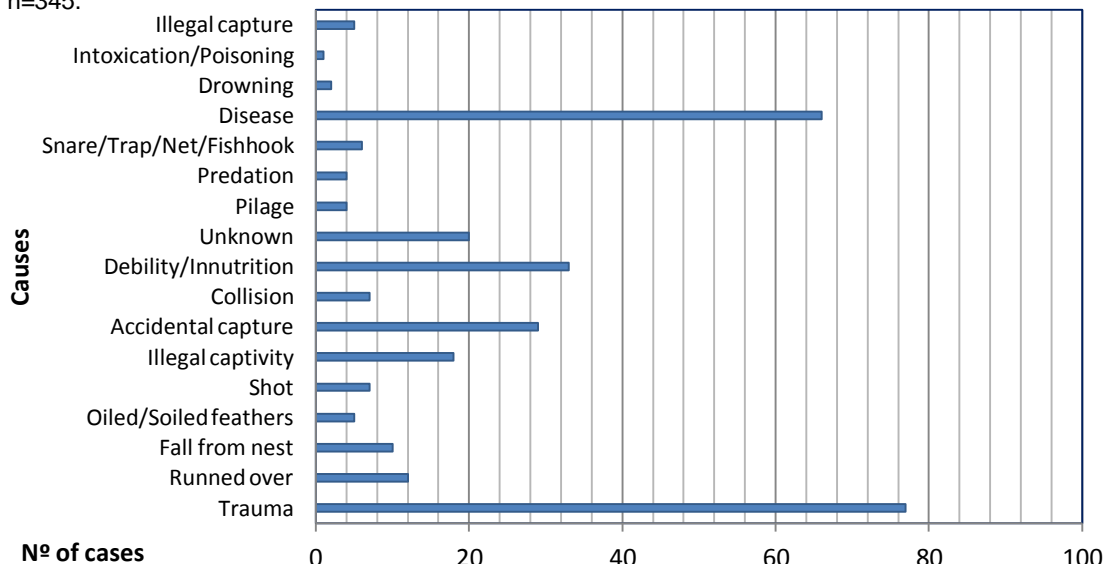
Table 3 – Temporal distribution of the entries, in RIAS during the 6 month training period (September 2011- March – 2012)

Months	Nº of animals admitted n=299
September	85
October	63
November	57
December	21
January	39
February	29
March	5

The distribution by month of the 299 animals that were admitted in RIAS during this training period was unequal, with a tendency to decrease towards the arrival of the coldest season (table 3). One possible explanation for this fact could be the arrival of winter and consequent migrations (including other survival techniques displayed in this season) and/or a diminished probability of retrieval by the common citizen and tourists.

During this period, the author assisted in and gained knowledge and practice in several procedures in 6 main areas: Clinical workout, Laboratory techniques, Rehabilitation, Diagnostic Imaging, Surgery and Necropsy examination. Initially was supervised closely during the course of these by the clinical director, biologists and technical personnel, but with the progression of the days and weeks autonomy was stimulated and developed in all fields.

In the clinic, the procedures followed or performed included: the physical examination; collection of the medical history (when possible); assistance in the physical restrain; determination of the cause of entry; emergency clinical procedures, like fluid therapy, wound debridement, disinfection, stabilization of fractures; institution of the appropriate therapies; maintenance of digital records of the entry, clinical examination findings and therapeutic protocols; and collection of samples for haematology, cytology, microbiology, parasitology and toxicology analysis intended for diagnostic purposes, but some also meant for projects in development in the centre or in partnerships with other institutions or professionals (e.g. dosing of heavy metals). Every Wednesday, a review of all the clinical cases of the week was made in the presence of the director with the aim to: motivate its discussion; the clarifications of doubts; the evaluation of the procedures and therapies; alterations of the nutrition plans, as well as the management of the transfers of animals, to the following stage of rehabilitation. Simultaneously in the recovery room the daily clinical assessment of the animals was performed: weighting, administration of medication or fluids (by oral, subcutaneous, corneal, intramuscular, intravenous or intraperitoneal routes), forced feeding (with flexible or rigid feeding tubes) were performed and physical therapy, changing dressings and bandages were also carried out, when needed. Animals kept outside in moulting or rehabilitation pens, lake and the flight tunnels, were examined visually everyday in the morning and when feeding.

In the laboratory was practiced the following techniques and subsequent diagnosis: blood smear followed by Diff-Quick ® coloration for haematology and parasitology; Gram' ® staining of samples collected in alive and dead animals with the purpose of a microbiology and cytology examination and assessment of the outcome of antimicrobial therapies by light microscopy; and microscopic direct examination of excreta and other techniques such as sedimentation and flotation for the microscopic detection and identification of parasites.

In the Imaging diagnostic sphere, the author gained knowledge and experience in the selection of the appropriate radiographic factors for exposure in different species with distinct problems (but within the medical fields of trauma and orthopaedics), correct positioning and restrain, and manual development of radiographic films.

The author assisted in five surgeries and the follow up of the recovery from the anaesthesia and subsequent full rehabilitation were accompanied.

Eighty seven necropsies were done in addition to forty outside the official training period to collect more data for this dissertation. Some were carried out with the intent to avoid damaging the skeleton or with additional caution to not lesion certain body parts (head, wings and hindlimbs), since they would be subjected to different procedures to cleanse and preserve these tissues for future display in environmental educational sessions organized by the centre, or were given to others institutions with the same goals. The observation and later execution of the necropsy examination of animals belonging to different classes enabled the acquisition of new skills and deepened the author's knowledge regarding the discrepancies in the anatomy of several kinds of living beings, but also the in different techniques applied in the necropsy evaluation. The correct reporting of the findings and collection of biological samples were other practices developed in this area, with special focus in the enrichment of the biological sample bank of RIAS with samples gathered with the aim to be used in the centre, like feathers for imping, or to be part of several studies conducted by numerous collaborators.

At times of low activity, training initiatives like air-sac endoscopy, reduction and fixation methods in traumatic orthopaedic surgery and sessions more specific and with the objective to prepare students to the predictable influx of high numbers of particular species on specific time intervals (like griffon vultures during winter migration) were organized in the centre´s facilities and attended by the author during these 6 months.

Also during this training period the author participated in several activities, courses and sessions organized by RIAS, for the common citizen and RIAS' personnel, and included:

- Several bird ringing sessions of Passeriformes and Wading birds in the centre;
- Official public field trips for bird watching (22nd of October 2011 in Ludo, Faro and 26th of February 2012 in the salt pans complex of Tavira) and numerous ones restricted to RIAS' collaborators in the Natural Park of Ria Formosa, Serra do Caldeirão, Castro Verde, Ludo, Tavira, among others;
- Avifauna Monitoring in the Wastewater Treatment Plant in Salgados, Faro Nascente, to prevent/detect/control outbreaks of botulism;
- Initiation course in the identification and ecology of bats organized in the PNRF on the 17th and 18th of September of 2011;
- Monitoring *Trachemys* sp.'s captures with traps in the project + Trachemys with the on-field responsible in several lakes of Algarve, on the 22nd of September of 2011;
- Initiation course to scientific ringing of birds on the 19th and 20th days of November in RIAS with a ringing session on the 20th of 2011;
- Lectures in the University of Algarve, organized by Biology students (NEBUA), where RIAS participated with 2 oral presentations: the Project Life + Trachemys and the work developed in the wildlife rescue centre, on the 5th of December of 2011.

- Volunteering in the environmental enrichment of the enclosures of the National Centre for the Reproduction of the Iberian Lynx on the 8th of December of 2011;

- Workshop – Pathology, Treatment and Rehabilitation of Great Bustard, Little Bustard and Lesser Kestrel, created by the Project Life Estepárias, in Castro Verde, 15th and 16th of February of 2012;

- Activities of environmental education; Release of rehabilitees and Fund-raising events: Beneficence party on the 10th of December of 2011 in Casa do Povo in the Municipality of Olhão; MOJU's walk in Pinheiros de Marim on the 5th of October 2011; 7th edition of the European Night of the Investigators organized by Ciência Viva in RIASshopping on the 24th of September of 2011;

2.3. RIAS' Clinical Laboratory protocols.

Clinical haematology protocol:

General procedure:

- Identify the sample (code of admission, species and date of sample collection);
- Prepare two blood smears on microscopic slides ("wedge method");
- Record data and findings.

Diff-Quick® and Hemacolor® method:

- Air dry the smears;
- Immerse the smear ten times (for one second each time) in solution number one, a fixative (Methanol);
- Without rinsing submerge six times in solution number two (Eosin);
- Withdraw the slide and immerse it ten times in solution number three (Methylene blue);
- Remove it and rinse it with a buffer solution;
- Air dry the smear;
- Microscopic examination.

Haemoparasites:

- Upon smear and colouring observe the slides for the presence of this kind of parasites with a magnification power of 200x or 400x, initially, then progressing to stronger objectives.

Coprology:

General procedure for the collection of faeces:

- Collect fresh excreta into an appropriate container (plastic sample cup);
- Identify the sample (code of admission, species and date);
- Record data and findings.

Direct Faecal Smear:

- Immerse a sterile cotton swab in physiologic saline;
- Tap it into the sample to collect a small portion of faecal material;
- Perform a smear on a clean slide after mixture;
- Place a coverslip on top and exert careful pressure until it lays flat;
- Microscopic examination (visualize with objectives 10x and progress to 40x).

Faecal Simple Flotation Technique:

- Take a portion of the sample from the container and place it in a test tube;
- Add 2 mils of a sucrose saturated solution (355ml of water plus 454g of simple sugar);
- Homogenate the sample;
- Filter this solution through gauze to another test tube;
- Add saturated solution to the test tube until completion with the formation of a meniscus;
- Place a coverslip on top of the tube, allow the tube to stand vertically in the rack with the suspended coverslip for 15 minutes;
- Carefully remove the coverslip and mount it on a clean slide;
- Microscopic evaluation.

2.4. Necropsy procedure.

Necropsy technique performed on paretic gulls:

 - External examination: main objective was to evaluate the animal globally.

 -Body score was evaluated observing the pectoral muscle development. Later, and in conjunction with the abundance of subcutaneous, mesenteric and coronary groove fat, the score was determined in a scale of 1-5 in accordance with the sequence of cachexy-emaciation-thin-normal-fat;

 -Integument was examined for: the presence of external parasites; its integrity (ulcers, lacerations, oedema, erosions, plaques, nodules, exudates, inflammation, foreign bodies); conservation aspect, cleanness and distribution of feathers and skin in the body and limbs, including the preening gland;

 -Wings and legs were examined by taking into consideration their several elements (bones, muscles, articulations, tendons, digital and metatarsal pads) for signs of: asymmetry, trauma, luxations, fractures, callus, inflamation, abnormal range of movement and tonus, effusions, oedema, deformations, bumblefoot…

 -Vertebral column was observed in order to identify lesions of trauma, malformations, inflammations, luxation and fractures;

 -Cloacae, its appearance: tonus, external sphincter patency and degree of soiling of the involving region with characterization of the exteriorized contents, principally nature, colour, consistency and odour, were the features analyzed;

 -Head was the next region to be the focus of this technique and deviations in the symmetry and fractures were looked for. Observation of the eyes and oral cavity was the following step. The beak was examined as to its colour, symmetry and articulation. The last external structures to be assessed in this group were the nostrils and nasal sinus by pressure.In the case of the nasal cavities, if there was a suspicion of a possible involvement of the respiratory apparatus, a transversal cut in the least resistant zone of the beak was equated.

- Opening of the body: with the animal in dorsal recumbency, 97% ethylic alcohol was used to moistened feathers in the sterna and ventral regions (decreasing contamination, limiting distribution of feathers during the procedure and aiding in the subsequent steps of the necropsy). In the next stage, pinching the keel's skin with forceps and cutting it with a scalpel, by digitoclasia, this initial cut was extended laterally and simultaneously cranially and caudally from the medial plane in order to expose the muscular body wall. This procedure is an alteration from the usual methods, and was applied to

prevent lesion of the viscera of the lower celoma – place where the first incision is frequently performed – allowing, additionally, the assessment of a possible dehydration by the detection of a strong adhesion of the skin to the adjacent tissues as Rose et al. (2006) state. In this phase, an evaluation of the subcutaneous fat tissue and muscular development was made (symmetry, colour, texture, consistency, surface and lesions) in addition to the conformation of the keel bone, confirming the previous body score attained. By extending the peeling of the skin to the knees joints, the thighs' adductor muscles dissection was carried out bilaterally to visualize part of the sciatic plexus (colour, dimensions, texture and thickness). After making a serial of transversal incisions of the pectoral muscles with a scalpel to look for lesions (the next step), a complete dissection of the same was performed. Afterwards the posterior portion of the celomic cavity was opened by a careful incision, with a scapel, of the skin and "abdominal" musculature (posterior to the keel). In this stage, the evaluation of the abdominal air sacs (walls – thickness and possibly the presence of anomalous contents), the abdominal organs, presence of intracelomic liquid/fluid, presence/absence of fat deposit, its degree of development and features (coloration and consistency) was possible. The keel was then taken out with any remaining muscle tissue, but always with cautious and trying to observe the thoracic air sacs as they were being destroyed in the process. While removing the keel, accidental severing of the heart was avoided by using a pair of blunt scissors, always cutting the cartilaginous portion of the ribs in order to aid this step (zone of least resistance) and prevent the incision of the lungs. In the coracoid and furcula region the cut was prolonged with shears.

- As soon as the celomic cavity was exposed, the observation of all the organs in terms of dimensions, relative positioning, adhesions and the presence of fluid and fat in the cavity and its features (formerly stated) could be clearly assessed and the previous recorded findings could be confirmed. Examination of the integrity of the celomic serosa and possible lesions was also accomplished in this step.

- Individual examination of the different organs, apparatus and systems.

The following sequence was executed:

1. Identification and evaluation of the thyroids and parathyroids in the branchiocephalic branch in close proximity to the carotids and jugulars (colour, dimensions, consistency, surface were evaluated), conjoint with the identification of the brachial plexus and assessment of its features (colour, dimensions, texture and wall aspects);

2. By pinching and severing the main vascular branches and fascias, with a pair of scissors, the heart was removed. Size, colour, consistency, surface, fat deposits, and pericardial sac (wall and content) were the characteristics noted. With a transversal cut in the apex, followed by two perpendicular cuts, each in one plane of the heart comprising the two ventriculi and atria, the interior was displayed and the endocardium visualized – surface, mural thickness, valves and chamber diameter and lesions when deviating from normality were recorded. The main blood vessels of the regions: carotids, pulmonary artery and aorta were also observed for alterations.

3. Liver: dimensions, surface, colour and consistency were assessed *in situ*, as well as the gall bladder (repletion, content and patency), and afterwards when exteriorized by incision of the adherences with a scissor, while gently supporting the organs with the fingers. With transversal cuts of the liver the analysis of the integrity of the hepatic parenchyma and biliary system was possible;

4. By holding the caudal portion of the oesophagus with forceps and with a rotation of the proventriculus on its long axis, the spleen was visualized (size, surface, consistency and colour were noted). Pinching the insertion of the rectum in the cloaca and the "thoracic" oesophagus and cutting the extremities with a scissor, the gastro intestinal tract and annexes were, almost completely, removed from the body for further examinations through incision of the normal adhesions to the cavity. Lining of the tract via incisions on the mesentery was performed afterwards. Dimension, colour, surface and features of the serosa were observed and by a longitudinal incision of the tract with a scissor the mucosa was studied (colour, surface and content). In this step the observation of the Fabricius' bursa (on the ceiling of the cloaca) was attempted. This last structure was only meticulously inspected, along with the cloacae, further on the necropsy procedure.

The examination of this tract by a longitudinal cut of its several portions could be made in this moment, if it was imminent that the waiting and consequent autolysis would hinder the observations, or if it was a case whereupon it was believed that the pathogenic agent could be present in these organs. However the author opted to proceed with this step in the finale of the necropsy, because the diarrhoea was abundant and she was concerned with the risk of dirtying the working field, but also because other differentials were in consideration and some could affect the nervous system - other tissue sensitive to the early effects of autolysis.

5. Observation of the lungs *in situ*: colour, surface, consistency and general appearance were features assessed. For a more duteous observation, its removal from the body was performed. This consisted in gently grasping with forceps the caudal portions of the lungs, where the adhesion to the costal wall was minimal due to the existence of a pleural space (Buckles et al. 2012), while gently peeling out them from the wall with scissors. Next, small incisions of the parenchyma were executed with a scalpel, while visualizing the principal bronchi to detect lesions. This was followed by a floatation technique (pneumodacimasia proof) to confirm potential findings of oedema or lung consolidation.

6. Kidneys, gonads, adrenal glands were regarded next. Dimensions, presence of lesions, surface, consistency and colour were regarded, and in the case of adults the determination of the sex was done, when possible. By cutting the adhesions and removing the cranial and medial portions of the kidneys with a scissor (part less adherent to the celomic cavity, Buckles et al. 2012), the lombo-sacrus plexus were exposed for evaluation (colour, texture, dimensions and thickness).

- The neck's skin was retracted and with a scissor, in the oral commissure, a cut was extended to the thoracic inlet to reveal the palate, palatine fissure, tongue, pharynx, and oesophagus (colour, size, surface and features of the: mucosa, contents and serosa, these last ones in the oesophagus when opened longitudinally, were taken into consideration). With a longitudinal incision of the trachea and syrinx the same criteria were noted as in the oesophagus. In the proximity of these two structures and next to the jugular veins, in the case of young birds, the thymic lobes were evaluated in terms of colour, dimensions, surface and consistency.

- Central Nervous System: with the animal on sternal recumbency, after the lateral retraction of the skin in the dorsum with the scalpel, the vertebral column was studied and possible lesions that may have been detected on the external examination or upon removal of nearly all the viscera in the dorsal

recumbence positioning were further examined. In the cases where evident alterations were present, the cut of the vertebra could be performed for a direct visualization of the medulla. By cutting the muscles in the area between the scapula and the column, the brachial plexus were observed and some of its aspects recorded when different from the normal findings, namely colour, dimensions, thickness and texture. Finally for the observation of the brain: the atlanto-axial disarticulation was accomplished; aided with a scalpel, the retraction of the skin followed; and by the use of a saw a superficial incisional line was created in the cranium. These were the preliminary procedures to expose the brain and with this line (diminishing the resistance) a knife was used to medially bisect the cranium and brain. Colour, size, consistency, the brain's surface and meninges, in addition to the emergence of the principal nerves, where studied for alterations.

- Joints.

Opening of the main articulations (wrist, shoulder, hock, stifle, hip) was executed, trying to assess by comparison with the normal and with the contralateral limb: dimension; articular capsule; synovial liquid – colour, quantity and viscosity; bone articulation zone and cartilage (colour and surface), tendons and ligaments. Mineralization of the bone was studied by exerting pressure over the tibiotarsus till the breaking point. Colour and consistency of the bone marrow, alongside with the thickness of the articular cartilage by percussion (with a scissor), were taken into consideration.

- The final step, as previously mentioned, was the observation of the cloaca and Fabricius' bursa (this one when was possible): dimensions, wall and mucosa - colour, thickness, surface, presence of lesions and/or content were examined.

Table 4 – Description of the normal appearance and features of several organs/tissues observed while performing a necropsy evaluation of healthy avian species.

Organ /tissue	Normal
Integument	Skin: Translucid and thin of variable pigments, without exudates and with few crusts. Normal moulting pattern. Beak and nails: integrity is maintained. Preening gland: from nonexistent to extremely well developed, like the case of birds of the family Laridae (Samour 2000).
Eyes, external acoustic meatus nostrils, oral cavitiy, nasal sinus and cloaca	Eyes:Translucid and convex cornea (recent death), white sclera, bright conjunctiva, round pupils, and irises are uniformly coloured. Nostrils: Discharge and content free. Oral Mucosa is pink without lesions. Nasal sinuses: without content and with shiny walls. Cloaca of variable sizes (depends on the content). Has two folds that separate the inner compartment in three chambers (urodeum, coprodeum and proctodeum) and its serosa and muscosa are smooth.
Nervous system	Cranium: smooth surface is white and shining (can be red due to post-mortem congestion or in cases of haematopoiesis). Meninges: semi-opaque. Brain: lissencephalus, creamy of a white/rosy colour, rostrally pointed and rounded posteriorly (symmetric). Nerves: bilaterally symmetrical, cream white, of smooth surface but also striated.
Muscles	Turgid, brownish red and symmetric.
Joints and Bones	Shining articular surface and the capsules are white and smooth. Synovial liquid clear and yellowish, slightly viscous and is present in a small quantity. Thin synovial membranes are a normal finding. Shining and smooth white to slightly bluish cartilage is seen. Ressonance at percurssion: dull sound. Bone Mineralization: when normal, under pressure breaks. Medulla is red in younger birds and yellow in adults, if red in the latter is indicative of haematopoiesis, but in either cases has always a gelatinous consistency.

Table 4 (continuation) – Description of the normal appearance and features of several organs/tissues observed while performing a necropsy evaluation of healthy avian species.

Oesophagus	Smooth to slightly wrinkled mucosa of bright brown to a tan tint. Larids do not have a crop, in accordance with Samour (2000).
Trachea	Semi-rigid structure. White to pale tan colour, internal and external surfaces are smooth but the external has regular series of ridges concordant with the presence of complete cartilaginous rings. Lumen is whitish and free of content.
Thymus	From pale rose to grey, multilobar (4-7) and bilateral. Regress with age, despite suffering hypertrophy after the reproductive season in some species.
Celomic cavity	Bright and glistening serosa. Nearly absence of fluid. Contains fat deposits.
Air Sacs	Translucent walls, without content, but can be slightly opaque after death.
Thyroid e parathyroid glands and ultimobrachial gland	Tyroids: 2 ovoid organs, dark red, of smooth surface and fine granular texture. Parathyroids: 2, ranging from pink to yellow, caudal to the thyroids, but smaller. Ultimobrachial gland (2) are not observable.
Heart	Uniformly dark red. Smooth external and internal surfaces. Thin, smooth, translucid pericardium with small quantities of fluid in the pericardial sac. Fat in the coronary sulks is firm and white/yellowish.
Lungs	Bright to dark pink, of fluffy consistency and collapsible. Autolysis alterations: congestion – dark red and wet consistency. Difficult to cut. Pneudocimasia proof: lung fragments float in water.
Liver and Gall bladder	Liver: 2 lobes with sharp edges. Are red to dark brown, although uniform. Firm and with a glistening smooth surface. Yellow greenish to dark green coloration of the hepatic tissue and duodeno in contact with the gall bladder is a normal finding owing to post-mortem imbibitions. Bladder: pyriforme and dark green in the species where it exists.
Proventriculus and Ventriculus	Proventriculus: with thin wall, muscular tissue is not pronounced, distensible organ when with contents and if empty is equal in diameter to the oesophagus. Mucosa is smooth and bright brown to a tan tint of a glandular aspect, can contain small quantities of mucus (white to brownish). Proventriculus and ventriculus junction: is almost imperceptible externally. Ventriculus in piscivores or carnivores: fusiform, thin walled, and there is not a demarcation from the proventriculus. Ventriculus in omnivores or grain-eaters: muscular wall well developed and koiline mucosa is frequently greenish due to impregnation by bile. Contents in these organs vary with the time after feeding: mucous to small portions of the food items.
Spleen	Dark red to purple, smooth surface and uniform. Several shapes are possible from round to elongated.
Small intestine	Serosa: Pale pink to brown. Smooth and glistening surface with visible blood vessels of red to purple colouring, but they are not prominent. Autolysis and post-mortem congestion can occur and turn them more reddish or even black. Rose to brown smooth mucosa. Presence of ingesta is possible and when it exists is bright bronze to mucous yellow in the proximal duodeno; in close proximity with the biliar ducts is greener; increased thickness is seen in jejunum and ileum; in the ceca the volume is superior and ingesta is dark green, thick and has an earthly odour.
Pancreas	Consistently whitish to rosy brown, firm and amorphous.
Large intestine	Large intestine is shorter than the small. Ceca: can exist or not, be composed by 1 or two strutures with dimensions that also vary with the species. When present: thin walled, same coloration of the other portions with greenish contents. Tonsils: almost undistinguishable.
Adrenals	Two globular structures, yellow and cranial to the kidneys.
Testicles and copulation organ	Two testicles with the format of beans ranging from elongated tubes to cylindrical shapes. The surface is white and smooth. Colour varies from light grey to black. Copulation organ: vestigial in most species

Table 4 (continuation) – Description of the normal appearance and features of several organs/tissues observed while performing a necropsy evaluation of healthy avian species.

Ovaries, oviducts and follicles	Mature birds still in activity: • Ovaries: of progressive increment in size that depends on the age of the bird and reproduction stage. Follicle: yellow with the same size pattern. Overall, has a "cluster of grapes" appearance. • Oviduct (left): prominent, increases in size, is flaccid, hollow, white, with visible vascularisation. Lumen has a wrinkled surface. Young or geriatric birds: • Ovaries and follicles: Quiescent. Follicles are hardly seen or are small with a granular appearance • Oviduct (left): white and of small calibre. (Right oviduct with the same features only exists in some Falconiformes and in the *Apteryx australis*).
Kidneys	Two, each with 3 lobes, are uniformly dark reddish brown and exhibit a superficial granular texture. Urates in ducts is normal (as long as they are not so prominent).
Fabricius's bursa	Regresses with age after a peak in its development between the 4 and 12 weeks, and so is undistinguished in adults. Has a spherical format or is spindle-shaped, of light colour with parallel mucosal wrinkles and of creamy white appearance.

Adapted from: Rose et al. (2006) and completed with data and findings of: Munson (2000); Samour (2000); Tully Jr., Dorrestein & Jones (2000); Work (2000); Davis & Morishita (2001); Australian Government – Department of the Environment and Heritage (2006); Coles (2007); and Buckles et al. (2012).

2.5. Samples and specimens sent to the Faculty of Veterinary Medicine of the University of Lisbon.

Table 5 – Specimens and samples sent to the faculty and diagnostic techniques used.

Specimen code	Specie	Age	Origin	Date of Admission-Death	Date of expenditure-examination	Samples	Procedures
V0664/11/A	*L.fuscus*	Sub	Portimão	4/10/11-5/10/11	Nov/11-Jan/12	carcass	Necropsy Toxicology
V0666/11/A	*L.michahellis*	Juv	Albufeira	5/10/11-7/10/11	Nov/11-Jan/12	carcass	Necropsy Toxicology
V0716/11/A	*L.fuscus*	Juv	Portimão	4/11/11-6/11/11	Nov/11-Jan/12	carcass	Necropsy Toxicology
V0717/11/A	*L.fuscus*	Juv	Loulé	4/11/11-6/11/11	Nov/11-Jan/12	carcass	Necropsy Toxicology
V0772/11/A	*L.fuscus*	Sub	S.B.Alportel	5/12/11-10/12/11	Feb/12-Mar/12	carcass	None
V0010/12/A	*L.fuscus*	Sub	Faro	11/01/12-13/01/12	Feb/12-Mar/12	carcass	Necropsy Toxicology Bacteriology Parasitology
V0731/11/A	*L.fuscus*	Juv	Loulé	11/11/11-17/11/11	May/12-Jul/12	carcass blood smear	Necropsy Toxicology Parasitology
V0761/11/A	*L.fuscus*	Ad	Loulé	22/11/11-25/11/11	May/12-Jul/12	carcass	Necropsy Toxicology
V0071/12/A	*L.fuscus*	Ad	Olhão	02/03/12-04/03/12	May/12-Jul/12	carcass	Necropsy Toxicology
V0605/11/A	L.fuscus	Juv	Portimão	14/09/11-released	Nov/11-Nov/11	blood smear	Parasitology

Note: Juv, Sub and Ad stand for juvenile, sub-adult and adult, respectively.

Histopathology technique:

When macroscopic alterations were found during the necropsy examination and depending on the decomposition degree of the carcass, samples of several tissues or organs were collected and fixed in

10% buffered formalin and later embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopic observation.

Bacteriology and Parasitology procedures:

A fragment of the intestine of gull V0010/12/A was routinely cultured for the isolation and identification of aerobic and strict anaerobic pathogenic microorganisms, based on Quinn, Carter, Marker and Carter (1994) and *Salmonella* was searched for using the same protocol of Duarte et al. (2002), but with the exception of the use of the Selenite-cystine broth as a selective enrichment medium, which was replaced by the MKTT (Mueller-Kauffman Tetrathionate medium base). Parasitology examinations were performed in the necropsy and faecal samples collected of gulls V0666/11/A, V0664/11/A, V0716/11/A, V0717/11/A and V0010/12/A, with the same methodology used by Magalhães, Gonçalves, Afonso-Roque and Madeira de Carvalho (1998).

Toxicological analysis methods:

-Heavy metals: Copper and Lead were searched for in samples of liver of specimens V0664 and V0666.

After homogenization and weighting, liver matrixes remained in a muffle furnace at 500ºC, for 12 hours. When cooled, nitric acid at 65% was added. Samples were run with standard calibration curves and spiked specimens with a flame atomic absorption spectrophotometer (Perkin Elmer Analyst 700), whose detection and quantification limits were of 0,1 micrograms per mil and 0,2 micrograms per mil, respectively, for both metals. Results are in parts per million in wet weight (w/w).

-Pesticides: Carbamates and Organophosphorous pesticides were searched for in liver samples of specimens V0716, V0717 and Strychnine in stomach contents of V0664/11/A. Larids V0010, V0731, V0761 and V0071, in turn, were sampled for all the above.

All the samples were subjected to an initial phase of solvent extraction, which was followed by a Thin-layer chromatography in accordance with the next methodologies:

- Methiocarb: Belas, São Braz, Moreira & Carrapiço (2011)
- Carbamates and Organophosphorous compounds (active molecule: Malathion, Malaoxon, Fenthion, Ethyl-parathion, Methyl-parathion, Dimethoate, Diazinon, Dichlorvos, Mevinphos, Furathiocarb, Fenoxycarb, Carbofuran and Aldicarb): Rodrigues et al. (2005)
- Strychnine: Rodrigues, Moreira, São Braz, Carrapiço & Silva (2006)

2.6. Treatment protocols

General procedure:

The paretic gulls, upon admission, were kept in a calm environment in the recovery room inside transportation boxes, on top of artificial nests made of towels laid on top of a layer of journals, correspondingly to avoid recumbence lesions and decrease the soiling of the box. The doors of these containers were concealed by towels to avoid additional stress caused by the entrance of personnel in the room, when tending other animals. The room has a window that was kept open during the day to not obscure completely this room and compromise the circadian cycle of the birds in rehabilitation. In the winter a heater was turned on to compensate the low temperatures. Water was always present *ad*

libitum and food was placed in the enclosure when the period of acclimatization was over or when it was believed that the gull would try to eat (estimated daily intake of 100 grams).

Fluid therapy was performed for the correction of the dehydration deficit and with the administration of the volume of maintenance needed for the bird (approximately 100 ml/kg/day) by the subcutaneous route. Oral fluids were given, additionally, to maintain the correct function of the gastrointestinal tract, preventing it from drying, promoting its normal movements and possibly diluting the presence and effects of a potential toxin or other etiologic agent still unknown, or aid in its rapid expelling. These last ones were calculated as 5% of the body weight. In the oral administration of fluids, in all the protocols, once the total volume was calculated, half of the determined volume was composed of lactated Ringer solution, and the remaining was completed with a 5% glucose solution. While in the case of subcutaneous injection of fluids, once the volume needed was determined, one third of the total was represented by lactated Ringer solution, the second third was a 5% glucose solution and the remaining was completed with Duphalyte®. Subcutaneous fluids were additionally heated to approximately 38-39ºC in a microwave prior to administration in the inguinal region. For all the protocols, the number of times a day of fluid administration depended on the volume calculated and that could be injected or orally given at each time, thus in cases that required large volumes, frequently on the first day upon admission, the total was divided by several administrations (three times a day-TID, two times a day-BID or once a day-SID).

Protocol C

- Morning admission:

-Oral diluted activated charcoal at 4 grams per 15 millimetres of water BID is given in the first day, upon admission and at the end of the day

-Subcutaneous fluids (TID),

-Oral fluids (SID/BID).

Next day only oral and subcutaneous fluids (BID/TID) are administered.

On the third day, in the recovery room, oral fluids (BID/TID) are administered and food is introduced.

- Afternoon admission:

-Activated charcoal with the same dose (SID),

-Subcutaneous fluids (BID),

-Oral fluids (BID/SID).

Next day gull repeats the activated charcoal. Subcutaneous and oral fluids are prepared respectively for TID and BID/TID administration. On the third day just oral fluids are given plus food (same as previously)

- Night admission:

-Activated charcoal (SID),

-Subcutaneous fluids (SID),

Next day gull repeats activated charcoal, the subcutaneous fluids are TID and the oral ones are given SID/BID. While in the third day oral fluids are forced like in the other cases (TID) and food items are presented.

Protocol F

Subcutaneous fluids are given TID and oral ones BID (initially), then corrected/diminished in the number of times per day in the same manner of protocol C, and in agreement with the necessities and improvements seen.

Protocol T

Neurobion® is injected weekly intramuscularly in the pectorals with a dose of 0,9ml/kg (previously diluted in an equal volume of sodium chloride solution) until full recuperation of the ability to walk, eat, drink and gain weight. In addition, fluid therapy was performed as:

- Morning admittance:

Oral fluids BID and subcutaneous fluids BID

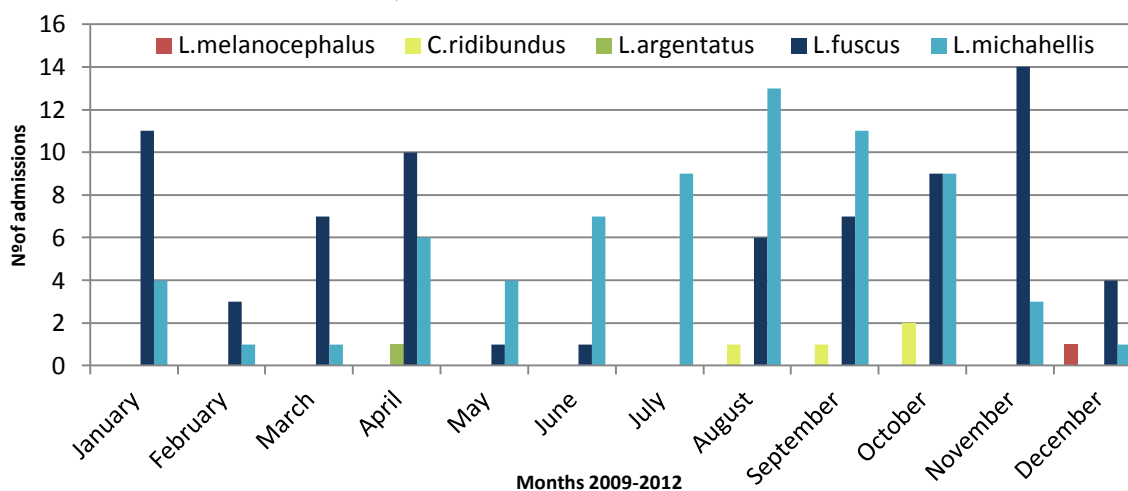- Afternoon and night admissions:

Oral and subcutaneous fluids SID

Appendix 3 – Results and discussion.

3.1. Temporal distribution of paretic cases and Migration.

Figure 103 – Number of cases with syndrome admitted monthly, sub-divided by species retrieved, organized in months of admission from 2009 to 2012, n=148.

3.2. Spearman's rank correlation test results for geographical variables (Table 7).

Variables: density x Proportion of Syndrome	Test value	P-value and S value
Agriculture and livestock holdings	Rho= 0,5067	S= 335,4572 p= 0,0452
Vegetal specialized holdings:	Rho= 0,5304	S= 319,3383 p= 0,0346
-Cereal, oilseed, protein crops;	Rho= -0,5781	S= 1073,075 p= 0,0190
-Other arable crops;	Rho= -0,4222	S= 967,119 p= 0,1033
-Sheltered Intensive gardening;	Rho= 0,2541	S= 507,2197 p= 0,3423
-Open air Intensive gardening;	Rho= -0,2165	S= 827,1938 p= 0,4207
-Other horti/floricultures and ornamental plants;	Rho= 0,1850	S= 554,222 p= 0,4928
-Vineyards;	Rho= 0,0415	S= 651,7918 p= 0,8788
-Fresh fruits and citrus trees;	Rho= 0,4208	S= 393,8884 p= 0,1046
-Olive groves;	Rho= 0,5052	S= 336,4602 p= 0,0459
-Diverse permanent crops;	Rho= 0,5723	S= 290,8438 p= 0,0205
Livestock specialized holdings:	Rho= -0,5170	S= 1031,595 p= 0,0403
-Dairy Farms	Rho= -0,1098	S= 754,6668 p= 0,6856
-Beef cattle holdings	Rho= -0,4151	S= 962,2895 p= 0,1098
-Beef and dairy cattle holdings	Rho= -0,2890	S= 876,553 p= 0,2776
-Diverse herbivores	Rho= -0,2815	S= 871,4127 p= 0,2909
-Pig industry	Rho= 0,1015	S= 610,9851 p= 0,7084

-Poultry industry	Rho= 0,0490	S= 646,6564 p= 0,8569
-Diverse granivores holdings	Rho= 0,0634	S= 636,8657 p= 0,8155
Mixed farms:	Rho= 0,2178	S= 531,907 p= 0,4178
-Polyculture	Rho= 0,0622	S= 637,6877 p= 0,8189
-Mixed livestock: herbivores	Rho= -0,3852	S= 941,9331 p= 0,1407
-Mixed livestock: granivores	Rho= -0,0416	S= 708,2915 p= 0,8784
-Arable crops and grazing animals farms	Rho= -0,3429	S= 913,1494 p= 0,1936
-Mix holdings of crop cultures and cattle farming	Rho= 0,3822	S= 420,0818 p= 0,144
Holdings not classified	Rho= -0,3582	S= 923,5821 p= 0,1731
UAA/km^2	Rho= 0,1437	S= 582,2788 p= 0,5954
Bovine	Rho= -0,2474	S= 848,2417 p= 0,3556
Caprine	Rho= -0,3333	S= 906,6729 p= 0,2071
Ovine	Rho= -0,0474	S= 712,2379 p= 0,8616
Horses	Rho= 0,0252	S= 662,8736 p= 0,9262
Swine	Rho= 0,0593	S= 639,7026 p= 0,8274
Commercial birds	Rho=0,4711	S= 359,6357 p= 0,0655
Rabbits	Rho= 0,1496	S= 578,2491 p= 0,5802
Waste water treatment plants	Rho= -0,5426	S= 1048,993 p= 0,0299
Landfills	Rho= 0,4584	S= 368,2545 p= 0,0741
Mines	Rho= -0,1518	S= 783,243 p= 0,5746
Iron	Rho=0,0528	S= 644,0967 p= 0,846
Copper	Rho= -0,2017	S=817,1863 p=0,4537
Manganese	Rho= -0,4945	S= 1016,265 p=0,0515
Barium	Rho=-0,4945	S=1016,265 p=0,0515
Lead	Rho= -0,3386	S= 910,2248 p=0,1996
Antimony	Rho= -0,3386	S= 910,2248 p=0,1996
Titanium	Rho= -0,3386	S= 910,2248 p=0,1996
Rock Salt	Rho=0,2539	S=507,3314 p=0,3426

3.3. Fisher's exact test p-value results of the association of the clinical signs with Age class and Specie (Table 9).

Presence, extent and severity	Age (n=144)	Species (n=148)
Mental Status	p=0,3641	p=0,6651
Body Score	p=0,04102	p=0,7298
Dehydration	p=0,3452	p=0,6575
Excreta	p=0,1215	p=0,2228
Cloaca	p=0,9818	p=0,1431
Infestation	p=0,2158	p=0,08116
Wing-drop	p=0,1434	p=0,7868
Weakness extent	p=0,5137	p=0,03836
Weakness degree	p=0,4146	p=0,4733
Ataxia	p=0,1767	p=0,2442
Paresis extent	p=0,493	p=0,2389
Paresis degree	p=0,5551	p=0,2
Paralysis	p=0,7814	p=0,4556
Dyspnoea	p=0,9652	p=0,8987

3.4. Necropsy findings in paretic gulls (Table 10).

	Findings and combinations seen	Nº of days in the centre				
		0 n=21	1-2 n=14	3-7 n=24	8-30 n=11	>30 n=3
External Lesions	- Skin abrasions/echimosis/crusts in distal articulations and beak	2	-	4	1	-
	-Bloody wrist and hemorrhagic flank	-	1	-	-	-
	- Extensive hematoma of the left flank	-	-	-	1	-
	-Laceration and perforation of the inguinal region with proliferation of fly's larvae	-	-	1	-	-
	- Subcutaneous vascular congestion	8	5	11	5	-
	-Subcutaneous oedema	-	2	-	1	-
Body score	1	1	1	4	6	1
	2	4	2	13	3	1
	3	6	9	3	2	1
	4	8	1	3	-	-
	5	2	-	-	-	-
Feathers	Dirtied with faecal matter	8	6	10	4	1
Fat	-None, vestigial or in small quantities with engorged vessels	6	4	5	4	1
	-Moderate	10	4	2	2	1
Cloaca	-Impactation, thin walled, congested, distended with faeces/diarrhoea and gas	15	5	8	3	1
	- Distended, wall oedema and active haemorrhage	-	-	2	1	-

**Table 10 (continuation)** – Necropsy findings in paretic gulls.

Skeleton	-Old fractures	-	-	1	1	-
	-Deformations: Keel, pelvis	1	-	-	1	-
	-Recent wrist Fracture	-	-	1	-	-
	-Recent sternum fracture	-	-	-	1	-
	- Recent rib fractures	-	1	-	-	-
	-Severed tendons on the right wrist	-	-	-	-	1
	-Intertarsic capsule and tendinous shealfts distension	-	-	-	1	-
	-Purulent synovitis in the left shoulder and haemorrhage in the left wrist	-	-	-	1	-
Muscles	-Abdominal hematoma	-	1	-	-	-
	-Atrophy	-	-	1	1	-
Celomic cavity	- Free Blood	2	2	2	-	1
	-Fibrin	-	-	-	1	-
	-Other fluids	-	1	1	-	-
	-Congestion	-	2	2	-	-
Air sacs	-Mildly opaque	2	-	-	1	-
	-Opaque	-	-	1	1	1
	-Whitish opaque irregular nodules (0,5-2mm) scattered on the surface	-	1	2	3	1
	- Masses of two cm in cranial air sacs	-	-	-	-	1
	-Black small nodular-like structures scattered in the surface	-	-	-	1	-
	-Perforation of air sacs	-	1	-	-	1
Trachea, bronchi and Lungs	-Vascular congestion of the trachea and bronchi	3	-	-	-	-
	-Blood in trachea or/and bronchi	-	1	1	-	1
	-Lung emphysema	1	-	-	-	-
	-Pale lungs	1	1	-	-	-
	-Congested/Haemorrhagic lungs	5	5	6	2	1
	-Lung congestion and atelectasy	3	-	1	-	-
	-White-yellowish small spherical lesions in bronchi's lumen, lungs and celomic serosa (elastic cartilaginous coalescent and hollow at the cut with haemorrhagic focal points)	1	-	2	3	1
	-Black viscous fluid in trachea and lungs	-	-	1	-	-
	-Plaques with velvety grey-wittish surface in the clavicular air sacs	-	-	-	-	1
Heart	-Pale with unclothed blood	1	1	1	-	-
	-Pale, flaccid with dilated chambers	3	3	2	2	-
	- Haemopericardium	1	1	-	-	-
	-Fluid in pericardial sac	1	-	-	-	-
	-Myocardial congestion	3	1	2	1	-
	-Heart atrophy	2	-	1	-	-
	-Cardiomegaly	-	8	3	-	-
	-White yellowish thickened layer of dry material attached to a thickened pericardium	-	1	-	1	-
	-Adhesions between pericardium and myocardium	-	-	-	1	-
	-Several nodules adherent to the ribs and heart	-	-	-	1	-
Liver	-Hepatomegaly, darkened parenquima with yellowish/brownish spots	1	-	-	2	-
	-Hepatomegaly	-	-	1	-	-
	-Congestion	3	-	-	-	-
	-Necrosis	-	1	-	1	-
	-Atrophy	-	-	1	1	-
	-Pale	-	-	-	1	-
Stomach/intestines contents	-Food	1	1	1	-	1
	-Dark fluid (charcoal)	-	1	1	-	-
	-Blood	1	-	1	1	1
	-Foreign body	1	1	-	-	-

Table 10 (continuation) – Necropsy findings in paretic gulls.

|						
Gastrointestinal Tract	-Oedema, vascular congestion and distension of all the tract with fluid and gas	10	6	9	5	2
	-Hemorrhagic walls with plaque/nodular-like lesions (white and cartilaginous/elastic, with a velvety green surface)	-	-	-	-	1
Pancreas	-Pale	4	-	1	-	-
	-Congestion	1	-	-	-	-
	-Haemorrhagic	-	2	-	-	-
	-White precipitates	1	-	-	-	-
Gall bladder	-Distended with bile	3	3	2	1	-
Thyroids, adrenals	- Pale with haemorrhagic foci	1	-	-	-	-
	-Adrenal hypertrophy	-	1	-	2	-
	-Adrenal congestion	-	-	-	1	-
Spleen	-Pale	-	-	1	1	-
	-Increased	-	1	1	1	1
	-Hypertrophy and congestion	-	1	1	1	-
Kidneys	-Pale	2	1	4	-	-
	-Congested kidneys with engorged vessels	2	2	1	-	-
	- Hollow dark brown and dry nodules, spherical of 4 mm, partially adjacent to the right kidney	-	-	1	-	-
	-Hypertrophy	1	2	1	1	-
	-Pale, hypertrophied, vascular congestion with small white opaque dots of <0,5mm scattered on the surface	-	-	-	1	-
	-Hypertrophy and engorged vessels	-	-	-	1	-
	-Atrophy	-	-	1	-	-
Gender	-male	7	5	6	5	2
	-female	7	7	9	5	1
Decomposition		11	5	12	3	0

3.5. Sightings of RIAS' ringed paretic rehabilitated gulls.

Figure 135 – Maps of the distribution of rehabilitated gulls upon release with ring codes a) Overall map of sightings and flows **b)** Details of the beginning of migration by a *L.fuscus* **c)** Details of Spring migration of *L.fuscus* **d)** Details of *L.fuscus'* Winter dispersions **e)** Details of the dispersions of *L.michahellis* gulls **f)** Details on the possible exploitation of a Landfill by a *L.fuscus* specimen. Sources: DIVA-GIS (2013) and RIAS database.
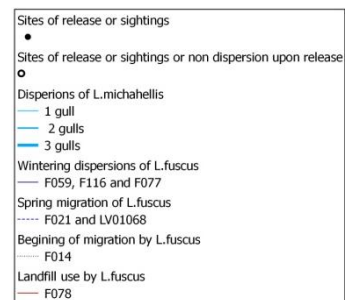
3.6. Interdictions for the harvest and capture of bivalve molluscs for sale and consumption in estuarine and coastal zones of production in Algarve between 2009-2012 and co-ocorrence of paretic cases.

Figures 136 – Zones interdicted for the catch and capture of molluscs for sale and consumption in Algarve (2009-2012), due to the presence of toxin producing organisms or detection of biotoxin's concentrations above the legally implemented values for safety by the European regulation nº853/2004. Source: CAOP(2013) and IPIMA (2011).
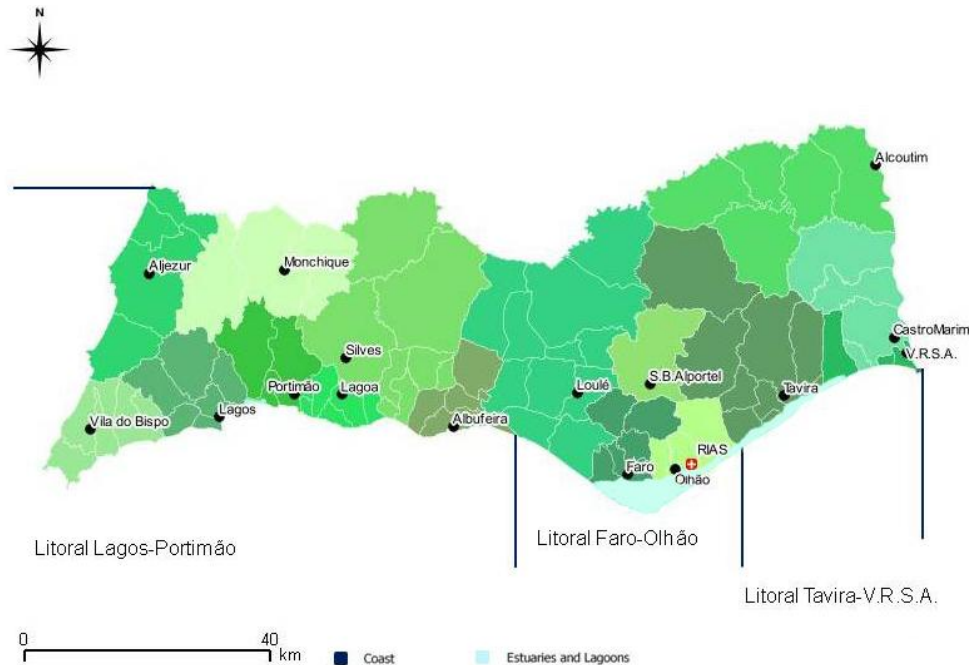

Note: Littoral zones encompass the coast line and the bathymetric line of the 40 meters (not depicted). Estuarine or lagoon regions correspond to the Ria of Alvor (Western) and Ria Formosa (Eastern)

Table 11 – Zones interdicted for the catch of molluscs in Algarve (2009-2012), toxin detected, period of interdiction and number of paretic gulls found during those periods in the affected municipalities. **Sources**: IPIMA (2011/written communication) and RIAS's database.

Year and number of paretic gulls retrieved	Production zone	Interdiction period	Toxin detected	Nº of paretic cases found and rescued
2009 (6)	Ria Formosa	14/04-20/04	ASP	-
		11/08-24/08	PSP	
	Litoral Faro/Olhão	7/04-14/04	ASP	
		13/07-10/08	DSP	
		10/08-24/08	PSP	
	Litoral Tavira/V.R.S.A.	14/04-20/04	ASP	
2010 (42)	Litoral Faro/Olhão	05/05-12/07	DSP	1
	Litoral Tavira/V.R.S.A.	13/07-27/07		-
2011 (84)	Litoral Lagos/Portimão	04/04-26/04	DSP	11
		14/09-26/09		3
	Ria Formosa	30/03-27/04		-
2012 (16)	Litoral Lagos/Portimão	08/08-31/10	PSP and DSP	1
	Rias Alvor	04/09-24/10	PSP	-
	Ria Formosa	21/08-26/10	PSP and additionally DSP in the last 2 months	1
	Litoral Faro/Olhão	16/05-4/06	DSP	-
		04/06-19/06	ASP	1
		06/06-18/06	DSP	-
		01/08-16/11	PSP and DSP	1
	Litoral Tavira/V.R.S.A.	06/06-19/06	ASP	-
		17/08-10/12	PSP and DSP	-

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3.7. Annual distribution of the relative frequencies of the all gulls admitted in RIAS between 2009 and 2012 for all categories, n=780 (Figure 137).
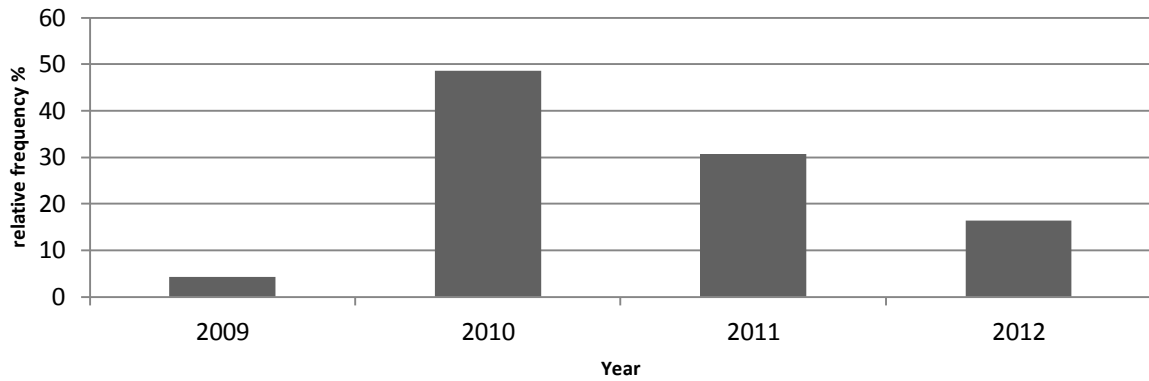

3.8. Different diagnosis confirmed by the physical examination records of all the gulls admitted alive in the 4 years, n=717 (Figure 138).
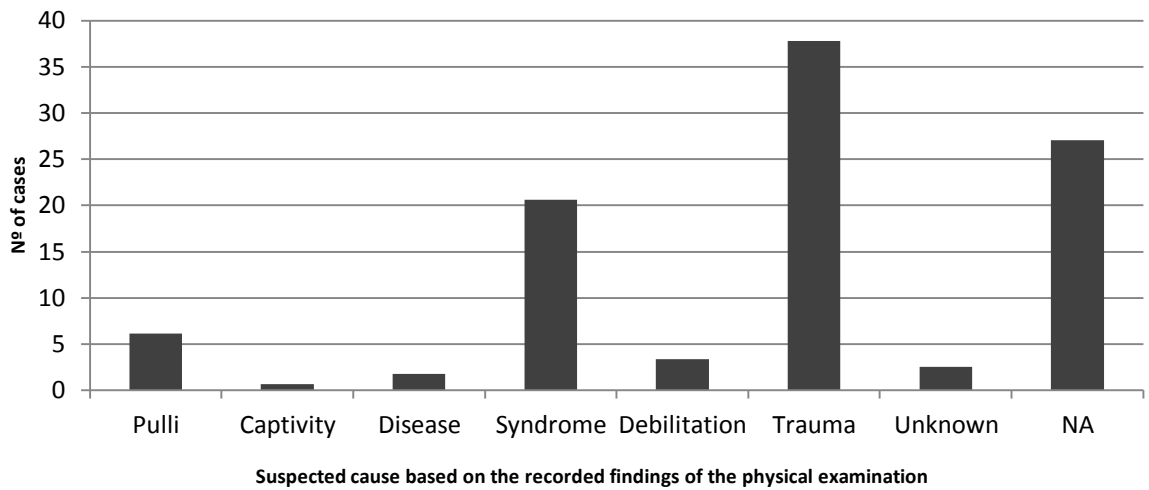

Note: Dead admissions excluding the ones with traumatic evident lesions responsible for the death were excluded. NA stands for cases where there is an absence of logged data of the physical examination

3.9. NA gulls classified with the suspected cause of admission logged, n=194 (Figure 139).
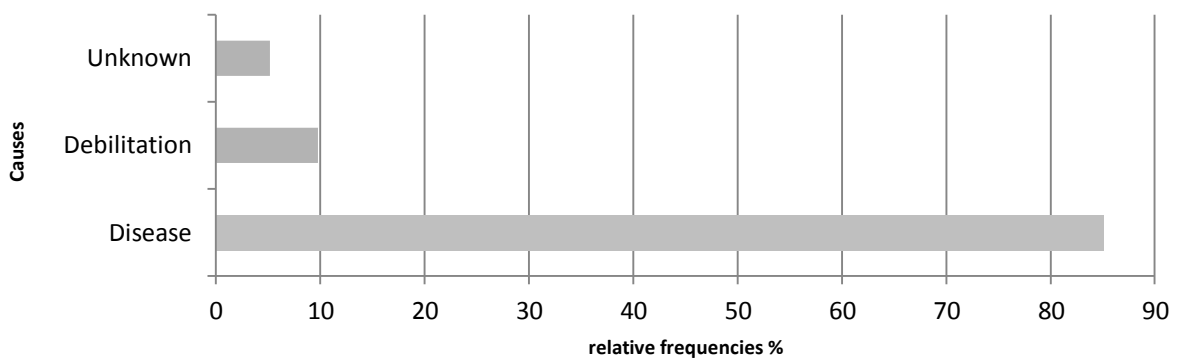
Note: Were excluded NA of pulli, captivity and trauma because they were not considerer dubious.

3.10. Yearly and Monthly distribution of the NA gull cases, n=194, 2009-2012 (Figure 140).
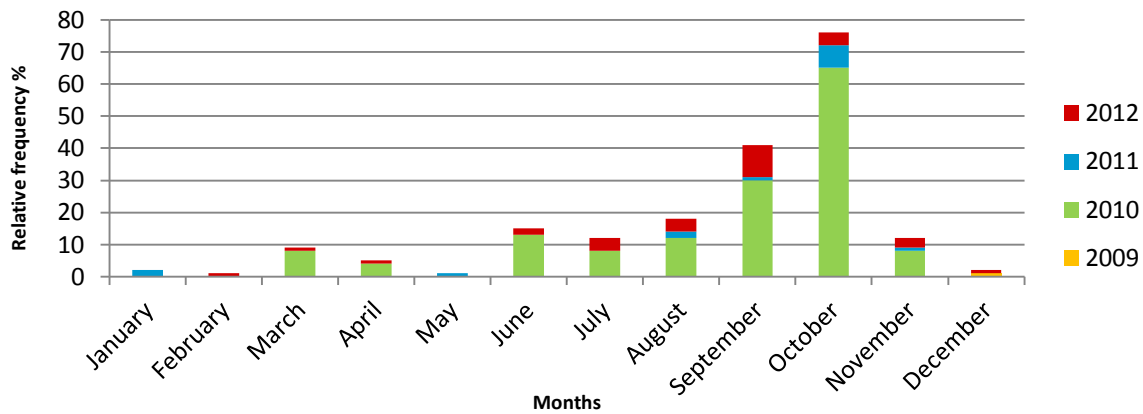
3.11. Temporal distribution by month and year of all the animals rescued or collected admitted in RIAS, 2009-2012, n=3210 (Figure 141).