UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

THE MOST PREVALENT RESPIRATORY AND GASTROINTESTINAL PARASITES IN HERRING GULLS (*Larus argentatus*) ADMITTED IN A WILDLIFE REHABILITATION CENTRE IN SOUTH EAST ENGLAND

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ORIENTADOR:

Drª. Amy Colling

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LISBOA

Dedicatory

To my parents, for everything.

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Parasitas respiratórios e gastrointestinais mais prevalentes em Gaivotas Cinzentas (*Larus argentatus*) admitidas num centro de reabilitação de animais selvagens no Sudeste de Inglaterra

Resumo

A gaivota-cinzenta *(Larus argentatus*) é uma das espécies mais amplamente distribuídas na orla costeira britânica. Nos últimos 40 anos, houve um declínio de 30% na população mundial, seguido de uma diminuição nas zonas naturais de reprodução e um aumento acentuado nas populações de nidificação urbana.

RSPCA Mallydams Woods, é um centro de reabilitação de animais selvagens situado no sudeste Inglês, que tem recebido um número crescente de gaivotas cinzentas que acabam por ser submetidas a eutanásia devido à presença de sinais respiratórios com a suspeita de persistência de um agente parasitário (*Cyathostoma* sp.), previamente identificado em 2004. Até à data, nenhum outro estudo parasitológico na espécie foi realizado pelo centro. Deste modo, um total de 65 necrópsias e análises coprológicas foram efetuadas para avaliar os principais parasitas respiratórios e gastrointestinais envolvidos, bem como as principais alterações patológicas sugestivas de outros agentes infeciosos.

Os resultados gerais mostraram que 32% (21/65) eram positivas à presença de parasitas. Estes resultados incluíam gaivotas que não receberam desparasitante, apresentando 60% de positivos (12/20) e gaivotas desparasitadas, que ainda assim apresentavam 20% de positivos (9/45). Cerca de 24,6% (16/65) apresentavam ovos do tipo dos observados na família Syngamidae, incluindo 2 casos de infeção mista, um com ovos de *Porrocaecum* sp. (1,54%) e outro com ovos de *Contracaecum* sp. (1,54%). Entre estes 16 casos, 44,8% também apresentaram parasitas visíveis na zona infraorbitária da cavidade nasal. Os nemátodes da espécie *Cyathostoma lari* foram detetados em 16,9% (11/65) dos casos e *Syngamus* sp. em 1,54% (1/65). Relativamente ao método de McMaster, demonstrou-se que 23,1% (15/65) das gaivotas tinham >50 EPG, incluindo 9,2% (6/65) das gaivotas desparasitadas. Durante as necrópsias, os sinais de inflamação aguda nos pulmões foram o achado mais prevalente (30%). Períodos de reabilitação mais longos e maior número de doses de desparasitante foram os únicos fatores que mostraram uma associação significativa com a menor prevalência de parasitas.

Este estudo demonstrou que a ineficácia do protocolo de desparasitação, a elevada densidade de animais no espaço existente e a presença de outros agentes infeciosos no centro de reabilitação, são as causas mais prováveis da persistência de sinais respiratórios nas gaivotas admitidas. No entanto, este parece ser o primeiro estudo parasitológico sobre gaivotas cinzentas realizado num centro de reabilitação britânico e há uma diferença de 40 anos entre este estudo e os previamente realizados em populações selvagens, o que demostra a elevada necessidade de mais projetos nesta área.

Palavras-chave: *Larus argentatus;* parasitas; *Cyathostoma* sp.*; Syngamus* sp.; inflamação aguda dos pulmões; Sudeste de Inglaterra.

The most prevalent respiratory and gastrointestinal parasites in Herring Gulls (*Larus argentatus*) admitted in a wildlife rehabilitation centre in South-East England

Abstract

Herring gull (*Larus argentatus*) is one of the most widespread species throughout the British coastal areas. In the last 40 years, there has been a decline of 30% in the global population followed by a decrease in the natural breeding sites and sharp increase in the urban nesting populations.

RSPCA Mallydams Woods, is a wildlife rehabilitation centre situated in the South-East of England that has been receiving increasing numbers of Herring gulls that end up being euthanized due to respiratory signs with the suspicion of the persistence of a parasitological agent (*Cyathostoma* sp.), previously identified in 2004. In the meantime, no further parasitological studies in this species were performed by the centre. Therefore, a total 65 necropsies and coprological analysis were performed in order to assess the main respiratory and gastrointestinal parasites as well as the main pathological alterations suggestive of other infectious agents.

The general results showed that 32% (21/65) were positive for the presence of parasites. These results included gulls that had not received worming treatment, who showed 60% of positives (12/20) and dewormed gulls, who still showed 20% positives (9/45). Around 24.6% (16/65) presented Syngamidae eggs including two mixed infection cases, one with *Porrocaecum* eggs (1.54%) and the other with *Contracaecum* (1.54%). Among those 16 cases, 44.8% also presented visible worms in the infraorbital area of the nasal cavity. Nematodes of the species *Cyathostoma lari* were detected in 16.9% (11/65) of the cases and genus *Syngamus* in 1.54% (1/65). As to the McMaster method, it was shown that 23.1% (15/65) gulls had >50 EPG counts, including 9.2% (6/65) within the dewormed gulls. During necropsy procedures, signs of acute inflammation on the lungs were the most prevalent findings (30%). The longer rehabilitation periods and higher number of doses of wormer were the only factors to show significant association with the lower prevalence of parasites.

This study demonstrated that the inefficacy of the worming protocol, the high animal density and presence of other infectious agents are the most probable causes of the persistence of respiratory signs within the admitted gulls. However, to the best of the author's knowledge, this is the first parasitological study on Herring gulls performed in a British rehabilitation centre and there is a difference of 40 years between this study and the previous ones performed in wild populations, which shows that there is a serious need for more projects in this field.

Key Words*: Larus argentatus;* parasites; *Cyathostoma* sp.*; Syngamus* sp.; acute lung inflammation; South-East of England.

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List of Abbreviations and Symbols

< - lower than

≥- higher or equal than

%-percentage

ºC- Celsius degrees

©-copyright

®-registered brand

µm-micrometer

AERC- Association of European Records and Rarities Committee

AOU- American Ornithologists' Union

BOU – British Ornithologists' Union

CI-Confidence Interval

cm- centimetre

cont.-continuation

DNA-Deoxyribonucleic acid

e.g.-*exempli gratia*

EPG- Eggs Per Gram

g-gram

GIT- Gastrointestinal tract

h- hour

IUCN – International Union for Conservation of Nature

Kg-kilogram

L1/L2/L3/L4- 1^{st} , 2^{nd} , 3^{rd} and 4^{th} larval stage

m^2- square meter

Max.- Maximum

mg- milligram

min- minute

Min.- Minimum

mL- millilitre

mm -millimetre

p- p-value

PI- Post-infection

PLM- Posterior Left Member

PPE- Personal Protection Equipment

PRM- Posterior right member

RSPCA- Royal Society for the Prevention of Cruelty to Animals

UK- United Kingdom

USA- United States of America

SEM-Scanning Electron Microscope

W- Wilcoxon test result

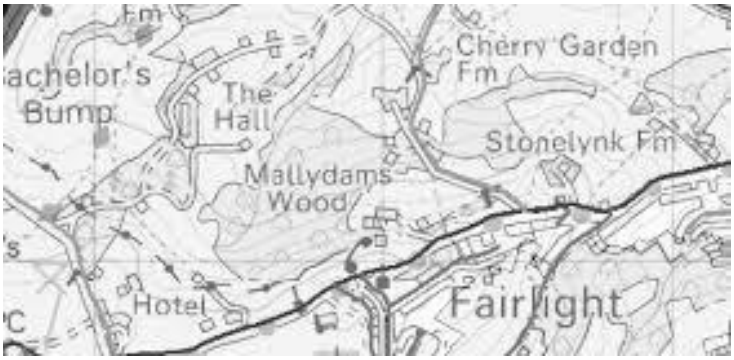
I. Curricular internship at Mallydams Wood Centre

As part of an Integrated Master's in Veterinary Medicine, a 3-month externship in the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Mallydams Wood Wildlife centre took place between September and December 2018. During this period, the centre received 325 animals of 59 different species. The most common admissions were Wood Pigeons (*Columba palumbus*), Hedgehogs (*Erinaceus europaeus*), Herring gulls (*Larus argentatus*), Feral/town pigeons (*Columba livia domestica*) and Collared Doves (*Streptopelia decaoto*).

1.1 Mallydams Wood Nature Reserve and Rehabilitation Centre

Mallydams Wood centre is located close to Fairlight village (Figure 1), in the South-East of England, serving the counties of Kent, Sussex and Hampshire (RSPCA Mallydams Wood, 2019).

Figure 1- Mallydams Wood reserve map (Source: East Sussex- UK Government)



The wildlife centre is part of a 55 acres piece of historical woodland that started making its contribution to wildlife rehabilitation in 1962, when John Goodman started to accommodate injured animals in his own bungalow with cages and pens built on-site. Throughout the years, the number of admitted animals increased exponentially demanding the expansion of the facilities (Thomas, 2011). Part of the new facilities were conceived to treat the numerous cases of oiled birds that the centre received by that time, being a pioneer in the successful rehabilitation of those birds.

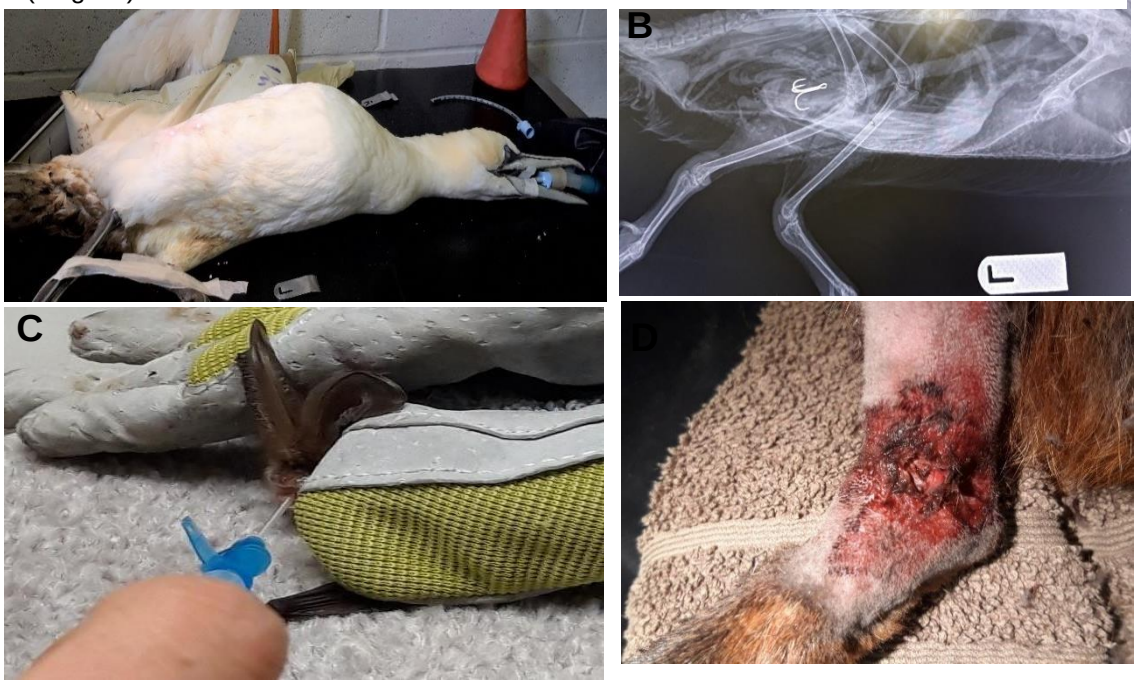
Nowadays, the centre receives more than 3000 animals per year and is mainly divided in 10 different areas: Examination room and X-ray room, General Care (for intensive care cases in small birds and mammals), Holding Room (less intensive care), Isolation cubicles (isolated areas for bigger birds and mammals), Outside pools (for marine mammals and aquatic birds), Mammal pens, Outside aviaries (pre-release areas with bigger dimensions), Bat aviaries, Drying room and Washing room.

1.2 Curricular Internship activities

1.2.1 Clinical practice

During the internship period, the author assisted Dr. Amy Colling in the clinical evaluation of the admitted animals, helping with the physical examination, some diagnostic techniques (radiography; coprological techniques), sedation and anaesthesia monitoring and minor surgeries (such as removal of ingested hooks and fishing lines entangling marine birds, removal of shotgun or air rifle pellets, and removal of necrotic extremities due to previous entanglement). Assistance was also provided in orthopaedic injuries in birds (wing bandaging), drugs and fluid therapy administration and euthanasia in animals with poor prognosis. In the images presented below (Fig. 2), some examples of the followed clinical cases are presented.

Fig. 2 - Examples of clinical cases followed during the internship: A) Preparation and anaesthesia of a Northern Gannet (*Morus bassanus*) for radiographic exam; B)Left Lateral radiographic projection of the Northern Gannet *(M. bassanus)* with a fishing hook inside the guizzard; C) Administration of oral fluids to a Brown Long-Eared Bat (*Plecotus auritus*); D) Surgical debriding in an open wound in the transition between tibia-fibula and tarsus in a Red Fox (*Vulpes vulpes*) – (Original).



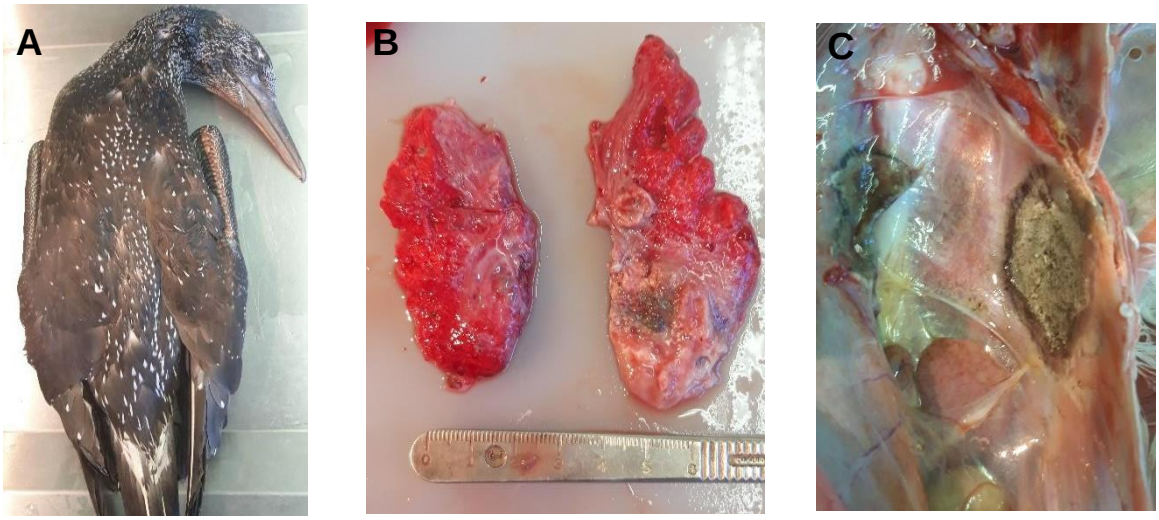
On the other hand, the author also performed daily husbandry work cleaning different rooms and cages, replacing substrates, preparing environmental enrichment and fresh food (including tube feeding when necessary) for the resident animals. These procedures were also used to check on the progression of the health condition of these animals causing a minimum of stress by minimizing their handling. Once animals were ready to be returned to their habitat, there was also a chance to plan and take part in the releases.

1.2.2 Necropsies

As part of the dissertation project, 65 Herring gulls were necropsied in the washing room facilities in order to look for the presence of adult parasites, eventual pathological alterations and to collect faeces for further analysis. In two refrigerated carcasses (5º-8ºC), it was possible to collect lung and liver samples and send them to histopathological analysis.

There was also opportunity to perform a necropsy in a juvenile Northern Gannet *(Morus bassanus*) admitted in the centre that ended up being euthanized due to poor body condition without other symptoms. The post-mortem analysis revealed several pale circular nodes harsh to the touch and greenish mould on the lungs and air sacs (Fig.3), and according to the veterinary staff, it was suggestive of Aspergillosis.

Fig. 3 - Necropsy and post-mortem finding to a Northern Gannet *(Morus bassanus)* A) Carcass of a Northern Gannet *(Morus bassanus)* B) Lungs of the animal presenting pale circular nodes harsh to touch and greenish mould C) Air sacs with greenish mould (Original)



1.2.3 Parasitological analysis

For the coprological analysis, the author performed the Willis Flotation technique and Natural Sedimentation method for each of the 65 Herring gulls' faecal samples. The slides were then examined using the Light Microscope to determine presence of eggs or larvae. The McMaster method was used to calculate the faecal egg count.

The author was also asked for assistance in performing Willis floatation and eggs per gram (EPG) counts in faecal samples from European Hedgehogs (*E. europaeus)* and adult parasites identification present in faecal samples of Harbour Seals (*Phoca vitulina*).

II. Introduction

It is now widely acknowledged that we are in the midst of a mass extinction event, the sixth such episode in our planet's history, but the first to be driven by the actions of humanity. Scientists estimate that species are disappearing at a rate 100 to 10,000 times faster the baseline level. Agricultural expansion, logging, overexploitation, urbanisation, pollution and climate change, are some of the main threats that these populations are facing (Allinson & Vovk, 2018).

According to Paleczny, Hammill, Karpouzi & Pauly (2015), seabirds' populations have declined 69,7% since the middle of 20th the century. European Herring Gulls (*Larus argentatus*) followed the same trend, having entered the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (BirdLife International, 2019). These are worrying facts regarding the important role that these birds play in the ecosystems by depositing marine nutrients inland, providing guano as fertilizer, controlling populations of invertebrate and vertebrate pests, or dispersing seeds that may be adherent to the body (Kaiser, 2017;Sekercioglu, 2006). They are good bioindicators of marine ecosystems, helping to determine the effects of disturbances (e.g. climate change) and contamination (e.g. pollutants, organic substances) on the habitats (Rajpar, Ozdemir, Zakaria, Sheryar, & Rab, 2018). However, the proximity of gulls' populations to human activities rises concerns regarding the dissemination of several potential pathogens, including zoonotic bacteria like *Salmonella* spp. and *Listeria* spp. (Duartea, Guerra, & Bernardo, 2002). This heightens the importance of performing continuous studies on the different infectious agents that may threaten this species.

The idea of the project resulted from the increasing numbers of Herring Gulls' admissions in Mallydams Wood centre in the last years, most of them nestlings/fledglings/juveniles. The centre has also registered peak numbers of euthanized/dead Herring gulls in the Summer months (Appendix I), many of those presenting respiratory signs (such as nasal discharge, cough, dyspnoea or/and sneezing with occasional expelling of adult parasites). In 2004, those adult parasites were identified as *Cyathostoma* sp. and since then, an antihelminthic preventive protocol has been put into practice. However, despite the efforts of the veterinary staff, the respiratory signs among the admitted gulls were still very common, resulting in high numbers of euthanized animals. Hence, the main objective of this project was not only to assess the respiratory parasites responsible for the described symptoms, but also some of the most prevalent and widespread gastrointestinal parasites that might cause concomitant infections and deteriorate gull's health condition (specially in confinement). As a complement, necropsy procedures were used to evaluate the presence of parasites and/or other macroscopic alterations that could suggest other diseases. Finally, the influence of several factors in the obtained results was also tested and analysed.

III. Literature review

1. Herring gull (*Larus argentatus*)

1.1. The problem of taxonomic classification

Despite being included in Charadriiformes order and Laridae family, the classification of genera, species and subspecies of gulls has been largely discussed in studies considering differences in plumage (Dwight, 1925), behaviour (Moynihan, 1959; Tinbergen, 1953) morphological characteristics (Chu, 1998) and most recently, DNA molecular markers showing an evident degree of taxonomic complexity. Unlike it was previously believed, studies suggest that Laridae are not a monophyletic clade and should be divided into 10 different genera, according to their phylogenetic, morphological and behavioural characteristics: *Larus, Chroicocephalus, Creagrus, Hydrocoloeus, Ichthyaetus, Leucopaheus, Rissa, Pagophila, Xema and Saundersilarusi* (Crochet, Bonhomme, & Lebreton, 2000; Pons, Hassanin, & Crochet, 2005).

Some important studies developed by Sangster, Collinson, Knox, Parkin & Svensson (2007) and Crochet et al. (2010) led to the recognition of the Yellow-legged Gull (*L. michahellis*), Armenian Gull (*L. armenicus*), American Herring gull (*L. smithsonianus),* Caspian Gull (*L. cachinnans*), and Lesser Black-backed gull (*L. fuscus*) as new separated species from the European Herring gull (*L. argentatus*). This classification is accepted by the British Ornithologists' Union (BOU) and Association of European Records and Rarities Committee (AERC), but not by the American Ornithologists' Union (AOU), that considers *L. smithsonianus* a subspecies of *L. argentatus* (Sangster, 2007;Crochet et al.,2010).

Therefore, according to BOU committee, there are only 2 subspecies of European Herring gull*:* the *L. a. argentatus* (Pontoppidan, 1763), also known as Scandinavian Herring gull, a large gull who breeds in Scandinavia and North-West Russia and tends to winter in the British islands; and the *L. a. argenteus* (Brehm & Schilling, 1822), known as the Western European Herring gull who breeds in Western Europe and can be found in the United Kingdom (UK) throughout the year. Generally, in a large zone between Denmark and Western Europe, the distinction of these subspecies is often impossible given the high number of hybrids (Olsen & Larsson, 2004).

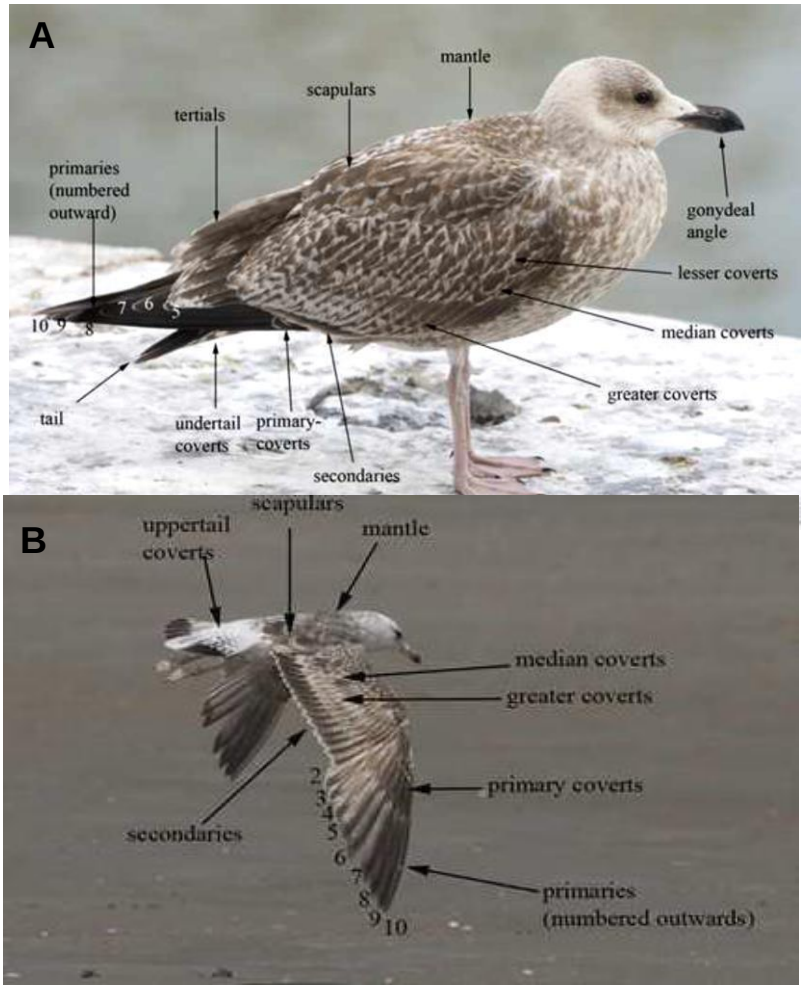
In fact, systematics of the Laridae family represents one of the most complex challenges in ornithology since several studies (Pons et al., 2005; Sangster et al., 2007) show phylogenetic evidence of the species' subdivision but there is still need for scientific support with more morphological, biogeographical and taxonomical studies that bring consensus between the specialists' panel.

1.2 Morphology, moulting and ageing

Herring gulls are medium-sized gulls (male 60-66 cm long and 1.05-1.25 Kg; female 56-62 cm and 0.8-0.98 kg) who generally present a square head, heavy yellowish bill with subterminal red spot and small beady eye. The wings are moderately long (wingspan of 125-155cm), the body is rather full usually with a prominent tertial step. Fully-grown males are larger and about 20% heavier than the female, showing a heavier, more bulbous tipped bill, flatter forehead and squarer neck than the females. Between the two subspecies *L.a. argentatus* is generally larger with extensive white on the wing-tips and heavier than *L.a. argenteus* (Pierotti & Good, 1994 ;Olsen & Larsson, 2004).

When examining the moulting phase, it is essential to bear in mind the terminology of different parts of the plumage. On the upperparts of the wings, the feathers are arranged in rows and each row has its own nomenclature (Fig. 4). The outermost, longest feathers (primaries) of the wing can also be conveniently numbered in outward direction till the 10th outermost feather - although gulls actually have 11 primaries, the last one being vestigial (Bosman, 2009).

Fig. 4-A- Nomenclature of the gull's plumage (standing position); Fig. B- Nomenclature of the gull's plumage (flight position); (Source: Bosman, 2009).

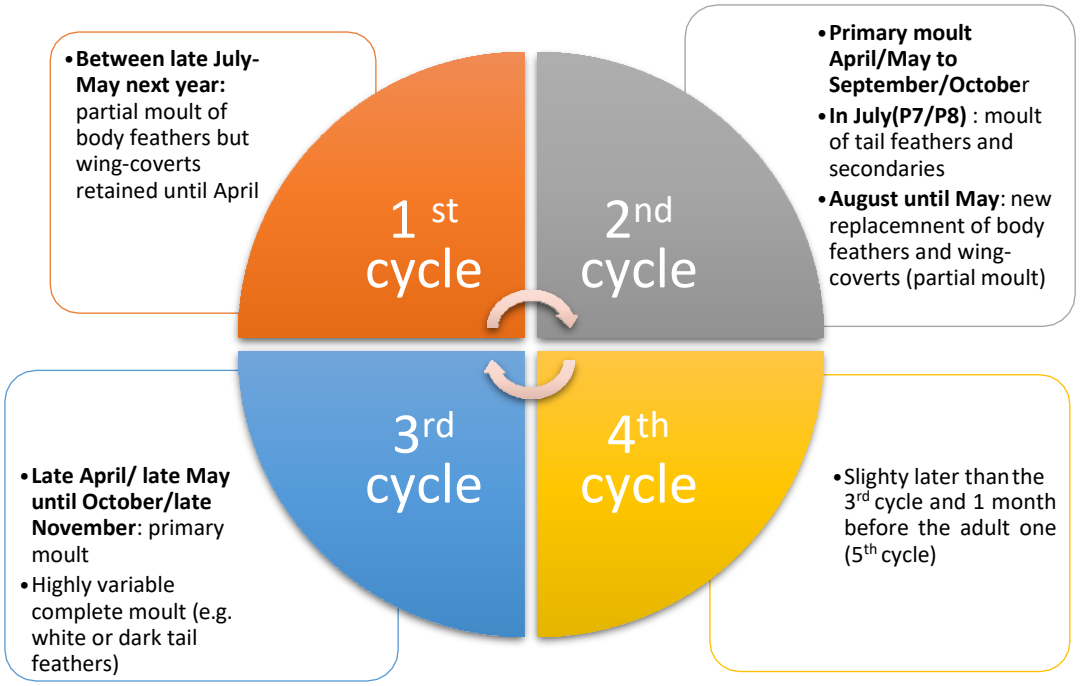


From the approximate age of one year on, large gulls replace their entire plumage once every year. The transition between plumages through moult is a method used to age gulls, but true precision can only be achieved in categorising first year and adult birds (Bosman, 2009).

The moult terminology introduced by Dwight (1925) has been largely used to describe the moulting process and consequently to help identify and age Herring Gulls. However, this system considered two moults per year: a first partial one, limited to body feathers and wing coverts (between late winter and early spring) and a second complete one, including the remiges and rectrices (between late spring and early/late Autumn), which can be rather uncertain since moulting is often an extended process, taking about six months. Moreover, these two moults may overlap between seasons which makes it difficult to distinguish how many moults occur during the year (Bosman, 2009).

For these reasons, the Humphrey-Parkes terminology (1959) based on clear plumage cycles and not in seasons ended up solving some of the problems of the previous system. The first cycle starts when the juvenile plumage is acquired and ends when the first primary (P1) is shed, which marks the beginning of the second cycle. Under this terminology, the two moults that occur after the first plumage cycle are known as: the pre-alternate moult (partial) which leads to the alternate plumage (previously identified as breeding plumage) and the pre-basic one that leads to the basic (non-breeding) plumage (Leukering, 2010). The following diagram summarizes the sequence of the first 5 cycles of moulting in Herring Gulls (Fig. 5).

Fig. 5 - Sequence of the first 5 moulting cycles (Adapted from Bosman, 2009)



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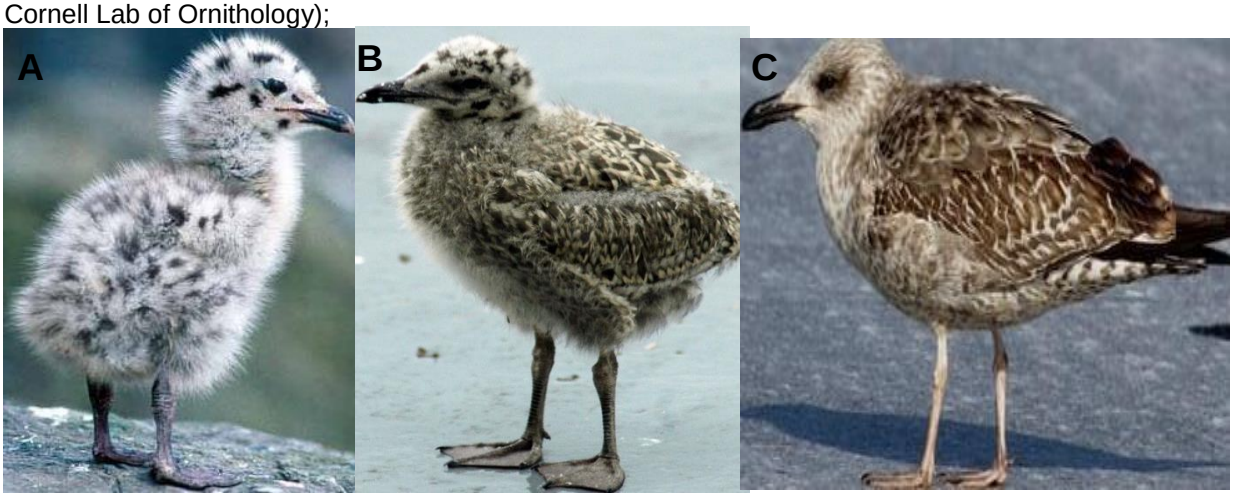
Winter plumage in adults includes dark streaking on head and neck, with a duller beak and orbital ring (Thompson, 2013)

Based on the characteristic plumage of each moulting cycle we can age a gull in the first 4 years. However, diverse factors may contribute to change those patterns and hinder this task. On one hand, as it was previously shown, the plumage appearance changes continuously through the first 5 years, depending also on different internal factors such as the bird's health, mal-nutrition and the hormonal and reproductive state (e.g. moults tend to start immediately after breeding activity ceases). On the other hand, external factors such as daylight period and wintering areas can also influence moulting processes, stimulating them in places with longer photoperiods (Dawson, King, Bentley, & Ball, 2001; Bosman, 2009). That is why gulls living in higher latitudes (Northern hemisphere) start moulting later in the course of the year. In spite of those difficulties, the shape and colour of the primaries and the deterioration caused by feather damage (causing frayed edges and tips and general blanching) are still the most consistent features to age a gull (Bosman, 2009).

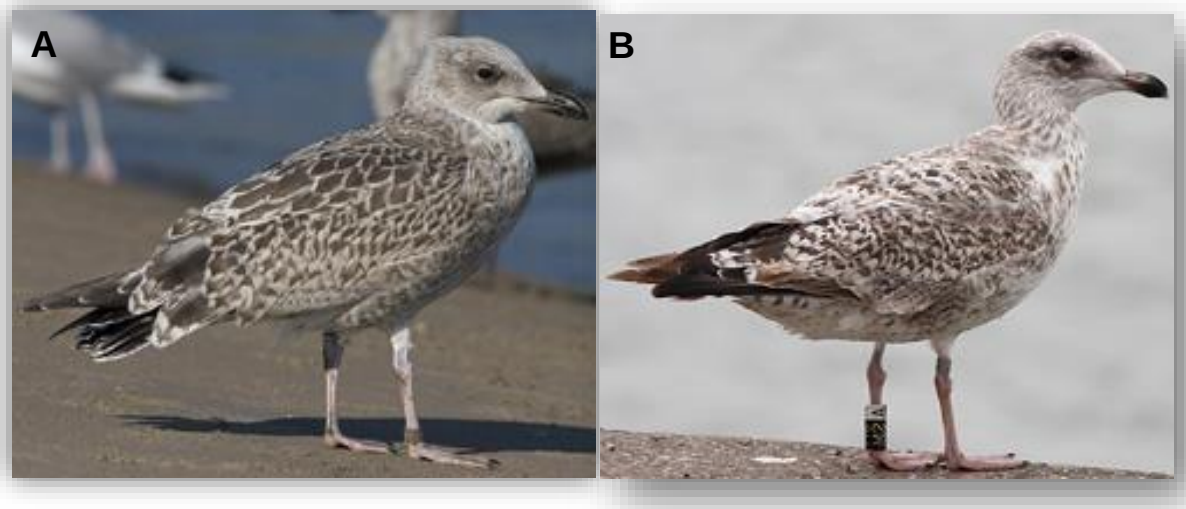
The typical first cycle plumage includes an initial nestling plumage (Fig. 6A) in which birds just present grey soft plumage with dark spots (until day 15-20) and a fledgling plumage, which ends around day 40-50 when feathers are fully emerged (Pierotti & Good, 1994; Bosman, 2009). Mallydams Wood wildlife centre sub-categorises fledglings in 2 phases to facilitate the management and care of the birds at the centre: fledglings phase 1 (Fig. 6B), presenting a soft nestling plumage throughout the body (mainly in the head) showing growth of some greater, medium and lesser coverts and mantle, scapulars and tertial feathers; and fledglings phase 2 (Fig. 6C), which no longer present soft nestling plumage, but instead are mostly fully feathered, including the presence of the first brownish-black primaries. In practical terms, fledglings phase 1 need less intensive care than nestlings, but their feathers are not completely waterproof yet, contrasting with fledglings phase 2 (Bosman, 2009; Richard Thompson, 2018, personal communication)

Fig. 6 - Typical plumages of the juvenile phases A) Herring gull nestling (Source:Dorset Life); B) Herring gull fledgling phase 1 (Source: Everyday nature trails); C)Herring gull Fledgling phase 2 (Source: The Cornell Lab of Ornithology);

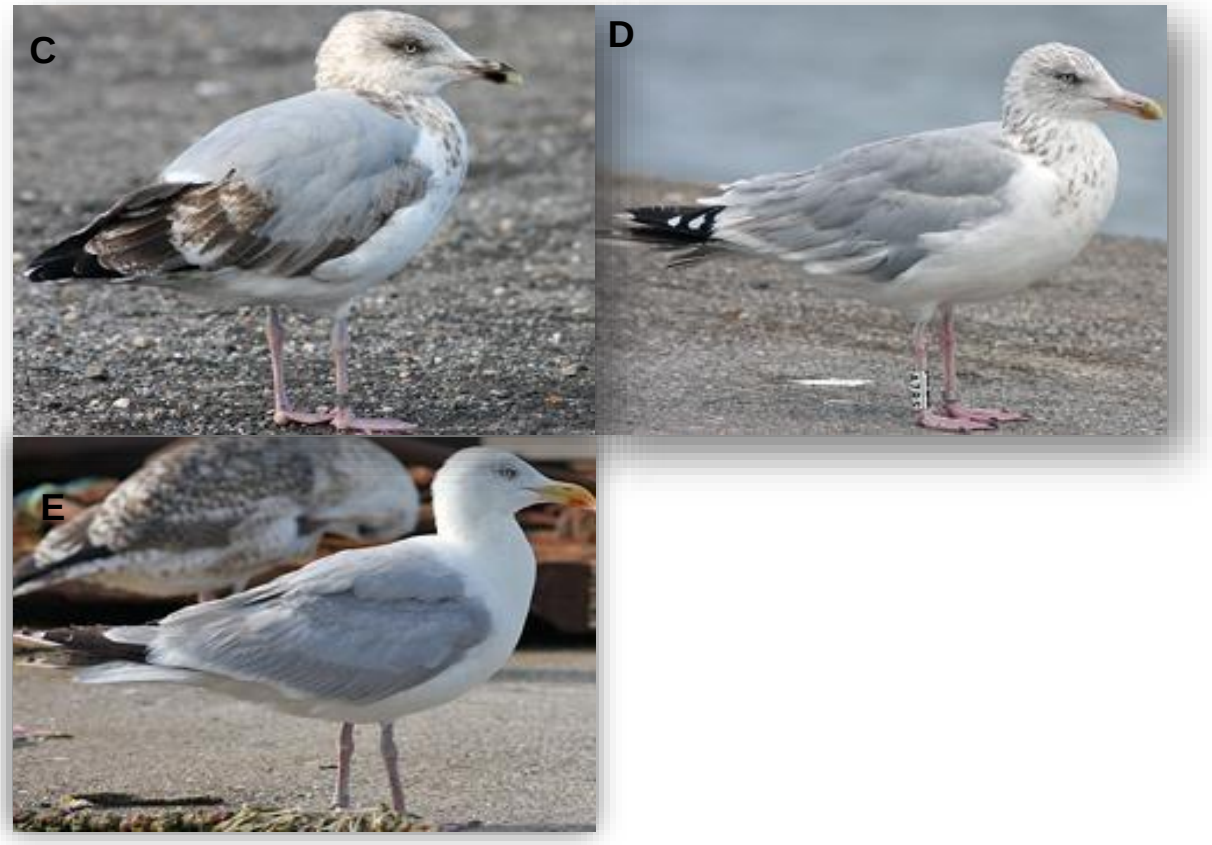


The following pictures (Fig. 7), show the description of the typical plumage on each one of the first five moulting cycles.

Fig. 7- Description of plumage in each moulting cycle of Herring Gulls A)- 1st Cycle Plumage - Primaries are more brownish-black and present a thin and pointed shape. The rest of the plumage shows a regular brownish and white pattern (greater coverts, for instance, show neat dark bars that run parallel to each other). The iris remains dark and the bill usually lacks a pale tip; B) 2nd cycle - Primaries are broader and more rounded than 1st cycle ones (the outer ones are quite blackish, while the inner primaries look more greyish or brownish). Generally, the plumage looks less regular, consisting in different patterns, and some gulls may acquire adult-like (greyish) mantle feathers and scapulars. The bill often shows a wide pale tip; (Adapted from Bosman, 2009; Images source: Gull Research)



(continuation) C) 3rd cycle- primaries generally present prominent, rounded white tips. The wing coverts, tertials and bare parts may appear more adult-like . A few birds may show two white mirrors already on the outer primaries (P9-10).The most precocious may be difficult to differentiate from the 4th cycle.; D) 4th cycle- the outer primaries have prominent, rounded white tips and at least one mirror (on P10) although they can retain more black than usual in adults. Also, the primary coverts still retain blackish markings (also found near the bill), but the rest of the plumage and bare parts are usually adult-like; E) Adult Cycle - The upperparts are bluish-grey, without any brown markings. The head, chest, tail feathers and underwing coverts (including primary coverts) are all-white and there are usually no dark markings on the bill nor primary coverts (Adapted from Bosman, 2009; Images source: Gull-Research)



1.3.Global distribution and UK population numbers/migration patterns

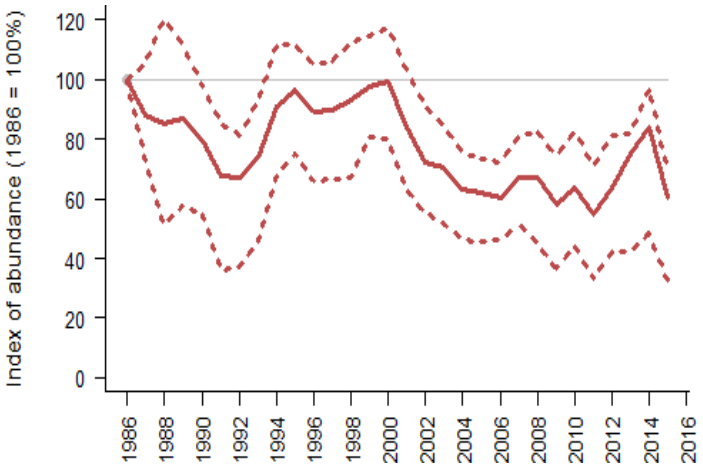
Until 1970, Herring Gull population was growing exponentially all over Britain specially near coastal areas due to poor waste management, availability of anthropogenic food and adaptation to human-altered environments (Belant, 1997; Ratcliffe, 2009). Between 1970 and until 2014, there has been an estimated decrease of 72% in the coastal nesting population in UK (Graph 1; Joint Nature Conservation Committee, 2018). This has mainly affected the natural breeding sites and might be linked to several different factors: general decline in commercial fishing, a reduction in opportunities for scavenging (due changes in fishing techniques and household waste management) and high rate of avian botulism. In Ireland, this infection has caused a dramatic drop of 90% in Herring gulls' population just in the last decade of 20th century (Mitchell, Newton, Ratcliffe, & Dunn, 2004).

At the same time, it is curious to find that the number of gulls nesting in British cities' rooftops did not follow the same trend. Usually, they provide safe nesting sites often situated right next to an abundant domestic or commercial food source and the number of birds has increased sharply (Rock, 2005). Although the numbers of roof nesting pairs in Sussex are not well documented, it was estimated that the they increased from 23 pairs to 1872 between 1965 and 2000 (Thompson, 2013).

Globally, despite being one of the most numerous bird species (2,060,000-2,430,000 individuals), the population has declined 30% in just 40 years which may be part of a longer-term fluctuation following previous increases according to some specialists (BirdLife International, 2019).

Dispersal and migration of these birds throughout Europe (Fig. 8) begins in late July with birds returning in April. However, few gulls (mainly nonbreeding ones) appear migratory since most adults remain near breeding grounds throughout year. Some juveniles remain with the parents for several months post-fledgling and other first-year birds winter in southern areas with second- and third-year birds moving to intermediate distances (Pierotti & Good, 1994).

Graph 1- Trend in abundance index (solid red line) of natural-nesting Herring gulls in the UK (Source: JNCC, 2018); Fig. 8 - Map with dispersion of Herring Gull populations in Europe (Source: IUCN-Red List,2018)



1.4 Habitat and feeding

Herring Gulls are known for its typical coastal-inshore sea distribution during the breeding period which results in a choice of the intertidal zone as a feeding habitat (Schreiber & Burger, 2002). According to a Kubetzki & Garthe (2003) study of pellets and faecal samples in two colonies of Herring gulls, bivalves and crustaceans represent the principal food sources in this natural habitats. However, the shortness in food resources has forced this species to exploit and adapt to new habitats such as mud flats, landfills, agricultural fields, fish-processing industries, garbage dumps, public beaches, parking lots or city rooftops (open areas with good visibility for spotting predators). In these areas, they are opportunistic scavengers on earthworms, invertebrate prey, fish, carrion and anthropogenic waste and even feed themselves on eggs, smaller seabirds or some gulls chicks of the same colony (Cornell Lab of Ornithology, 2018).

Such a wide variety of food resources makes it difficult to meet the nutrient needs in captive seabirds in rehabilitation centres. So, it is important to guarantee that adequate amounts of amino and fatty acids, vitamins A, D, E, carotenoids and minerals are offered as part of the diet (viscera are important sources of these nutrients) or with supplements since they contribute to the optimum function of uropygial gland and reproductive system (McWilliams, 2008).

1.5 Social Behaviour and Breeding

These species often patrol shorelines and open ocean, in widely dispersed groups that comprise their own loose hierarchy based on size, aggressiveness and physical strength of the gulls (Cornell Lab of Ornithology, 2018). Adult males are usually dominant over females and juveniles in feeding (Fig.9A) and territorial disputes, while adult females are typically dominant when selecting nesting sites (Spencer, 2008). Despite being flock seabirds, apart from female-male pair and parental relationship, gulls do not engage in social grooming, keeping a minimum distance from other individuals. Adults spend most time loafing and sleeping in large groups on the territory, especially during incubation period (Pierotti & Good, 1994).

Naturally, one of the main social interactions is mating and the subsequent breeding stage. *Larus argentatus* begin breeding around 4 years old and do it seasonally, pairing up around mid-March and laying eggs around mid-May (Spencer, 2008). The most common mating relationship is monogamous with rare cases of males that bond to a secondary female but with lower reproductive success (Fitch & Shugart, 1983). If the pair is successful in hatching eggs and chicks survival, it is likely they maintain the relationship and nesting site in the following breeding seasons (Graves, Whiten, & Henzi, 1986).

Generally, females take between 4-6 days to lay three egg clutches that are posteriorly incubated by both parents during 28-30 days. Synchronization of the incubation activities

between the pair is essential to its success and although females spend more time incubating the eggs, males spend more time searching for food (Pierotti & Good, 1994). When chicks finally hatch (Fig. 9B), they do it asynchronously with the first and second chick hatching 1 day earlier than the third one (making it the most vulnerable one). This leads to a hierarchical competition for food between siblings and gives advantage to the larger sex (males) of the first hatched chick, impacting on the broods' survival when there is food shortage (Kim & Monaghan, 2006).

Fig. 9- .Social Behaviour and chicks' hatching in Herring Gulls; A- Herring gull feeding nestlings with regurgitated food (Source: Everything is Permuted: Wildlife photography); B- Herring Gulls chicks' hatching (Source: Wildscreen archive).

These constitute semiprecocial species born with open eyes that are able to leave the nest on foot just one day after hatching and start running out the nest territory in 1 week period. Fledging occurs when all feathers are fully emerged around 6 weeks of age and juveniles are fed in parental territory up until 11-12 weeks old with regurgitated small fishes, insects or earthworms (Pierotti & Good, 1994).

When newly fledged chicks start flying, they gather in groups with parents or other fledglings from the colony and tend to concentrate in areas where food is predictably obtained such as intertidal areas, fishing activities and refuse dumps (Pierotti & Good, 1994).

1.6 Interaction with humans and Importance of conservation

Some members of the public, often consider Herring gulls as nuisance species with conflicting attitudes towards their urban nesting colonies. This is mainly explained by their high pitched vocalizations during courting and breeding season, unexpected food snatching, bin bags rupture and accumulation of gull excrements through the urban areas (Thompson, 2013; Rock, 2005). In fact, as previously stated, the *Larus argentatus* population (mainly in urban areas) increased exponentially until 1970, leading to the implementation of a series of measurements to deter gull's nesting, including: appliance of rooftop spikes, tensioned wires, netting, fake models of birds of prey, broadcasting of predator sounds, the removal of nests and eggs or

even gull culling projects (Rock, 2005). One classic example is the gull control measurements that took place between 1972 and 1986 in Isle of May Natural Reserve where 45,500 gulls were killed, eggs crashed and nests removed with the establishment of “gull free” areas. The main objective was to reduce the population to a sustainable level unlikely to have a significant impact on the breeding success of other species of the island (Pickett & Luurtsema, 2014). However, those methods were only considered successful if permanently applied since the species showed several adaptations such as the decrease in the age of first breeding, increase in gulls’ seasonal migratory movements to breed in the island and nest rebuilding in other areas (Rock,2005;Calladine, Park, Thompson, & Wernham, 2006). The majority of control and deterrence methods were proved ineffective since gulls have proved to be very intelligent species readapting at every new situation (Bounter & Shah, 2016).

Despite the previous tendency, European Herring Gulls in UK have decreased at an alarming rate entering the IUCN “Red List” of threatened bird species to the “Least Concern” group in 2009. This lead to a change in policies in 2010, removing this species from the list of birds covered by general licenses, which had previously authorized people to kill the birds under certain circumstances without requiring additional permission (Royal Society of the Protection of Birds, 2009).

Herring gulls play an important role in the ecosystem contributing to its correct functioning as urban cleaners that keep urban areas free from plagues (Coulson, 2015) and soil enrichers. They are valuable bio-indicators or environment sentinels that cannot be dismissed. On the other hand, they contribute to the dissemination of infectious diseases and drug-resistance pathogens like *E. coli* and *Salmonella* sp. to humans and consequently, should be kept under close vigilance (Duartea et al., 2002; Soares, 2010).

1.7 Main diseases and gross pathology findings in Herring Gulls

Several agents such as bacterial, fungal, viral and toxins have been described in necropsies performed in *Larus argentatus*. One of the most frequent descriptions is the intoxication by chemical substances such as organochlorines (Karstad, Frank, Holdrinet, & Addison, 1977;Hario, Hirvi, Hollmén, & Rudback, 2004) and organophosphates (Whitney, 2004). However, specific gross necropsy lesions are not usually found in acute and chronic exposure and the diagnosis relies on residue analysis of brain samples (Glaser, 1999). Just like in many other marine birds, pieces of plastic have been commonly found in their alimentary tract or on analysed food boluses (Lindborg, Ledbetter, Walat, & Moffett, 2012; Seif et al., 2017).

Some studies also refer to amyloidosis as one alteration found mainly on adult and captive gulls. Vascular deposits of amyloid were found in the spleen, liver, kidneys, myocardium and most commonly in the gastrointestinal tract (Jansson et al., 2018). Aspergillosis lesions are also frequently described in the lungs, air sacs and intestinal tract with plaques of whitish

caseous material with greenish mould on it. This disease is correlated with the cumulative stress of confinement and nutritional deficiencies (Friend & Trainer, 1969; Cacciuttolo, Rossi, Nardoni, Legrottaglie & Mani, 2009).

Since the first report of a botulism outbreak in gulls in Britain by Macdonald & Standring (1978), several reports have followed detecting mainly *Clostridium botulinum* type C, but also types B, D and E which are linked with gulls' scavenging habits of feeding on landfills. At post-mortem exam, they can present an empty and bile stained proventriculus and ventriculus, a full gall bladder, distended cloaca and dry subcutaneous tissues (Ortiz & Smith, 1994; Neimanis, Gavier-Widén,Leighton, Bollinger, Rocke, & Mörner, 2007).

Although salmonellosis is usually not considered an important disease in free-ranging wild birds, gulls have been associated with isolated outbreaks (Milton, 1999; Quessy & Messier, 1992; Palmgren et al., 2006). They have also been pointed out as vectors to humans, cattle and sheep (Coulson et. al. , 1983; Duartea et al., 2002). At necropsy, wild birds commonly show paratyphoid nodules (small white nodules or yellow, cheesy plaques) often seen in the liver, breast muscle or surface of the oesophagus. Congestion of the mucosa of small intestine and adherent fibrinous material can also be detected (Milton, 1999).

2. Respiratory parasites

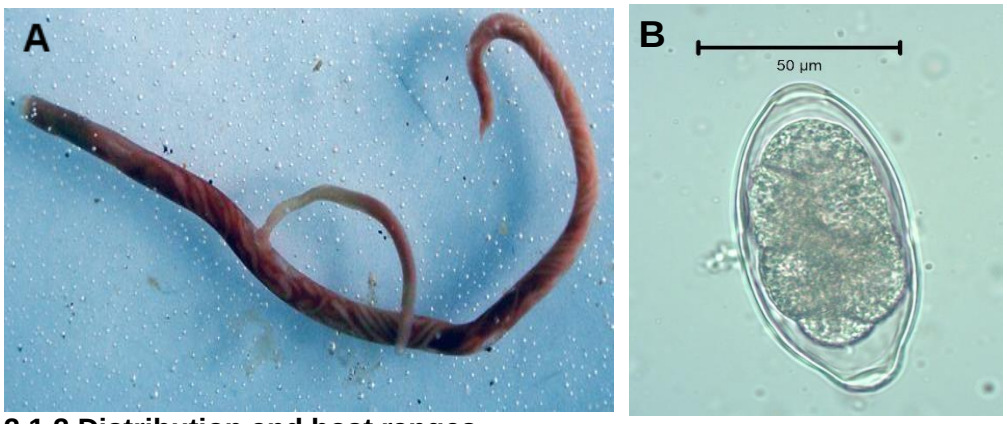
2.1 *Syngamus* sp.

2.1.1 Morphology

Syngamus sp., also known as gapeworms, are strongylid nematodes that infect the respiratory tract of wild and domestic birds. Although there are multiple species inside this genus, it is extremely difficult to differentiate the morphology of the adult worms. Both male (2-6mm) and female (5-40mm) are bright red, permanently in copula, forming a Y shape (Fig.10A). The buccal capsule is large and well developed with eight (sometimes nine) teeth at the base, which allows them attaching to the wall of the trachea (Fernando & Barta, 2008).

The eggs (80–110 µmX40-50 µm) are ellipsoidal and bipolar with distinct opercula at each end. When fresh in the faeces, they contain a morula (Fig.10B)(Zajac & Conboy, 2012; Fernando & Barta, 2008).

Fig. 10- *Sygamus trachea* female, male and eggs: A) *S. trachea* female and male (smaller one) in permanent copula; (Source: Loftgest); B) *S. trachea* egg with bipolar distinct opercula (Source: Edinburgh Datashare).



2.1.2 Distribution and host ranges

Syngamus sp. have been commonly described in ground-feeding fowl from Phasianidae family (turkeys, grouse, chickens and pheasants are particularly susceptible) and in invertebrate feeding birds from Ardeidae (herons), Gruidae (cranes), Muscicapidae (robins) families (Fernando & Barta, 2008). In several studies on Laridae (gulls) family, *Syngamus trachea* has been one of the identified species (Pemberton, 1963; Ellis & Williams, 1973 ;Threlfall, 1967). *Syngamus* sp. has been described not only in Europe (including some cases with pheasants, crows and song thrushes in Britain), Russia, but also in USA, Canada, and Papua New Guinea (Fernando & Barta, 2008).

Gapeworms are not usually responsible for clinical infections in wildlife. However, confined rearing of susceptible hosts may exacerbate the transmission, making these serious parasites for young birds (Fernando & Barta, 2008). In fact, studies conducted by Campbell (1935) showed the prevalence and intensity of infection in juvenile birds is much higher. Also, some wild birds may act as reservoir hosts, having been implicated in outbreaks on game bird farms (e.g. pheasants) as well as free range poultry (Leamaster, 2007).

Gapeworms are not known to infect mammals, including humans and therefore pose no public health concerns, but other tracheal worms infecting mammals (e,g., *Mammomanogamus laringeus*) are relatively common and have been reported as cause of accidental infections in humans (Fernando & Barta, 2008).

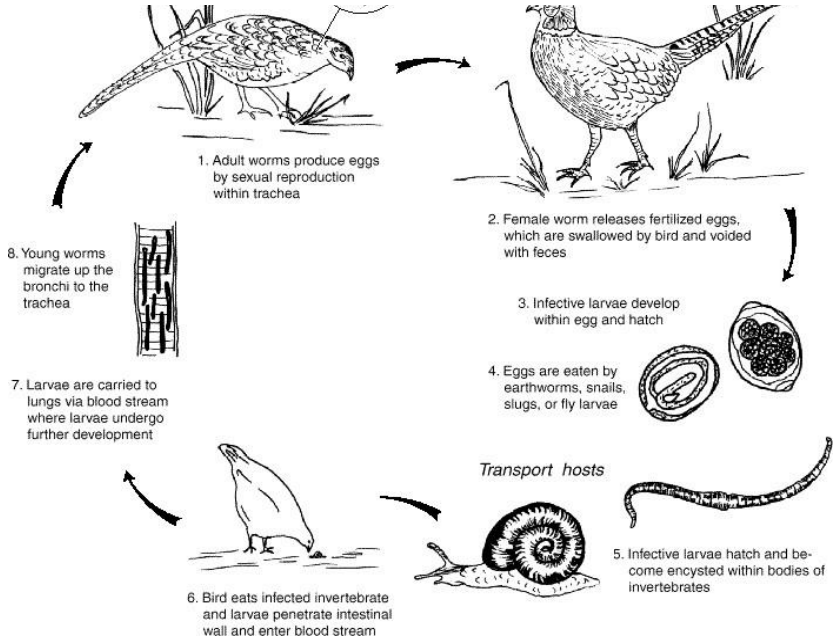
2.1.3 Life cycle

Syngamus sp. have typical direct life cycles (monoxenous) although they frequently incorporate a paratenic host (earthworms, slugs, beetles) that may be important to the larval survival overwinter. In fact, *S. trachea* larvae have been found in the body wall of earthworms, deeply embedded inside the muscle in a thin hyaline cyst (Fernando & Barta, 2008)

Eggs laid by the female within the trachea are coughed up and usually swallowed passing to the faeces of the host where they take around 1 week to reach the infective third stage within the eggs. They may then hatch spontaneously in the environment or within the intestine after ingestion (both eggs and larvae are infective). After passing from the intestine to the liver via bloodstream, infective larvae reach the pulmonary connective tissue and air sac capillaries 4h post-infection (PI) and the atria of the lungs by 24 h (Fernando, Stockdale, & Remmler, 1971; Fernando & Barta, 2008).

Worms can be recovered from the lungs up to 7 days PI and from the trachea between 7-11 days PI. Male worms are permanently attached to the tracheal mucosa and females are attached to them in permanent copula, feeding at multiple locations around the attachment site of the partner. The first eggs are found in the faeces 17–20 days PI (Fig.11)(Fernando & Barta, 2008).

Fig. 11-Tracheal worms life cycle (Source:Friend & Franson,(1969) - Manual of Wildlife Diseases, General Field Procedures and Diseases of Birds)

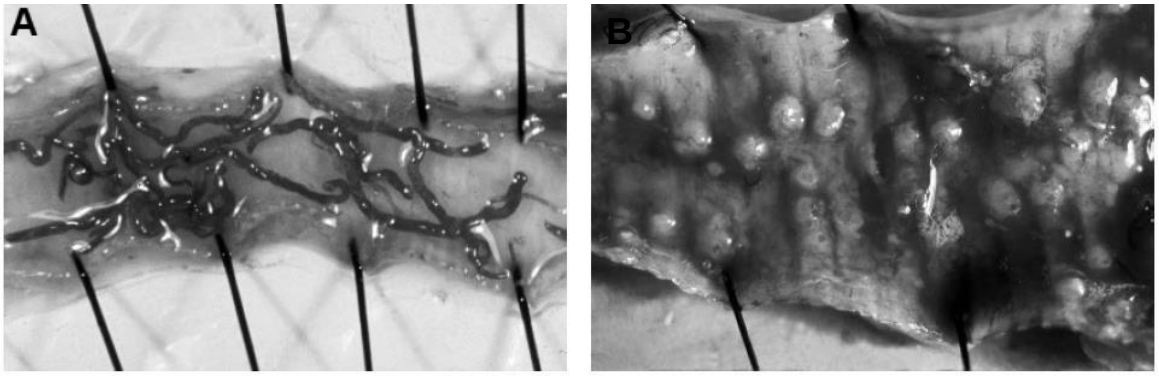


2.1.4 Clinical signs and pathogenic alterations

Clinical signs depend on the size of the bird and the intensity of infection. Juvenile birds tend to be severely affected by migration of larvae through the lungs and may develop pneumonia. Approximately 2 weeks after infection, adult worms can block the trachea (Fig. 12A) leading to the typical clinical sign of “gaping” or gasping for air. Head shaking and bouts of coughing are seen in some young birds but adults show few other signs than an occasional cough. Death may result from asphyxia or progressive emaciation and weakness (Leamaster, 2007; Fernando & Barta, 2008) .

Most pathological alterations are associated with an inflammatory host response to the presence of larvae and their antigens causing haemorrhagic tracheitis and bronchitis. Male worms sometimes penetrate so deeply that they can cause rupture and perforation of the tracheal rings (Fernando &Barta,2008; Fernando et al., 1971). Male worms are permanently attached to the tracheal mucosa leading to the formation of nodules around the anterior end of the male worm (Fig.12B)(Clapham, 1935; Fernando &Barta,2008) .

Fig. 12- Pathological alterations caused by *Syngamus trachea* - A) *S. trachea* worms blocking the trachea; B) Nodules of the mucosal surface of the trachea at the attachment point of the male parasites (Source: Fernando & Barta,(2008)- Parasitic Diseases of Wild Birds)



2.1.5 Treatment and control

Benzimidazole antihelminthics such as fenbendazole mebendazole and flubendazole have demonstrated efficacy against gapeworm infections in various studies (Mitterpák & Vasil',1976; Ssenyonga,1982; Kirsch,1984; Draycott, Woodburn, Ling, & Sage, 2006). Tetramisole showed moderate efficacy against 3-4 day larvae in pheasants and turkeys but poor efficacy against infection associated with disease (7 day old larvae or more; Connan &Wise,1977). Pyrantel, levamisole and piperazine 17% (in birds <6 weeks age) were also described for treatment of gapeworms in poultry (Leamaster, 2007).

In Peru, a broad-spectrum anthelmintic containing ivermectin, fenbendazole and praziquantel is used to control this parasite in poultry, particularly in younger birds. Off-label use of injectable ivermectin has also showed variable success in treating syngamosis in poultry and wild birds. In case opportunistic secondary bacterial or fungal infections are present, additional specific treatment is needed (Lamka., Svobodová & Slézková, 1997; Fernando & Barta, 2008)

Restricting access of young birds to heavily contaminated yards, open ground (risk of infection by earthworms), older birds or other wild bird reservoir hosts might be a preventive measure to reduce the intensity of infections and therefore, the clinically significant infections (Fernando & Barta, 2008). Also, studies developed by Gethings, Sage, & Leather (2015) highlighted the contribution of certain climatic variables (high temperature and humidity) to larval abundance as well as certain pen characteristics (soil moisture, pen age and stoking density of previous years).These variables can be controlled to prevent Syngamosis.

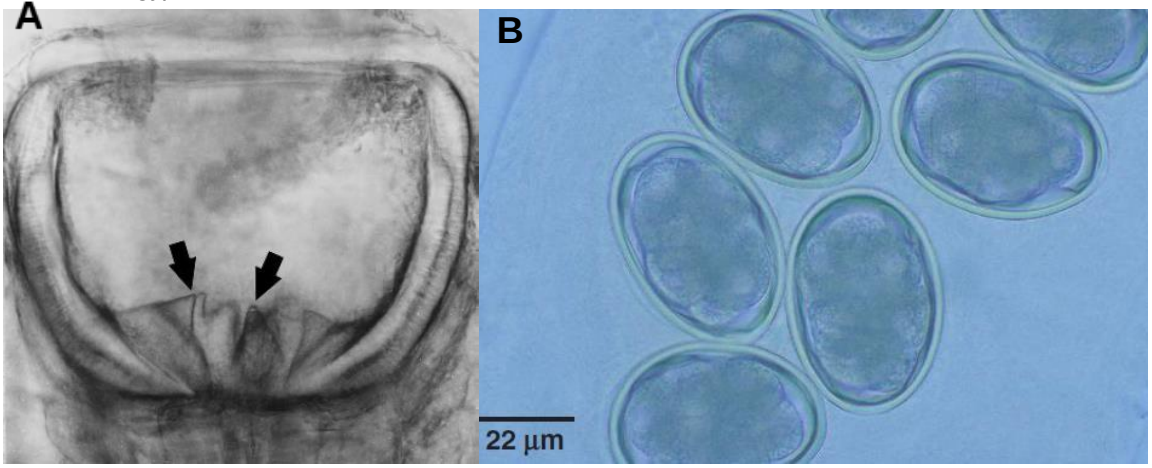
2.2 *Cyathostoma* sp.

2.2.1Morfology

The *Cyathostoma genus* was first described in the orbital cavity of a Black-headed gull (*C.ridibundus*) in Sicily (Burt & Eadie,1958). This is a nematode from the Syngamidae family that infects the respiratory tract of wild and domestic birds. Within the most prevalent species (*C.lari, C. americanum, C.variegatum*), *Cyathostoma bronchialis* is the most commonly studied and frequently used to describe the species morphology. Both male (4-6mm) and female (16-30mm) specimens of *C. bronchialis* present a reddish colour, with males presenting a very well-developed bursa and spicules with 0,5 mm in length. Unlike *S. trachea*, they are not in permanent copula and their buccal capsule is cup shaped with 6-7 teeth at the base (Fig.13A). The conspicuous buccal capsule and highly muscular oesophagus is actually responsible for the attachment to the mucosa and blood feeding (Colam, 1971; Fernando & Barta, 2008).

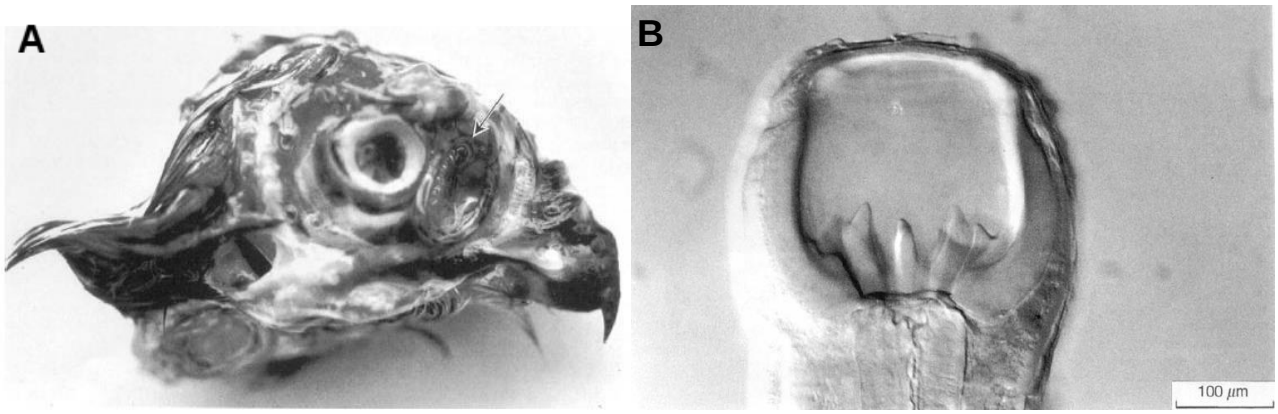
The eggs of *C.bronchialis* (75–83 µm X 50–62 µm) are typically ovoid and morulated with a single indistinct operculum at the narrow end (Fig 13B; Fernando & Barta,2008). However, some studies suggest that other *Cyathostoma* species' eggs may actually present two opercula at each pole which complicates the distinction from *Syngamus* eggs (Burt & Eadie, 1958; Lavoie et al., 1999).

Fig. 13- *Cyathostoma bronchialis* morphological features and eggs A) Buccal capsule showing triangular teeth at the base of a *C.bronchialis* male; (Source:Fernando et al.,1973) B)- *C. bronchialis* without prominent polar plugs (Source: Zanjac & Conboy, 2012- Veterinary Clinical Parasitology)



Another important parasite species that has been widely described in Herring gulls is *Cyathostoma lari* (Pemberton, 1963; Threlfall, 1967; Threlfall et al., 1968). This syngamid is normally found inside the orbital and nasal cavities of domestic and wild birds (Fig.14A). Females are moderately robust with two long ovaries that are convoluted and twisted on themselves and males present one single testis, which is twisted on itself and its duct. The buccal capsule is thick-walled and cup-shaped with 9-12 teeth situated on its base (Fig.14B). The relative scarcity or absence of male specimens of *C. lari* has been noticed in some studies and it is known that they do not persist in the avian host as long as females (Burt & Eadie, 1958).

Fig. 14 - *Cyathostoma lari* morphological features and usual location: A) *C. lari* present in the infra-orbital cavity (arrow); B) Buccal capsule of *C. lari* with 9-12 teeth (Source:Simpson & Harris,1992).



2.2.2 Distribution and Host range

C. bronchialis, is likewise found globally and it is common in Anseriformes (Fernando & Barta,2008) and Charadriiformes (Threlfall, 1967, 1968). It is also normally found in predatory birds that acquire infection by eating rodents or smaller birds with infective larvae in their alimentary tracts (Lavoie et al., 1999; Simpson & Harris, 1992). *C. (Hovorkonema) variegatum* species has been recovered from wild birds in respiratory distress, suggesting that these parasites may be an unrecognized cause of air sacculitis and wild bird mortality (Krone, Friedrich, & Honisch, 2007). Regarding *C. lari*, this species has been found in the most wide variety of hosts such as gulls, rooks (*Corvus frugilegus*), Eurasian Jackdaw (*Corvus monedula*), European Starling (*Sturnus vulgaris*), Grey Heron (*Ardea cinereal*), as well as several raptor species (Falconiformes and Strigiformes) (Lavoie et al., 1999; Fernando & Barta, 2008). According to Threlfall (1966), it is mainly found in juvenile birds and the transmission between domestic and wild birds can happen easily.

2.2.3 Life cycle

The life cycle of C*. bronchialis* is very similar to the previously described for *S. trachea* although larvae or eggs containing third-stage larvae are not directly infective. Consequently, earthworms are obligate intermediate hosts (Fernando & Barta, 2008). After they are ingested, larvae migrate into the lungs through the peritoneal cavity and air sacs. They moult twice in

the lungs and then reach the trachea. By day 13 PI, most males and females have already copulated and consequently, their eggs are present in the tracheal mucus. Eggs are shed in the faeces shortly thereafter (Fernando, Hoover, & Ogungbade, 1973).

Threlfall (1966) conducted several experiments in vitro on the development of *C. lari* larvae. These studies showed that in contrast with *C. bronchialis*, but similarly to *Syngamus* sp.*, C. lari* has a direct life cycle that may include earthworms as non-obligatory paratenic hosts. It also appeared that relatively low temperatures were necessary to the larvae development and that paratenic host may contribute to their overwinter survival. In other experiments, Threlfall (1966) described that this species is normally found attached to the wall of the orbital and nasal sinuses by a plug of mucosa that the parasite lacerates with its teeth to feed on the hosts' blood.

2.2.4 Clinical signs and pathological alterations

Even though *C. bronchialis* can be a significant pathogen in young captive waterfowl, most wild birds infected with *Cyathostoma* genus show no signs of disease (Fernando & Barta, 2008). In rare instances, the clinical signs include emaciation and/or dyspnoea. Eggs, worms or cuticular material shed by developing worms may lead to pyogranulomatous airsacculitis, bronchitis or pneumonia. In some cases, Cyathostomosis may be associated with fungal (Aspergillosis) and bacterial infections (Lavoie et al., 1999).

In *C. lari*, the alterations are hardly noticed even when these nematodes are present in big size and numbers within the orbital cavities. In some cases, only emaciation, anaemia, reddish-black mucus in the gizzard, pale liver and extensive swelling in orbital cavities were described. It may be speculated whether numbers of large nematodes, moving around the back of the eyes, could have affected the visual acuity of some birds (Simpson & Harris, 1992).

2.2.5 Treatment and control

Just like in Syngamosis, Cyathostomosis can be treated using a wide range of anthelminthics such as fenbendazole, mebendazole and flubendazole (Mitterpák & Vasil',1976; Ssenyonga, 1982; Kirsch,1984; Draycott et al., 2006).

In heavy infections, treatment may produce fatal pneumonia from the aspiration of dead worms and consequent inflammatory responses. Opportunistic secondary bacterial or fungal infections may require specific treatment (Fernando & Barta, 2008).

3. Gastrointestinal parasites

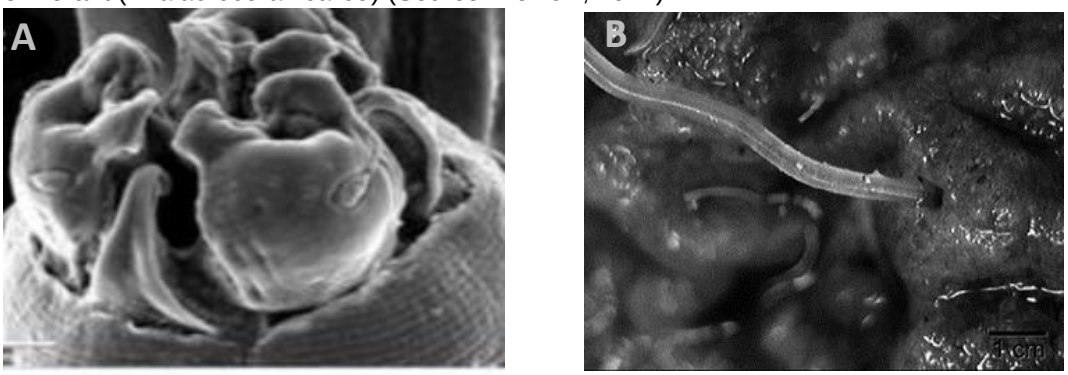
3.1 Ascaridoidea superfamily

3.1.1Morphology

The Ascaridoidea superfamily presents more than 50 genera but this chapter focus on two of the most important in avian species: *Contracaecum* sp. and *Porrocaecum* sp. *Contracaecum* sp. are medium sized anisakids that contain over 100 species (Shamsi, Norman, Gasser & Beveridge, 2009) of slender parasites of the proventriculus, gizzard, and intestines of birds,

seals, and dolphins (Fagerholm & Overstreet, 2008). The morphological characteristics of *Contracaecum rudolphii*, one of the most widespread and studied species, include three lips and well developed interlabia that present a non-bifurcated triangular shape in the anterior end (Fig 15). In the posterior part, males possess numerous preanal papillae (69-79 pairs) arranged in single sub-ventral rows and a conical tail that ends with a sub-pointed tip (Kim et. al., 2010; Al-moussawi, 2017). Despite using a number of structural features (e.g., the distribution papillae in the male), it is extremely difficult to differentiate the morphology of the adult worms given their intraspecific variability (Fagerholm & Overstreet, 2008).

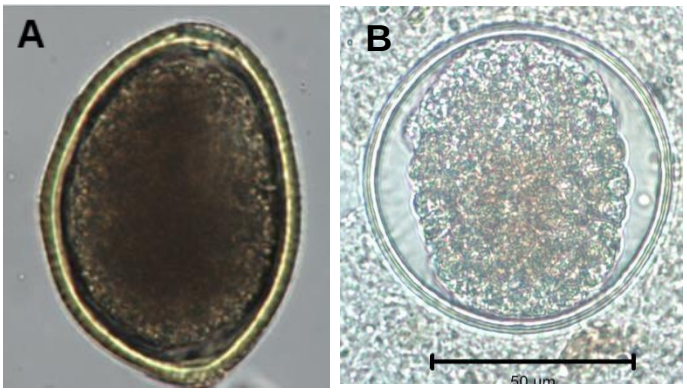
Fig. 15- *Contracaecum rudolphii* morphological features and usual location A) *C. rudolphii* cephalic extremity with three lips and well developed interlabia (SEM) (Source: Al-Moussawi, 2017); B*) C. rudolphii* attached to the proventricular mucosa of a Black Cormorant (*Phalacrocorax carbo*) (Source: Rokicki, 2011).



As to *Porrocaecum* sp., they are ascaridids that infect the intestine of birds and include around 40 species (Li & Scholz, 2019). This species differ from *Contracaecum* sp. by the presence of small interlabia, a delicate denticulated ridge on the lips and absence of a ventricular appendix. The spicules of male specimens of *Porrocaecum* sp. are usually short (1 mm), while those of *Contracaecum* sp. range from 2 to 12 mm in length. *Porrocaecum* sp. have been differentiated in relation to the presence or absence of cervical alae and on the projections of the lip pulp (Fagerholm & Overstreet, 2008)

Eggs of Ascaridoidea transmitted in terrestrial habitats are thick-shelled (Fig.16A) (*Porrocaecum* sp.) whereas those in species transmitted in aquatic habitats are generally thin-shelled (Fig.16B) (*Contracaecum* sp.). They are round/oval (64-58 µm), unembryonated when laid and only develop when passed to the external environment in faeces of the definitive host. In the external environment, eggs embryonate to first-stage larvae (L1) which grow and moult to the second (L2) or third stage (L3),ending these eggs with L2 or L3, the infective stages for the definitive hosts (Anderson, 2000).

Fig. 16: *Porrocaecum* sp. and *Contracaecum* sp. eggs: A) *Porrocaecum* egg with its thick-shell; (Source: Edinburgh Data share); B) *Contracaecum* egg typically unembrionated and thin-shelled (Source: Bowman, 2014)



3.1.2 Distribution and host range

Species of *Contracaecum* sp. are generally very common in fish-eating birds and have a wide host range and geographic distribution. In some aquatic birds, at least four different species may occur concurrently in the same individual. *C. rudolphii*, has been reported in the Neotropics, Nearctic, Palearctic, Ethiopian and Australian regions in several studies. Many species of fish-eating birds serve as definitive hosts, with some species of *Contracaecum* sp. being highly specific to their avian host and others infecting a wide range of hosts (Fagerholm & Overstreet, 2008).

These species have been described in numerous families, such as Laridae, Gaviidae (divers), Ciconiidae (storks), Phalacrocoracidae (cormorants) , Pelicanidae (pelicans), Anatidae, Ardeidae and even Spheniscidae (penguins) (Fagerholm & Overstreet, 2008).

Just like the previously described species, *Porrocaecum* sp. present a cosmopolitan and wide distribution among different birds. Some of the most common species include: *Porrocaecum ensicaudatum*, mostly found in the intestine of passerines, *Porrocaecum semiteres* described in studies with Laridae and *Porrocaecum crissum*, present in Anseriformes (Anderson, 2000).

Avian Ascaridoidea species are generally not a significant risk to public health, although it has been suggested that juvenile worms may be able to acclimate to high temperature or develop to the 4th larval stage (L4) and infect humans (Vidal-Martinez, Osorio-Sarabia, & Overstreet, 1994). Regarding domestic animals, *Contracaecum* sp. nematodes may present a potential risk to aquatic birds reared commercially, on display in zoos or used in research/recovery. Infections can rapidly accumulate in birds that are confined in small amounts of water with fish that will serve as intermediate hosts. As for *Porrocaecum*, these may represent a significant risk to domestic birds especially when earthworms are present and/or paratenic host have access to these. However, the apparent host specificity of some species may reduce the relative risk of infection to domestic hosts. Little is known about the risk that wild birds pose to the domestic ones (Fagerholm & Overstreet, 2008).

3.1.3 Life cycle

A number of researchers has continuously studied the life cycle of these species. In the life cycle of *Contracaecum*, fully embryonated eggs containing L3 are passed in the faeces of definitive hosts. In the aquatic environment, those species that parasitize aquatic vertebrates have free swimming larval stages (Bowman, 2014). These are ingested by a crustacean intermediate host and penetrate into the body cavity of these invertebrates. Fish can act as paratenic hosts by ingesting crustaceans and once that happens, L3 larvae encyst within the intestinal wall, mesentery, liver or other internal organs. When fish (or crustaceans) are ingested by a suitable avian host, the L3 are released and undergo two final moults to L4 and adults within the proventriculus of the avian host (Fagerholm & Overstreet, 2008). Parasites survive by consuming the hosts ingesta and when not feeding, they attach to the proventriculus wall (Anderson, 2000). Under some conditions, fish may serve as a true intermediate host and directly transmit the infection. An additional important way of infection is the direct "accidental" transmission of adult worms along with regurgitated food from the parent birds to the chicks (Fagerholm & Overstreet, 2008).

In contrast, the life cycle of *Porrocaecum* shows their adaptation to terrestrial existence, including earthworms as intermediate hosts and various migrations through transplacental and transmammary pathways. Another adaptation was the development of an eggshell capable of surviving to chemical and physical insults and remaining infective in soil for many years (Bowman, 2014; Fagerholm & Overstreet, 2008).

After earthworms ingest larvae from the embryonated eggs, these reach the main blood vessels and remain there. Some species found in birds of prey (e.g. *P. depressum*), use small mammals as paratenic hosts and the larvae are found encysted in the mesentery and intestine (Fagerholm & Overstreet, 2008).

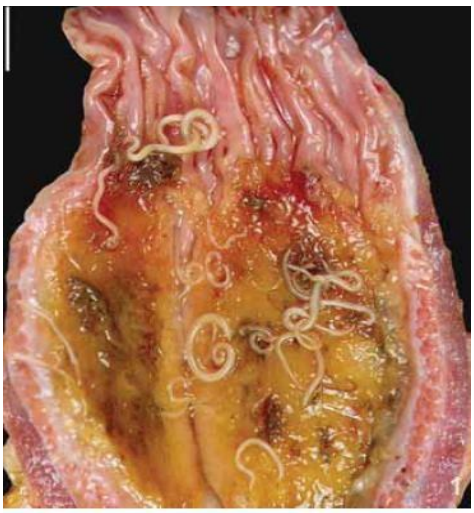
3.1.4 Clinical signs and pathogenic alterations

Infections with *Contracaecum* and *Porrocaecum* adults usually produce no severe disease or clinical signs in birds. In fact, numerous studies show or suggest that the host nutritional, immune and stress status may directly/indirectly affect the severity of infection. However, starvation, ruffled feathers, inability to maintain balance, severe inflammatory response in alimentary tract (related to migrations within the proventriculus, oesophagus or intestine), peritonitis and anaemia were described in several cases (Fagerholm & Overstreet, 2008). *Contracaecum* parasites are often found lying unattached to the lumen of the proventriculus (Fig.17) or embedded in its mucosal wall causing petechial haemorrhages, ecchymosis and erosive ulcerations. The tissue interface is usually a nodular area showing signs of necrosis, penetration, granulomatous inflammation and abundance of bacteria (Huizinga, 1971; Fagerholm & Overstreet, 2008).

There is evidence that prevalence and intensity of infection with *Contracaecum* sp. declines with age. This may be related to the reduced exposure to worms once birds are old enough to select their own prey as well as age immunity (Humphrey, Courtney & Forrester,1978).

In terms of public health, *Contracaecum osculatum* was previously associated with irritation of the oesophagus and stomach (nausea, vomiting and diarrhoea) or even abdominal pain in humans (Buchmann & Mehrdana, 2016).

Fig. 17 A- Neotropical cormorant (*Phalacrocorax brasilianus*) proventriculus showing *C. rudolphii* loose individuals, bar=10mm (Source: Amato, Monteiro & Amato, 2006)



3.1.5 Treatment and control

Despite not being usually responsible for serious infections, fenbendazole (20 mg/kg orally) has been advised for *Contracaecum* sp. treatment in waterfowl and seabirds (Keeble, 2017). In other studies, albendazole and fenbendazole were determined effective against *Contracaecum multipapillatum* in the Brown Pelican (*Pelecanus occidentalis*) and a single dose of 1-tetramisole also showed good results (Fagerholm & Overstreet, 2008). Buchmann & Mehrdana (2016) used thiabendazole successfully against symptoms re-ocurrence of a human *C. osculatum* infection after surgical worm removal.

Control strategies should first consider eliminating or reducing access to the intermediate hosts, which means that *Contracaecum* hosts must be prevented from feeding on infected fish and from defecating in confined water systems. In addition, other measures should be taken in order to reduce compounding stress triggered by lead shot, pesticides and other contaminants. To prevent transmission through infected food, these should be exposed either to high temperatures (63ºC for 17 min) or freezing temperatures (-20ºC or below for 7 days) that will kill all stages of *Contracaecum* and *Porrocaecum* (Fagerholm & Overstreet, 2008).

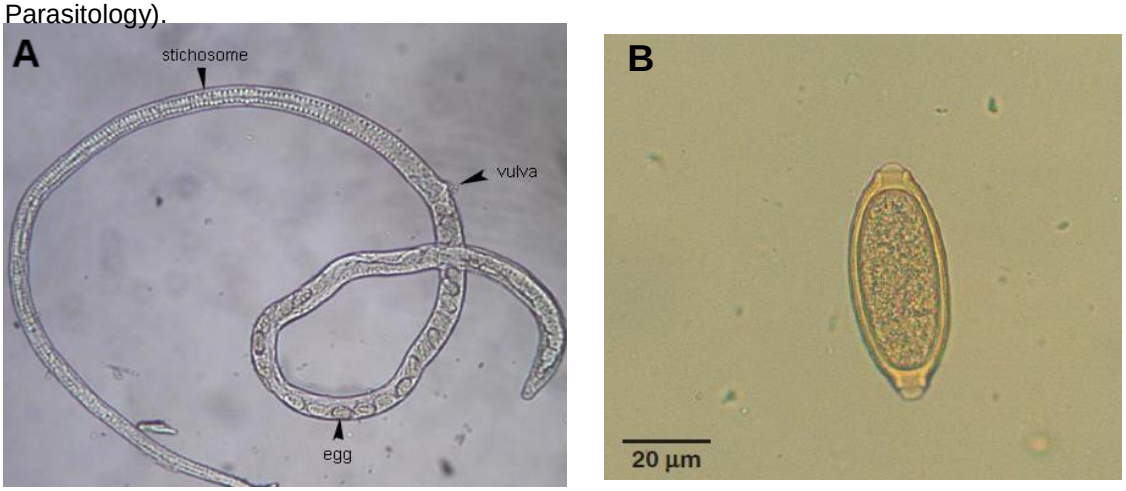
3.2 Capillariidae family

3.2.1Morphology

Capillariidae family comprises a very large group of 24 genera that infect all classes of vertebrates, 7 of these (*Baruscapillaria, Capillaria, Echinocoleus, Eucoleus, Ornithocapillaria, Pterothominx,* and *Tridentocapillaria*) affecting the gastrointestinal tract of birds. The species *Eucoleus contortus* has been found in the pharynx , oesophagus and crop of birds from Laridae family (Yabsley, 2008; Bowman, 2014).

Capillarids are small nematodes often found partially embedded in mucous membranes of the organs and showing a characteristic oesophagus with a short muscular anterior part and a long glandular posterior portion (stichosome) (Yabsley, 2008; Bowman, 2014). Identification of species depends on knowledge of the site of infection, examination of the vulvar region of the female worms and a detailed study of the posterior and anterior ends of adult worms of both sexes(Fig.18A). Capillariidae eggs have bipolar plugs and their colour may vary between nearly clear to a deep golden colour (Fig.18B). The surface ranges from smooth to variably textured (Yabsley, 2008).

Fig. 18 – Capillariidae morphological features and eggs: A) Female *Capillaria* sp. showing the stichosome, vulva and eggs (Source: Faculty of Medicine of Chiang Mai); B) *Capillaria* eggs with their bipolar plugs (Source: Zajac & Conboy, 2012- Veterinary Clinical Parasitology).



3.2.2 Host and distribution

Capillariidae are highly adaptive species with a cosmopolitan distribution because of the wide geographic range of the numerous hosts they infect (e.g., Anseriformes, Galliformes, Passeriformes, Charadriiformes, Ciconiformes, Falconiformes, Gruiformes).Those species have been reported from two or more related avian species and a few are known to infect a wide range of avian host species (e.g., *E. contortus* infects birds in nine orders) (Yabsley, 2008).

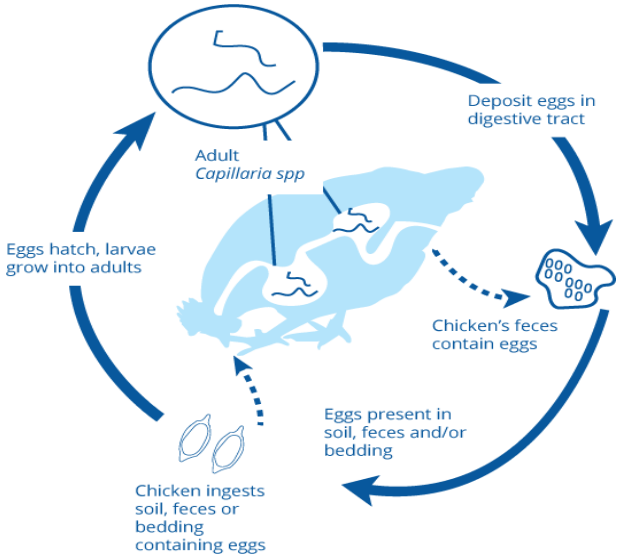
The only known avian capillarid that poses a zoonotic threat is *Capillaria philippinensis*, causing severe enteritis in humans when they ingest raw/undercooked freshwater and brackish-water fish (Yabsley, 2008).

3.2.3 Life cycle

Species from the family Capillariidae use a direct life cycle or a paratenic host (earthworm) as a mean of transmission between hosts. The eggs are shed in the faeces and develop into the infective stage. They can be ingested directly by a new host or by an earthworm and in this case, the parasite does not undergo any life cycle stages until it reaches the definitive host (Fig.19) (Hendrix & Robinson, 2012).

For *E. contortus*, the life cycle is direct and eggs take 24–40 days in the environment until they become infective. Under ideal conditions and no sunlight, embryonated eggs can remain viable for up to 15 months (Yabsley, 2008).

Fig. 19 -*Capillaria* life cycle (Source: Poultry DVM)



3.2.4 Clinical signs and pathological alterations

Although clinical disease among free-ranging birds is uncommon, there is high risk of transmission and disease among captive birds in poorly maintained facilities where worm burdens are high. Clinical signs and severity of lesions depend on several factors: host species, parasite species and their location within the host, intensity of infection and extent of tissue damage. Birds with large numbers of parasites may present emaciation, diarrhoea, ruffled feathers, anorexia, reduced water intake, ataxia and weakness (Yabsley, 2008).

Also, in high intensity infections, Capillariidae may be recovered from the wall of the upper alimentary tract and crop where they can cause inflammation, dilatation of the crop or oesophagus, thickening of mucosa, ulceration, bacterial colonization, exudation and fibrinonecrotic plaques (Hendrix & Robinson, 2012; Yabsley, 2008).

Low numbers of worms in an healthy host rarely cause any appreciable lesions, but when parasites migrate to the submucosa significant lesions and secondary bacterial infections can develop in aberrant hosts. Diagnosis of *Capillaria* infection can be performed by detection of eggs in a faecal sample, presence of characteristic clinical signs and/or lesions and absence of other pathogens (Yabsley, 2008).

3.2.5 Treatment and control

Fenbendazole, febantel, and levamisole have shown high efficacy in the treatment of capillariasis in numerous avian species including domestic fowl and falconiformes (Yabsley, 2008). Piperazine, mebendazole, thiabendazole, levamisole, tetramisole and ivermectin have also been used in Anseriformes and Gruidae family (Wallach & Boever, 1983; Olsen,1994; Tarello, 2008) .

Captive wild birds when exposed to stress and infective eggs/earthworms may develop clinical disease. Sanitation of living areas and raising birds on wire bottom cages are preventive measures that greatly decrease the risk of disease (Yabsley,2008).

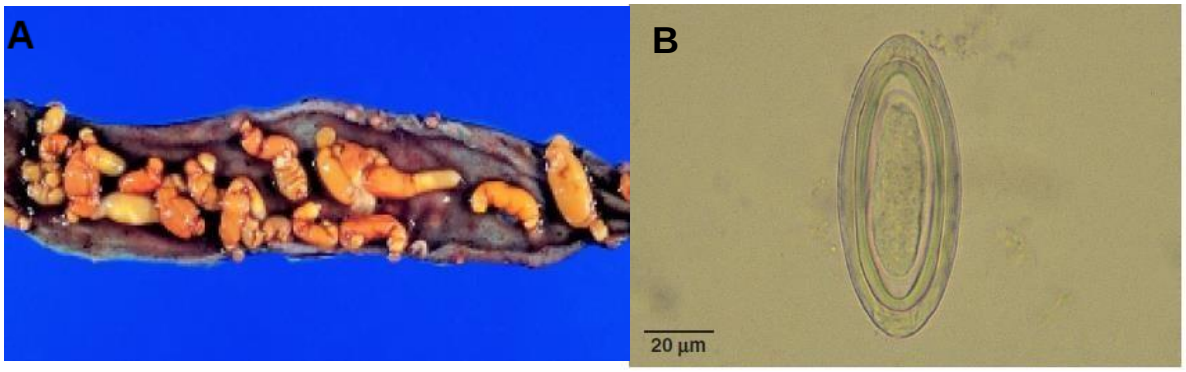
3.4 Acanthocephala phylum

3.4.1 Morphology

Parasites from the phylum Acanthocephala comprise more than 1.100 valid species. They are known as thorny-headed worms because of their retractable and invaginable proboscis (although some are not retractable) with sharp, recurved hooks used to attach to the intestinal wall of the definitive host (Fig. 20A). Acanthocephalans in birds vary greatly in size from a few millimetres to over 10 cm long, depending on the species (Cole, 1999; Richardson and Nickol, 2008). They do not present a digestive tract, with nutrients being ingested through the tegument (Bowman, 2014).

Acanthocephalan eggs (50-100 µm) have several internal layers surrounding the larva (acanthor) (Fig.20B; Zajac & Conboy, 2012).

Fig. 20- Acanthocephala adult worms and typical egg A) Acanthocephalans attached to mucosa of the intestine of a bird (Source: R. A. Cole- Field Manual of Wildlife diseases); B) Acanthocephalan egg of a Barred owl (*Strix varia*), presenting several internal layers surrounding the larva (acanthor) (Source: Dr. Ellis C. Greiner- Veterinary Clinical Parasitology)



3.4.2 Host and distribution

Acanthocephalans infect all classes of vertebrates and life stages are common in a few avian hosts. Some species from Anatidae family (ducks, geese, and swans) are considered to be the most commonly infected birds, along with birds of prey and some species of passerines (Cole, 1999). *Arhythmorhynchus* and *Plagiorhynchus* are the main genera of Acanthocephala in Charadriiformes although some authors, have also reported the presence of *Falsifilicollis* and *Profilicollis* (Threlfall, 1968; Pemberton, 1963; LaSala & Martorelli, 2007; Richardson and Nickol, 2008).

3.4.3 Life cycle

All acanthocephalans present an indirect life cycle in which the vertebrate definitive host becomes infected by ingesting the larvae (cystacanth), contained in the body cavity of an arthropod intermediate host. The intermediate hosts tend to be typical food of the definitive hosts (Cole, 1999). Those with terrestrial life cycles are usually infected when they ingest insects, but the ones with aquatic life cycles prefer microcrustaceans (e.g., *Amphipoda, Isopoda* and *Decapoda*). Fish, snakes and frogs have also been identified as paratenic hosts (Cole, 1999; Richardson and Nickol, 2008).

When the eggs are released by the definitive host, they contain a fully developed larva, the acanthor, which is ingested by the intermediate host. After penetrating in the wall of the alimentary canal, the acanthor develops through an acanthella stage into an encysted infective larva, the cystocanth. The cystocanth is capable of reencysting in the gut of a vertebrate paratenic hosts, or even reencysts in its normal definitive host instead of attaining its final stage of maturity. If ingested by a suitable definitive host, the cystocanth will attach to the definitive host's intestinal mucosa through its spined proboscis, mature, mate and produce eggs (Cole, 1999; Richardson and Nickol, 2008).

Some crustaceans infected with certain species of acanthocephalan register a change in colour, which is thought to be an evolutionary adaptation that increases predation by the definitive hosts and consequently the chances of completing its life cycle (Cole, 1999).

3.4.4 Clinical signs and pathological alterations

There is little understanding about the clinical signs in birds with acanthocephaliosis. In some cases, low intensity infections seem to have serious adverse effects but in other cases of high intensity infections, there is an absence of clinical signs. This situation is actually more common and it seems to be connected with food shortage periods, exhaustion and stressful situations (Cole, 1999; Richardson and Nickol, 2008).

Lethargy and emaciation are some of the nonspecific but common clinical signs associated with severe infections. In the mucosa or external surface of the intestine, fibrinous nodular

lesions caused by the attachment of the proboscis of the parasite might be present. In some cases, penetration of the acanthocephalan through the gut wall might lead to peritonitis and death (Cole, 1999; Richardson and Nickol, 2008). The fact that the parasite absorbs nutrients from the bird's intestinal canal, weakens the host and make it more susceptible to other diseases and to predation (Richardson and Nickol, 2008).

3.4.5 Treatment and control

Acanthocephalans that infect mammals have been successfully treated with Ivermectin or fenbendazole. Thiabendazole has been recommended for use in birds, but the treatment is acknowledged to be difficult with low rates of success (Cole, 1999; Brown & Cromie, 1996).

Prevention of acanthocephaliasis in wild birds seems impractical. However, captive populations can be managed in order to keep them away from environments that harbour infected intermediate hosts (e.g., crustaceans) and younger birds can be segregated on water areas which are not used by other birds (Cole, 1999; Richardson and Nickol, 2008)

IV. The most prevalent respiratory and gastrointestinal parasites in Herring Gulls admitted in a wildlife rehabilitation centre in South East England

1. Objectives

As previously mentioned, Herring gulls are one of the most commonly admitted species to RSPCA Mallydams Wood Centre, with peak numbers during the summer months (Appendix I). The main objectives of this dissertation are:

1) To determine the presence of some of the most common respiratory and gastrointestinal parasites in a group of Herring gulls admitted and euthanized in the centre, by using coprological methods;

2) To evaluate the parasite burden on the studied population using EPG counts;

3) To identify, macroscopically, the presence of parasites and to characterize gulls' major pathological alterations by performing necropsies;

4) To evaluate the relationship between positive cases and different variables (such as life stage, body condition, duration of stay, admission month, post-mortem pneumonia signs, and doses of fenbendazole…)

2. Material and methods

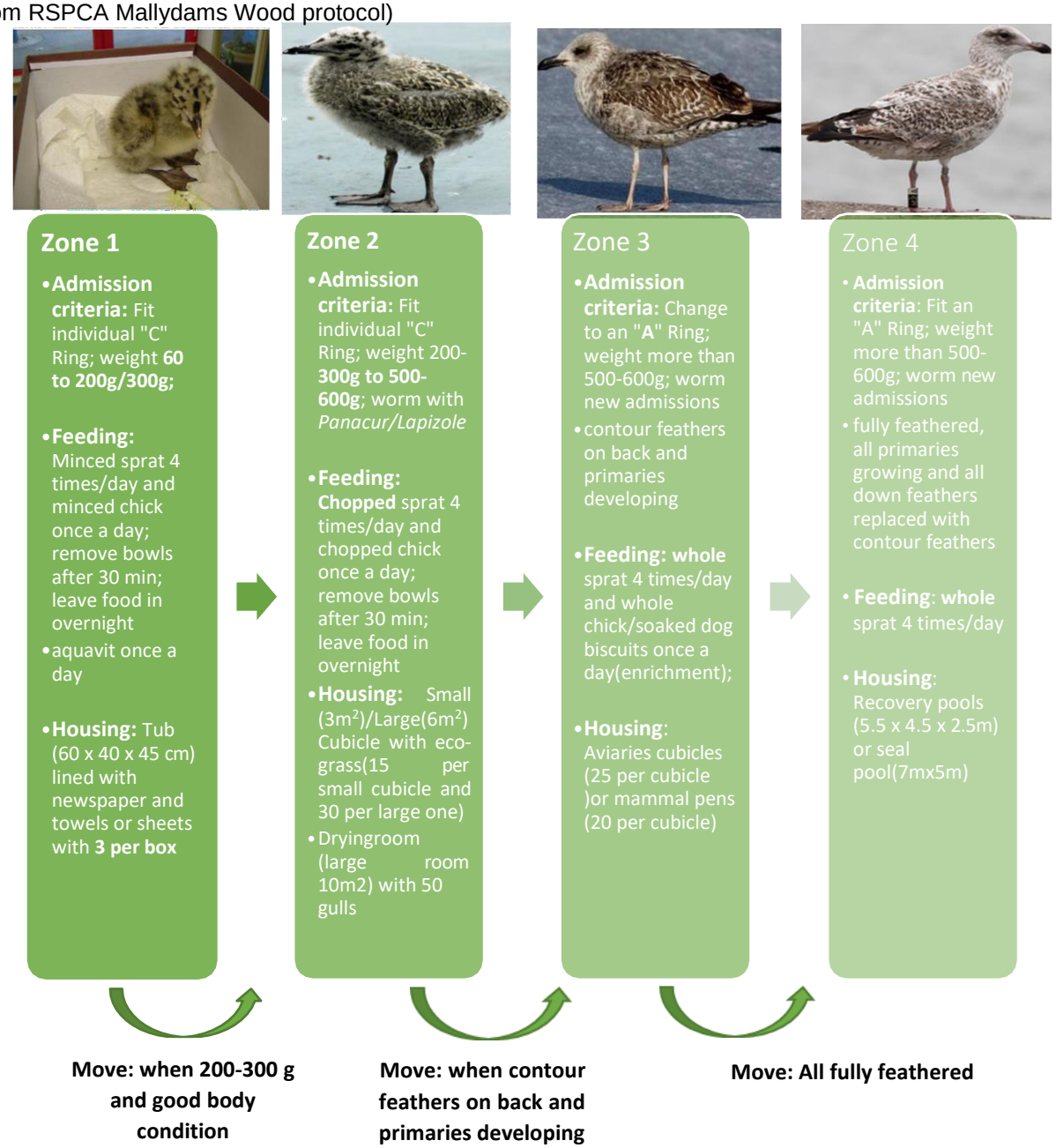
2.1. Characterization of environmental and husbandry factors

Seabirds are especially sensitive to environmental changes (Rajpar et al., 2018), so when studying them, the abiotic factors should definitely be considered. According to Meteorology Office (2018), summer temperatures in the UK were well above the average in 2018, being considered the warmest summer to date. July was considered the warmest month with a mean temperature 2.2 ºC above average and June was the driest month with less than 10% of average rainfalls in the southeast. After the first week of August, both temperature and amount of rainfall were close to the average (Met Office, 2018).

RSPCA wildlife centres developed several husbandry and rehabilitation protocols for the most commonly admitted animals, including gulls. However, in 2017, the veterinary staff readapted this protocol due to the high number of gulls with respiratory signs that needed euthanizing (especially among nestlings and fledglings - Fig. 21). The centre created four zones, which separated animals from different life stages that needed distinct management (e.g., frequency/type of feeding). This also had impact in the eventual transmission of pathogens between different stages. In that year, gulls were dewormed with 50mg/kg fenbendazole following RSPCA and EUROWA (European Oiled Wildlife Response Assistance) oiled wildlife response protocol (Thomas, 2016). The medications including Fenbendazole like Panacur® Equine oral suspension 10% 100mg/N or Lapizole® 20 mg/ml were used in every zone, but with the persistence of respiratory signs, the veterinary staff started considering other agents (e.g.,*Aspergillus* sp.).Therefore in 2018, the deworming frequency was reduced to just once on admission in gulls with minimum weight (200g at the starting of gull season with a re-adjust

of 300g mid-season) or twice if the respiratory signs persisted. This decision was also justified by the concerns with the stress handling to worm and fenbendazole effects on feather development. The staff also tried to fog gulls from zone 2 with F10® nebulizer, which is used to reduce the risk of aspergillosis infections.

Fig.21- RSPCA Mallydams Wood wildlife husbandry and rehabilitation protocol 2018 (in the beginning of season the minimum weight to deworm was 200g and minimum weight to fit an "A" ring was 500g but in mid-season the protocol was readjusted to 300g ang 600g, respectively) (Adapted from RSPCA Mallydams Wood protocol)



2.2 Storage of carcasses, necropsy procedures and sample collection

Summer months are the ones with the highest numbers of gulls' admissions and so, a random sample of 63 carcasses of different life stages was previously stored in the freezing chamber (-20ºC) until the beginning of the study. Each carcass was identified by its ring number (residents in the centre) or by a sticker with its case number (euthanized on admission) so that the author could have access to the individual recorded data. During the internship period, the

student had also the opportunity of performing two fresh necropsies (n=65) where histopathological analysis were performed in lung samples with acute inflammatory signs. However, it was not possible to resort to histopathology in the other 63 carcasses since the freezing process causes disruption of tissues' architecture and cellular lysis by ice crystals (Latimer & Rakich, 1994). The centre provided all the necessary material, such as full personal protection equipment (PPE), forceps, scalpel, blades and sample containers.

The necropsy procedures were performed after the elaboration of a risk assessment approved by RSPCA and following a necropsy protocol (Appendix II) adapted to the present study. Initially, an external examination was performed and the body condition score determined by palpating the keel and breast muscle on a scale of 1 (emaciation) to 5 (obesity). Then, the carcass was incised in the caudal edge of the keel and the skin removed. Once the body cavity was opened, liver, heart and lungs were examined for parasites and pathological alterations, especially acute inflammation signs on the lungs. For this purpose, the float test was used and in case the lung sample did not float that was suggestive of pneumonia (the presence of inflammation in the lower respiratory system leads to lung sinking). The upper airways and upper digestive tract were examined by cutting the commissure of the beak and continue through the oesophagus and then the trachea. A transversal cut in the upper beak was also very useful to look for the presence of parasites in the nasal cavity. Finally, after removing the digestive tract, faecal samples were collected for further coprological analysis and the digestive tract was cut open and observed looking for the presence of worms or alterations in the mucosa.

The collected faecal samples were stored in a refrigerator during 3 to 4 hours until the coprological methods were performed. When adult parasites were found, they were also stored in sample containers under the same conditions.

2.3 Coprological analysis

In order to identify parasite eggs' present in the faecal samples, two types of concentration methods were used: Faecal Willis Flotation and Natural Sedimentation. The McMaster technique was also used to determine the number of parasite eggs shed per gram of faeces. Other coprological methods such as Baermann method or Coproculture were not considered in the present study since both methods imply the presence of live forms of parasites, so that migration and larval growth can occur.

2.3.1 Macroscopic identification of parasites

All the adult parasites were observed using a magnifying glass and measured to allow the correct identification (up to genus level) in comparison with the morphological characteristics referred in other studies (Burt & Eadie, 1958; Zajac & Conboy, 2012; Fernando & Barta, 2008).

2.3.2 Willis Floatation and Natural Sedimentation

The Willis Floatation is a qualitative coprological method based on the specific gravity of eggs, cysts and larvae (between 1.1-1.2 g/mL) when added to flotation solutions. These solutions, such as concentrated sugar, saturated sodium chloride, zinc sulphate or magnesium sulphate (used in the present project) have higher specific gravity than most eggs (usually nematode and cestode ones) which makes them float, whereas the heavier ones sink in bottom of the column. It's worth mentioning that although magnesium sulphate is an inexpensive and readily available solution, it may form crystals on microscope slide (Hendrix & Robinson, 2012).

Around 2g of faeces were diluted in 15 to 20 ml of magnesium sulphate and then passed through a strainer into a tube until a meniscus was formed (Fig.22). After that, a cover slide was placed on the top of the tube during 10-15 min so the eggs have time to float and attach to the cover slide (Greiner & Ritchie, 1994). Finally, the glass cover slide was placed on a slide and observed on a microscope using 10x and 40x objectives.

Fig. 22 Willis Flotation method (Original).

As for the Natural Sedimentation method, it is usually used to detect eggs or cysts that have too high specific gravity to float or that would be severely distorted by the flotation solution (Hendrix & Robinson, 2012). In the present project, this technique was only used to identify acanthocephalan eggs although according to Bowman (2014), it is also appropriate for trematode eggs, amoebas, ciliates and formalin fixed *Giardia* cysts. Using the same Willis Flotation tube, the supernatant was decanted and the remaining sediment was stirred until 2 or 3 drops were collected by a Pasteur pipette and placed in a microscope slide. To facilitate the observation, one drop of methylene blue was added to the slide to stain the faecal debris blue, which contrast with the yellowish brown colour of the eggs (Zajac & Conboy, 2012).

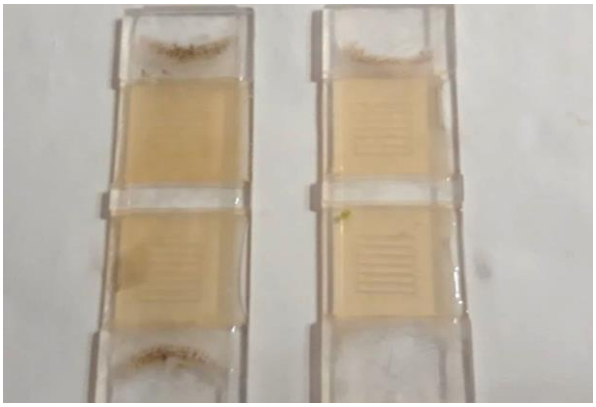
2.3.3 McMaster technique

The McMaster technique is used to determine the number of parasite eggs' shed per gram of faeces. For that purpose, 2g of faecal sample were added to 28 ml of flotation solution and

homogenized. After that, the solution was filtered through a strainer into a cup and both McMaster chamber compartments (with 0,15 ml capacity) were filled in with the mixture. After waiting 3 to 5 min, so that the eggs could float and attach to the top layer of the chamber, the preparation was finally observed under the microscope using 4x and 10x objectives (Fig.23) (Zajac & Conboy, 2012).

To determine the number of EPG on each sample, the eggs observed inside the limits of the grids are counted and multiplied by the correction factor of 50 (Madeira de Carvalho, 2002).

Fig. 23- McMaster quantitative technique(Original).



2.4 Statistical analysis and tests

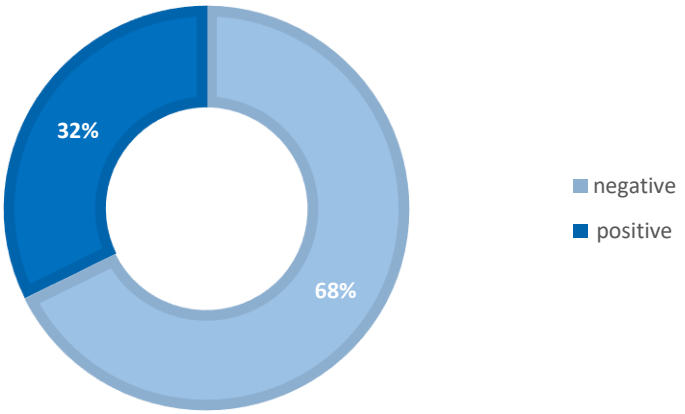
The results of necropsy findings and coprological analysis were registered in a spreadsheet of Microsoft Office Excel 2007 © software where the descriptive statistical analysis (Tables, Graphs) was made. Statistical tests were performed in the Software R © version 3.5.2., using the graphical user interface, R-Commander. Chi-square test for independence was used to determine significant differences between parasitism and different variables (parasitism vs. signs of pneumonia) and when the expected frequencies were lower than 5, Fisher's exact test was chosen instead (parasitism vs. life stage, parasitism vs. admission months). Some of the variables were not normally distributed and in these cases, the two-sample Wilcoxon non-parametric test was used to evaluate means differences between groups (parasitism vs. duration of stay; signs of pneumonia and duration of stay; parasitism and doses of fenbendazole).

3. Results

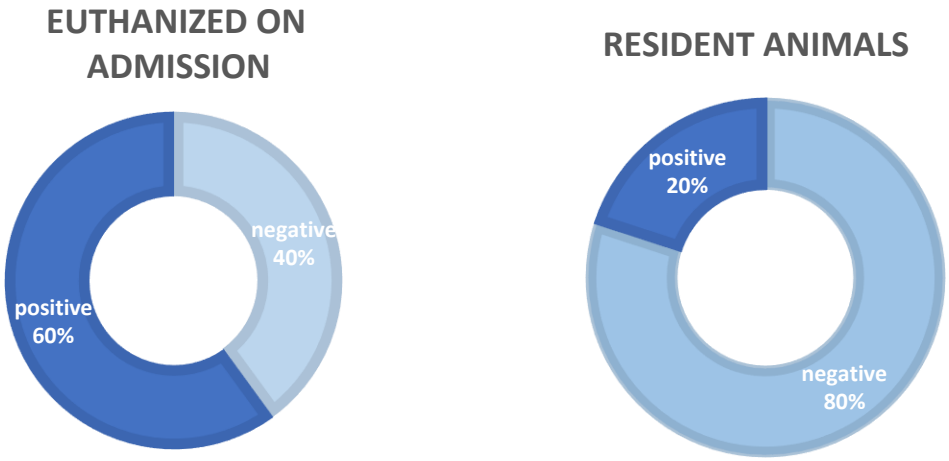
3.1 General results

The general results (Graph 2) of the present study showed that 21 of 65 of the animals (32%) were positive to the presence of parasites in at least one of the diagnostic techniques. Among the 45 resident animals (that received at least one dose of fenbendazole), 9 (20%) were still diagnosed as positive and as for the euthanized on admission ones (non-dewormed), 12 (60%) out of the 20 were found positive to parasites.

Graph 2- Graphic that shows the proportion of positive and negative samples (N=65) .



Graph 3 - The percentage of positive and negative within the euthanized on admission group (n=20);
Graph 4 - The percentage of positive and negative within the resident animals group (n=45).



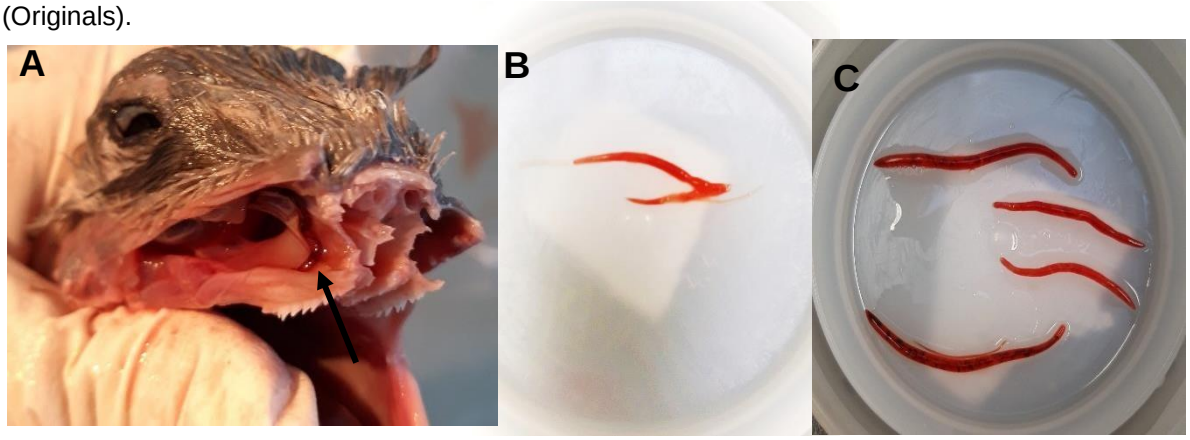
3.2 Parasite identification

3.2.1 Macroscopic identification

During necropsy procedures, 18.5% (12/65) of the individuals, all of them fledglings, presented worms in the nasal cavity close to the infraorbital area (Fig 24A). Considering their morphological characteristics (colour, size, shape) and localization, all the adults were diagnosed as syngamids. There was one case (1.54%) of two adult *Syngamus* spp. that although showing visible degeneration, was visibly formed by a large female (1.7 cm) and smaller male (1.2cm), forming an “Y” in permanent copula (Fig. 24B).The other 16.9% (n=11) were diagnosed as *Cyathostoma lari* due to its colour, localization, size (between 1 cm and 2,6 cm) and separation between males and females (Fig. 24C). The macroscopic distinction between sexes wasn´t possible due to autolysis (the convoluted and twisted ovaries or twisted single testis weren’t visible).

A total of 27 adult *Cyathostoma lari* individuals were found in the nasal cavities with a mean of 2.25 individuals per gull. Among the 12 cases who presented adults in the nasal cavity, 5 weren't positive to any other qualitative/quantitative method.

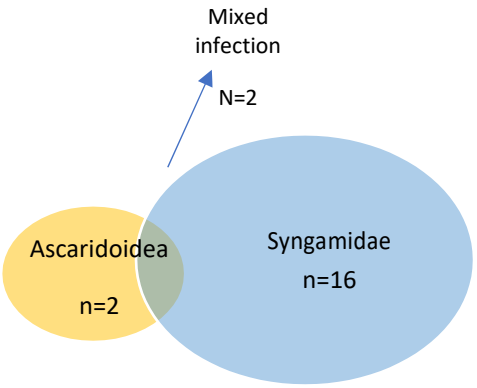
Fig. 24 - Macroscopic identification of parasites of the project: A) Adult parasites inside nasal cavity(arrow); B) Adult forms of *Syngamus trachea* showing the large reddish female permanently in copula with the small male forming a "Y" shape; C) 4 adult reddish forms of *Cyathostoma lari* (Originals).



3.2.2 Concentration methods: Eggs' identification

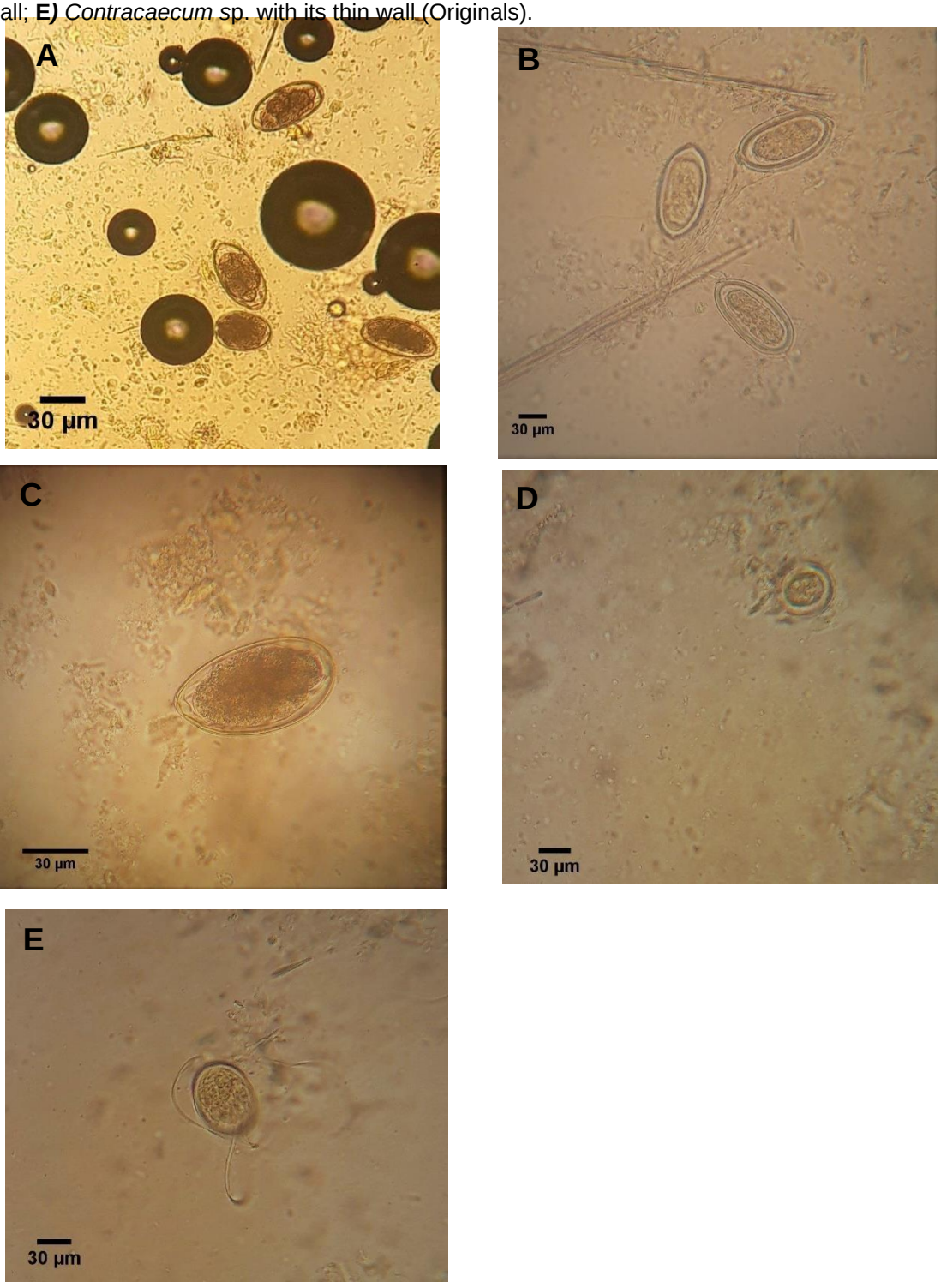
Using the Willis Flotation method, 24.61% (16/65) were diagnosed as positives to the presence of eggs. A total of 44.8% (7/16) within this group also presented visible worms. All these 16 cases presented Syngamidae eggs with ovoid/ellipsoid form, distinct operculum in both ends and presence of a morula, which are typical from *Syngamus* sp. but also from some *Cyathostoma* species (namely *C. lari*). In 3.07% (n= 2) of the total population (n=65), some of the analysed eggs presented typical characteristics of *C. bronchialis* with an ovoid form and a single indistinct operculum at a narrow end which points to co-infection by different species, although the eggs can only be classified with certainty as Syngamidae type. Similarly, 3.08% (2/65) other cases presented a mixed infection with eggs from the Ascaridoidea superfamily: 1.54% (1/65) with *Contracaecum* sp. eggs and 1.54% (1/65) with *Porrocaecum* sp. type eggs) (Graph 5). As to Capillariidae eggs, no parasitic forms of this family were found.

Graph 5- Parasite eggs species' with respective prevalence: Syngamidae (blue circle)=16; Ascaridoidea (yellow circle)=2 (Original).



Each sample (n=65) was also analysed using the natural sedimentation technique but Acanthocephala eggs were not found. Figure 25 shows the different parasitic forms identified through coprology techniques in this study.

Figure 25 -Different parasitic forms identified in coprology **A)** Presence of 4 Syngamidae eggs: 3 ellipsoid eggs with distinct opercula on each end and 1 ovoid egg without distinct opercula typical of *C. bronchialis*; B**)** *3* Syngamidae eggs with distinct opercula and ellipsoid form; C) Syngamidae egg with ovoid form without distinct operculum; **D)** *Porrocaecum* sp. with its thick wall; **E)** *Contracaecum* sp. with its thin wall (Originals).

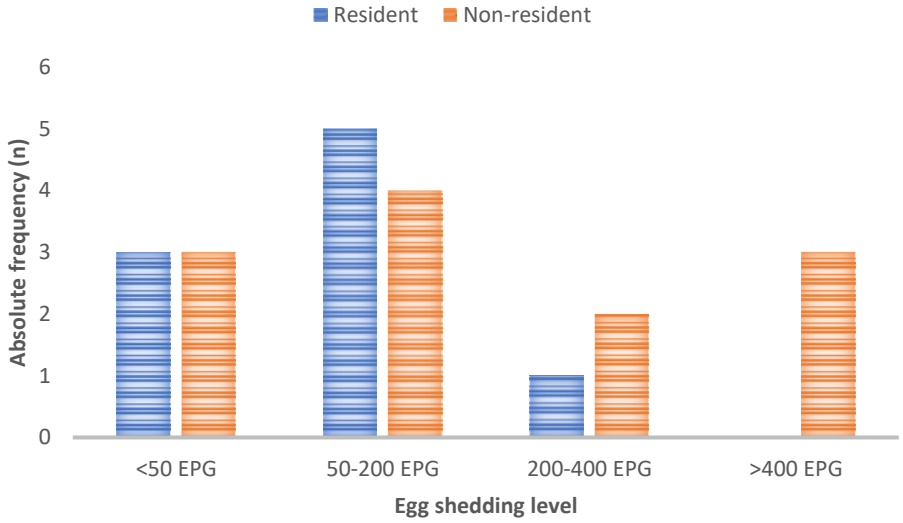


3.2.3 McMaster method

After performing the McMaster technique to the faecal samples, it was verified that 15 (15/65=23.1%) of the 16 positive samples to Willis flotation method (93.8%, 15/16) were also positive to this technique showing EPG counts ≥50 for Syngamidae eggs. The results (Graph 6) show that resident animals (who took one dose of dewormer), still showed 6 cases (9.2%, 6/65) with 50 to 400 EPG counts. The non-resident gulls also showed precisely 6 cases (9.2%, 6/65) with 50 to 400 EPG, but also 3 cases (4.6%, 3/65) with heavier faecal egg counts of 500, 800 and 1800 EPG .

As for the other studied parasite species (*Contracaecum* sp., *Porrocaecum* sp., Capillariidae, Acanthocephala), none was described through McMaster Method. There were still 6 other samples (9.2%, 3 from residents and 3 from non-residents) that although had been proven positive through other methods, were negative to this quantitative method.

Graph 6- Worm burden of Syngamidae in resident and non-resident animals.

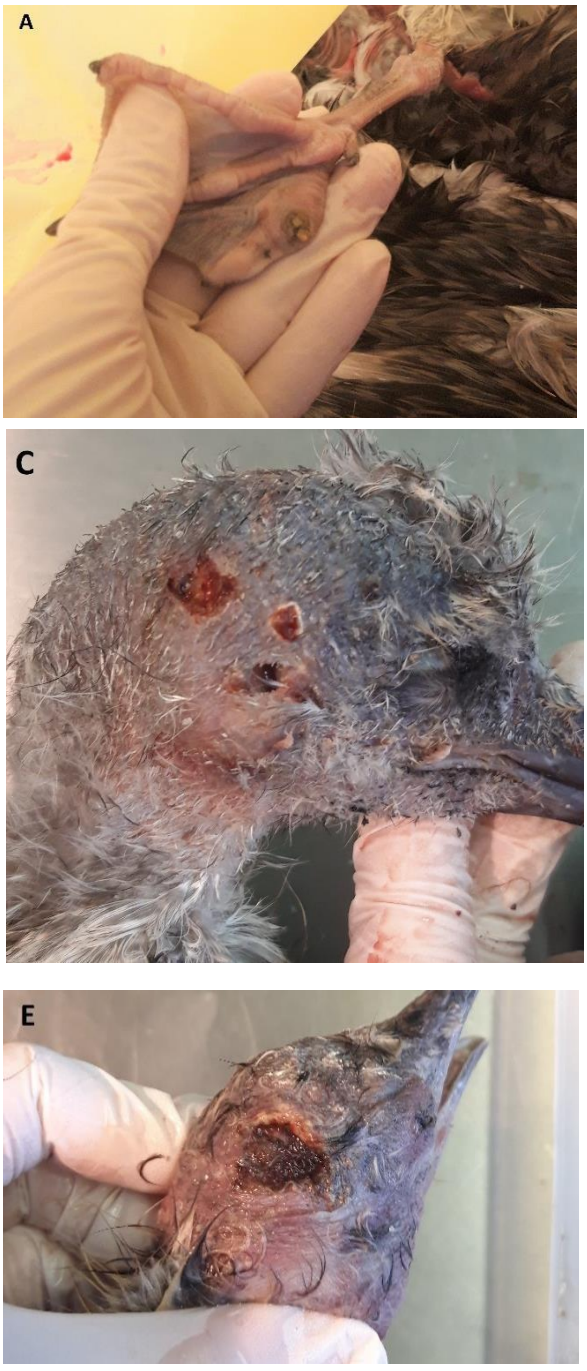


3.3. Necropsy findings

3.3.1External exam

In order to simplify data presentation, the external alterations were divided into several categories that can be found on Appendix III. Although 47 gulls did not show external alterations (72%), 13 out of 65 (20%), showed visible signs of pododermatitis. Some of the main external alterations can be found in the following pictures (Fig.26).

Figure 26 – Main external alterations at necropsy: **A)** 1st digit of the posterior left member (PLM) showing a digital nodular purulent and ulcerated lesion - pododermatitis; **B)** Left wing presenting loss of the feather's impermeabilization properties; **C)** Head's lateral right side showing open wounds above the auricular region; **D)** Posterior right member (PRM) with a chronical inflammatory process in a resolution phase with the presence of fibrotic tissue and signs of ulceration; **E)** Right lateral side of the skull presenting a fracture covered with coagulated blood (Originals).



3.3.2 Internal exam

The main internal alterations found at necropsy were also grouped in several categories that can be found on appendix III. The most prevalent alterations were lung congestion (23%, 15/65), lungs showing acute lung inflammation (30%, 20/65 sinked on the float test), presence of parasites from the Syngamidae family (18.46%, 12/65) and presence of foreign bodies (pieces of plastic, sticks) (17%, 11/65). There were also 5% (3/65) of the cases with chronic inflammatory alterations suggestive Aspergillosis. Some of the main internal alterations (Fig.27) can be found in the following pictures.

Fig. 27- Main internal alterations found at necropsy: A) Oesophagus with white isolated 2 mm node adherent to wall. B) Left lung showing a <2mm white isolated node with harsh consistency (compatible osseous metaplasia) and caudo-medial aspect of the right lung with a demarked congested area; C) Lungs with generalized signs of congestion; D) Celomic cavity with aqueous and haemorrhagic effusion; E) Left hepatic lobe with fissures in the central area and medial area of the right lobe presenting hematogenous pigment; F) Proximal area of the trachea presenting haemorrhagic fluid (Originals).

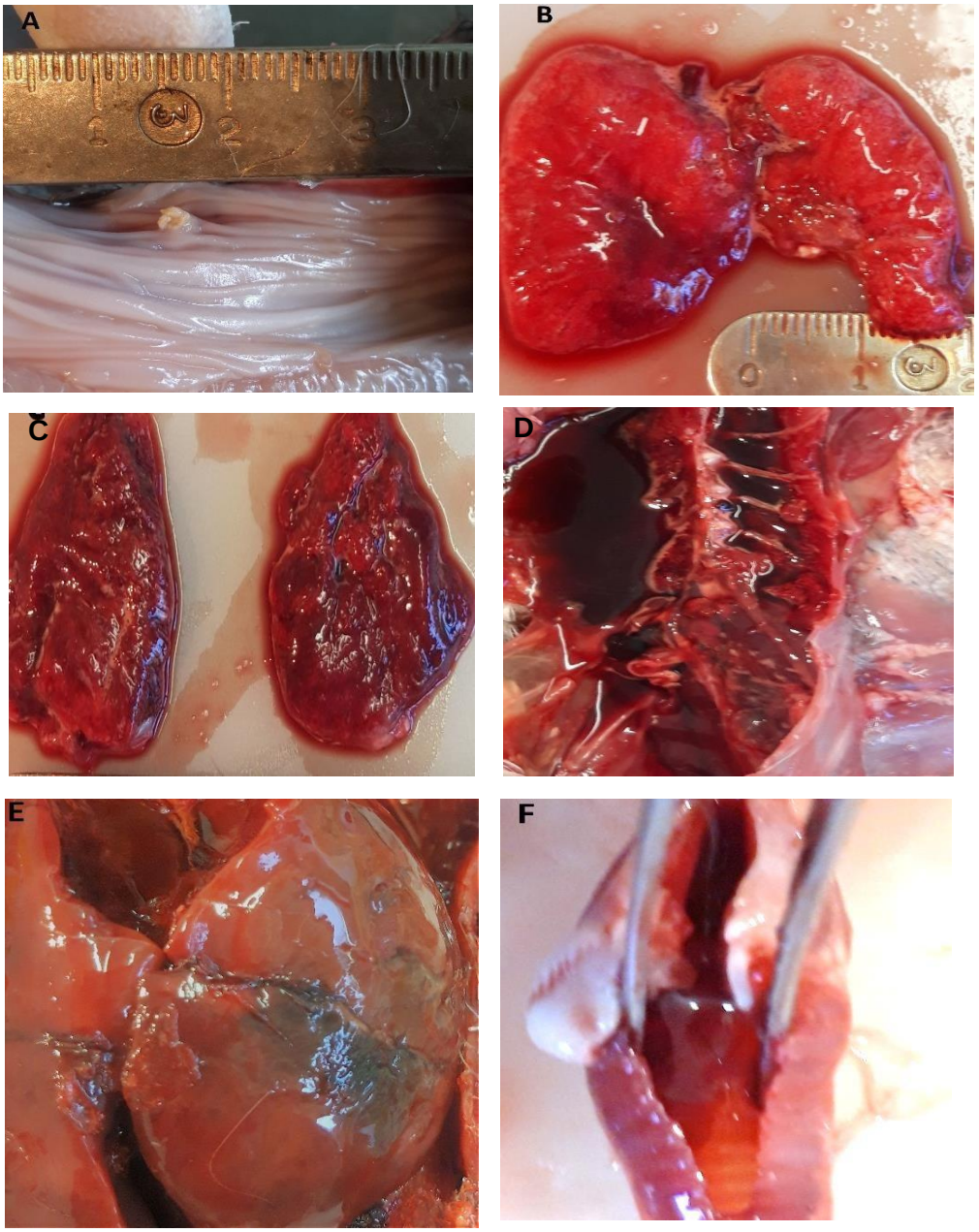
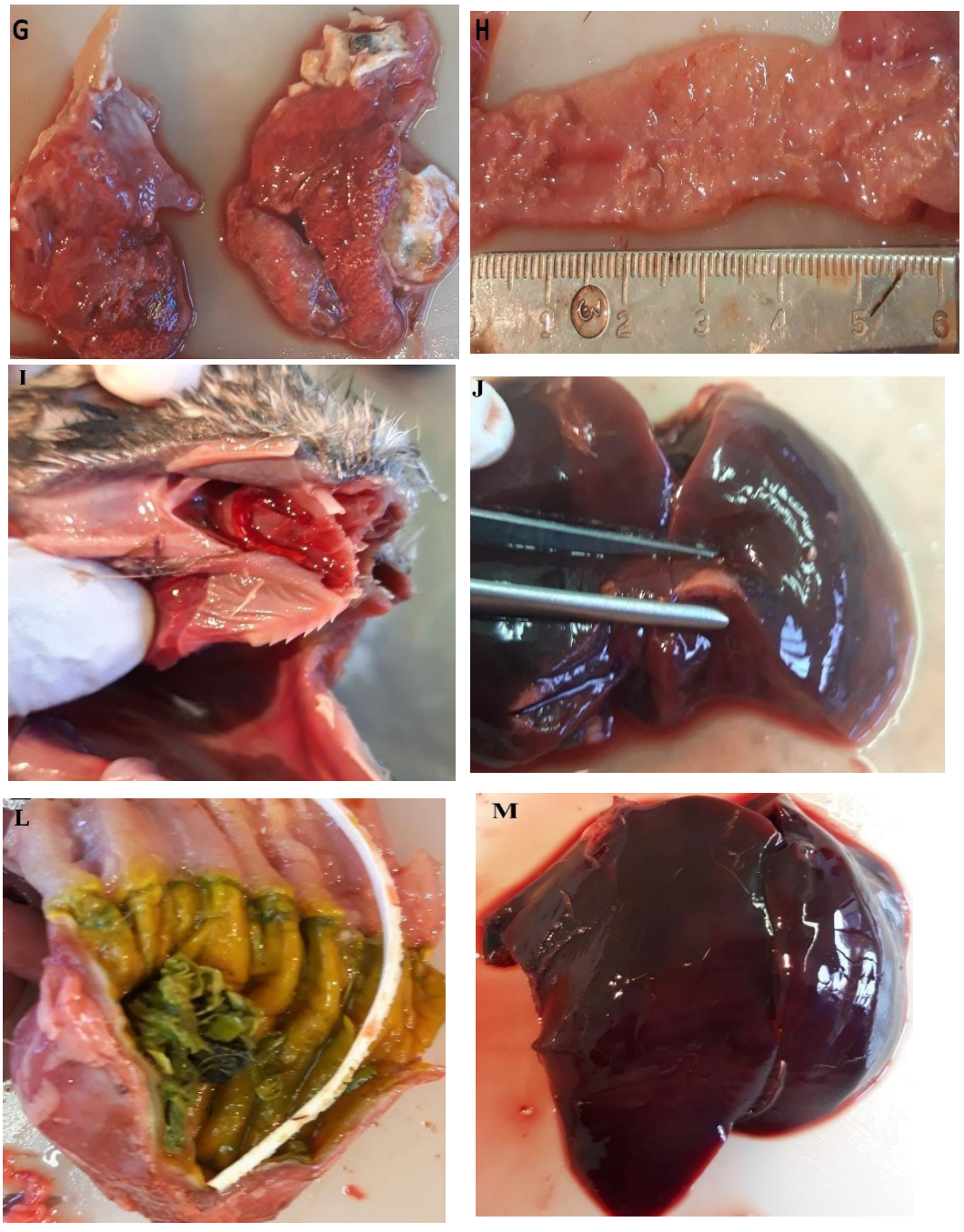


Fig. 27 (cont.) **G**) Lungs presenting white disseminated granulomatous lesions (suggestive of Aspergillosis) and congestion of the pulmonary parenchyma; **H**) Proximal area of the duodenum with erosive lesions (<1mm); **I)** Right infraorbital area of the nasal cavity presenting the parasites *Cyathostoma lari*; **J)** Right hepatic lobe with a sectioned lesion of a focal necrotic hepatitis; **L)** Gizzard with a foreign body (piece of plastic); **M)** Liver with signs of generalized congestion (Originals)

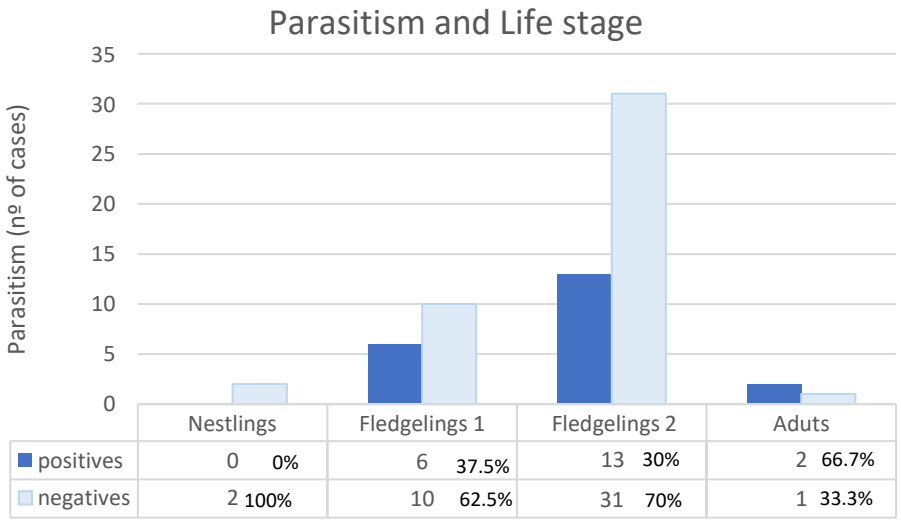


3.4 Statistical analysis

3.4.1 Relation between parasitism and life stage

Between the 4 ageing categories (Graph 7), the highest prevalence was found in adults with 66.7% [CI95%, 20.8%;93.9%] positives and fledglings type 1 with 38% [CI95%, 18.5%;61.4%] positives. As for the fledglings type 2, although this was the group with the highest number of researched gulls, only 30% [CI95%, 18.1%;44.2%] were diagnosed as positives. At last, none the 2 nestling gulls were positive. One can state that no significant differences were observed between the positive cases and the different life stages (p=0.3797).

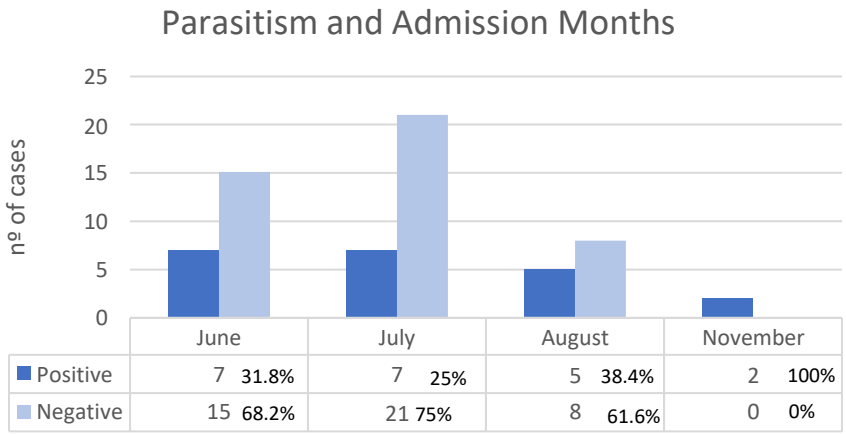
Graph 7- Percentage of positive and negative cases according to life stage.



3.4.2 Relation between parasitism and admission months

Most of the studied cases were admitted in the Summer months with exception of 2 cases admitted in the Fall (November) (Graph 8). The percentages of positives to parasites was 100% [CI95%, 34.2%;100%] in November, 38.4% [CI95%, 17.7%;64.5%] in August, 25% [CI95%, 12.7%;43.4%] in July and 31.8% [CI95%, 16.4%;52.7%] in June. The admission month (within the period of the study) does not seem to be associated with the number of positives (p=0.1848).

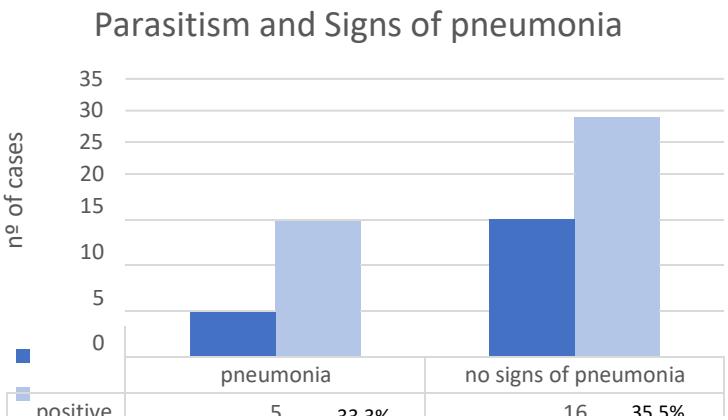
Graph 8- Percentage of positive and negative cases according to the admission month.



3.2.3 Relation between parasitism and post-mortem signs of pneumonia

As previously stated, 20 gulls (31%, 20/65) presented post-mortem signs of pneumonia. Within this group, 33% (21/65) [CI95%, 16.4%;52.7%] were also positive to the presence of parasites (Graph 9).The occurrence of pneumonia within the positive cases of parasitism was not statistically significant (p=0.401).

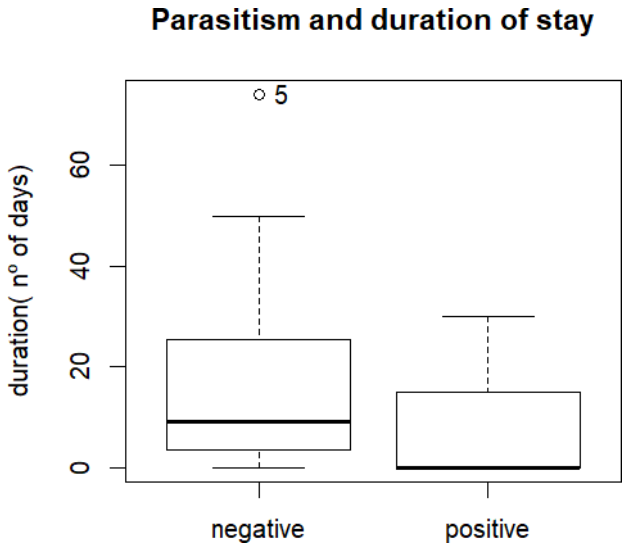
Graph 9- Percentage of positive and negative cases of parasitism and individuals with post-mortem signs of pneumonia.



3.2.4 Relation between parasitism and duration of stay

Globally, the duration of stay varied between 0 days Minimum (Min.) and 74 days Maximum (Max.) with a mean duration of approximately 14 days. For the positive cases, the values range between 0(Min.) and 30(Max.) and for the negative cases, they range between 0 and 50 (with an outlier value of 74) (Graph 10). Positive cases have actually a significant relation to shorter amount of time in the centre (W=632;p=0.0162).

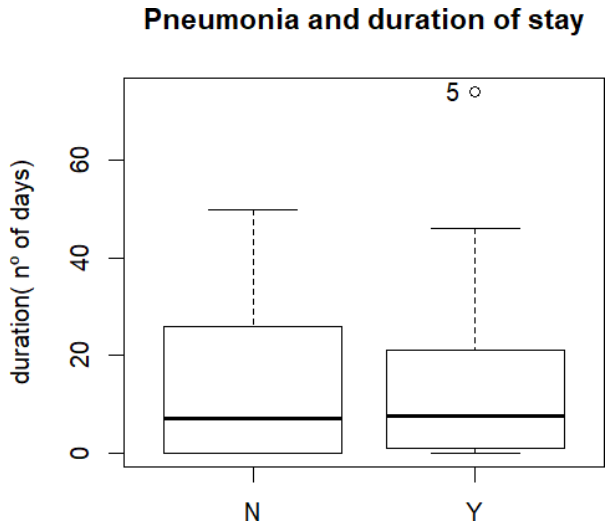
Graph 10- Box-plot of the relation between parasitism and duration of stay in the centre.



3.2.5 Relation between post-mortem signs of pneumonia and duration of stay

For the cases with post-mortem pneumonia signs, the duration of stay ranges between 0 (Min.) and 46 (Max.) days with an outlier value of 74 days and a median of 7.5 and for the negative cases, they range between 0 and 50 with a median of 7 (Graph 11) .There is not enough evidence to prove the association between the cases with post-mortem signs of pneumonia and the duration of the rehabilitation in the centre (W=431;p=0.7903).

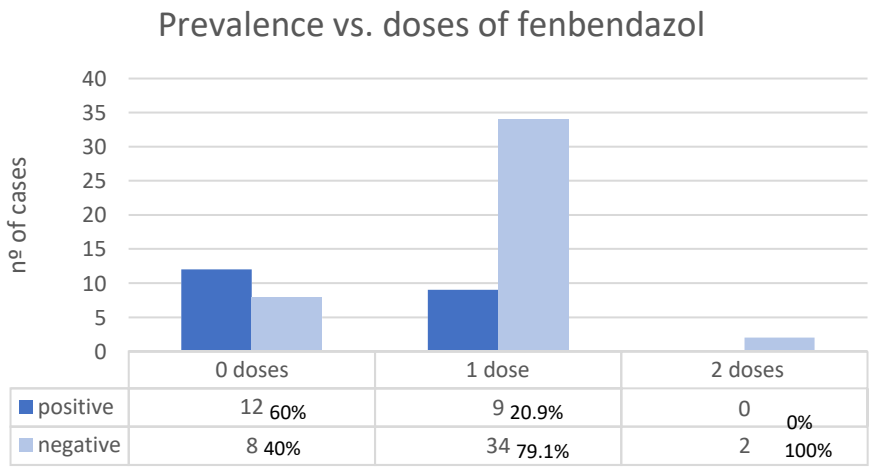
Graph 11- Box-plot of the relation between post-mortem pneumonia signs and duration of stay in the centre.



3.2.6 Relation between parasitism and doses of fenbendazole

The 20 gulls from the sample that were euthanized on admission did not receive any dose of fenbendazole. Within this group, 60% [CI95%, 23.2%;50.2%] were positive to parasites and as for the group that received 1 dose of fenbendazole, 20.9% [CI95%, 11.4%;35.2%] of the gulls were positive. In the group that received the 2nd dose of fenbendazole (when respiratory signs were still present), no positive cases were registered (Graph 12). The number of positive cases to parasites is lower when the number of doses of dewormer increases, showing a statistically significant relation (W=601.5;p=0.0024).

Graph 12- Percentage of positive and negative cases of parasitism and doses of fenbendazole.



According to the centre's database, 49 Herring gulls took a 2nd dose of fenbendazole when respiratory signs were detected. Within this group, 36 (73.5%) were successfully released and 12 (25%) were euthanized (16% due to feathers in bad condition, 6% due to respiratory problems, 2% by severe pododermatitis) and 1 died due to non specified cause (2%).

3.2.7 Relation between parasitism and body condition

On a scale 1 to 5, body condition of the sampled gulls varied between 1 (Min.) and 2.5 (Max.). The mean value of 1.74 for the body condition of the gulls which were negative to the presence of parasites, contrasts with the mean value of 1.85 for the positive cases.

4. Discussion

This parasitological study revealed a prevalence of 32% within the sampled population of Herring gulls admitted in the centre (n=65). The general prevalence includes 60% of positives within the group of gulls (n=20) that did not receive any dose of fenbendazole (euthanized on admission) and the 20% of positives that received at least one dose of fenbendazole as residents in the centre (n=45).

According to the author's research, this is the first parasitological study done in Herring gulls in a British rehabilitation centre. Before this, Pemberton(1963), Threlfall (1967,1968) and Buck, Cooper, & Crites (1976), had already conducted parasitological studies but in wild caught Herring gulls from UK and North America, living under distinct environmental conditions from the ones in the present project. In all these studies, the prevalence was of 100%, with exception of Threlfall (1968) study that presented a prevalence of 93.64% in adults and 62.99% in chicks. However, those studies only used macroscopic identification of parasites, but no coprological techniques, which compromises the comparison to the general results. Also, this is the first parasitological study conducted in this species in the last 40 years and the influence of this time gap, together with the changes that occurred in the ecosystem, must be considered when comparing the parasitological fauna. Still, Table 1 compares the prevalence of worms between those studies and the present one.

Firstly, we can see the presence of *C.lari* in fledglings (16.9%) is actually very similar to the 13.57% found in chicks/juveniles (fledglings included) in Threlfall study (1968) in Newfoundland, Canada. As to *Syngamus* sp. specimens, they were either absent or showed low prevalence on the cited studies (11% and 0.21%) in similarity with this study (1.54%). Capillariidae genus which was one of the most widespread in those early studies, was absent in the present one. Acanthocephala, which was present in low percentages in previous studies, was not detected.

Table 1- Prevalence of some of the most prevalent parasites in Herring gulls (chicks/juveniles and adults) parasitological studies (Original).

	C. lari	***Syngamus***	***Porrocaecum***	***Contracaecum***	**Capillariidae**	**Acanthocephala**
Pemberton (1963)	A= 33%	A=11%	A=11%	-	A=44%	A=2.7%
Threlfall (1967)	C/J=71.58%	-	C/J=3.83%	-	C/J=31.69%	
	A=55.7%*	A=0.21%	A=0.42%*	A=0.87%	A=77.93%	
Threlfall (1968)	C/J=13.57%	-	-	C/J=1.77%	-	C/J=3.89%
	A=7.57%	-	A=1.06%	A= 1.94%	A=2.18%	A=2.36%
Buck et. al. (1976)	-	-	-	C/J=13.33%	C/J=11.11%	-
				A=0%	A=47.67%	
Present study – Worm detection results *(2018)**	C/J=16.9%**	C/J=1.54%**	-	-	-	-
	A=0%	A=0%	-	-	-	-

*Adults including 1st year birds ** Percentage includes chicks, fledglings 1 and fledglings 2 *** These values do not include egg detection prevalence.

On the other hand and considering studies conducted with other gulls' species from different parts of the world (Table 2), Acanthocephala seems to be the most widespread and prevalent group, followed by Capillariidae family (with high prevalence in New Zeeland) and *Contracaecum* sp. (showing generally low percentages).The British study performed *on Larus fuscus* was the only one showing *Syngamus* sp. presence, suggesting that this respiratory parasite may be more prevalent in areas with cold/temperate and humid weather. This contrasts with the fact that high temperature and humidity lead to higher larval densities but can be explained by the possible survival overwinter of these nematodes inside paratenic hosts in these areas of the globe (Fernando & Barta,2008; Gethings et al.,2015).

The difference between the parasitological fauna of gulls from different areas is supported by Locke, Levy, Marcogliese, Ackerman, & Mclaughlin (2012) studies. They showed that both geographic distance (especially further away from the equator) and age difference between gull populations have a strong impact in the loss of similarity of parasite communities. Also, one must bear in mind each geographic location is related to different climatic conditions, feeding habits, availability of intermediate hosts, which altogether contribute to distinct parasitological communities.

Table 2- Most prevalent parasites in studies performed in different gulls' species (Original)

	Cyathostoma	*Syngamus*	*Porrocaecum*	*Contracaecum*	Capillariidae	Acanthocepha-la
Larus dominicanus[1] (Patagonia, Argentina)	---	---	---	3.85%	3.85%	6.9%
Larus fuscus[2] (Walney Island, Britain)	---	*2%*	---	*5%*	*6%*	---
Larus cachinnans[3] (Galicia, Spain)	---	---	---	0.9%	14.5%	4.9%
Larus novaeholandiae scopulinus[4] Otago , New Zeeland	---	---	---	---	88%	44%
Larus hyperboreus[5] Spitsbergen, Norway	---	---	---	---	---	50%
Larus atlanticus[6] Bahia Blanca, Argentina	---	---	---	---	---	100%

1. (Diaz, Cremonte, & Navone, 2011); 2. (Ellis & Williams, 1973); 3. (Sanmartın, Cordeiro, Alvarez & Leiro, 2005); 4. (Fredensborg, Latham, & Poulin, 2004); 5. (Sagerup et al., 2009); 6. (LaSala, L & Martorelli, 2007).

Considering the coprological analysis, Syngamidae eggs were the most prevalent forms found, representing 24.61% of positive cases, including 3.08% mixed infections with Ascaridoidea eggs (1.54% with *Contracaecum* sp. and another 1.54% with *Porrocaecum* sp.). These percentages for *Contracaecum* sp. and *Porrocaecum* sp. eggs are very similar to the ones found in Threlfall (1967,1968) studies. Despite two of the three adult gulls were positive to coprological techniques, no visible worms were found, probably due to the bigger size of the nasal cavities that can facilitate the expulsion of those worms (e.g., by sneezing).

It is clear that the general prevalence of 32% (and 60% in non-dewormed gulls) in this study is much lower from other studies. One important factor that might have influenced the results is the extended freezing period (2-2.5 months) of the carcasses, which decreases the detection of parasites and EPG value in the faecal samples (Jagła, Śpiewak, Zaleśny, & Popiołek, 2013). In fresh samples, higher prevalence and other parasite species could have been detected.

When comparing EPG counts between resident and non-resident gulls (dewormed and non-dewormed respectively), we can see that the non-dewormed have naturally some cases with heavier faecal egg counts (≥400 EPG). There were still 6 cases that despite being positive to other techniques proved negative to the McMaster method. Since the detection threshold is 50 EPG, those negative results represented individuals with faecal egg counts lower than this value (Madeira de Carvalho, 2002). What was not expected was for the dewormed group to

still show 9.2% of the cases with ≥50 EPG. In fact, the 20% positives within the dewormed group turned out a surprising result.

According to 2018 deworming protocol, gulls weighing more than 200g/300g were dewormed once with 50 mg/kg fenbendazole on admission with either Panacur® Equine oral suspension 10% 100mg/N or Lapizole® 20 mg/ml and the process was repeated if they showed any respiratory signs. Although Panacur® is used off-label and there is no advised protocol for birds in the information sheet, Lapizole® includes information for pigeons, passerines, psittacines and raptors. For these groups, the dosage varies between 10-20mg/kg during 3-5 consecutive days. The dosage used in the centre is higher because it is a one off dose.

Some authors suggest the use of 20 mg/kg of fenbendazole orally just once to deworm seabirds as an extrapolated dosage from waterfowl (Carpenter & Marion,2012;Bailey & Apo, 2015;Keeble,2017). In other species, fenbendazole revealed a 100% efficacy in deworming pheasants and partridges when used for 5 days (Kirsch, 1984) and 100% efficacy in eliminating *S. trachea* in poultry after 3 consecutive days of treatment (Ssenyonga, 1982).These studies support the idea that for an effective deworming, birds should be treated for at least 3 days. However, in Mallydams centre, the high numbers of admitted gulls during the summer may difficult the continuous treatment which can also increase stress and feather damage.

Therefore, there is a serious need for studies on the efficacy of these dosages of fenbendazole on Charadriiformes and seabirds in general. It is also recommended for the centre to test the efficacy of different protocols (e.g., extending the duration of treatment) and dosages (e.g., 20 mg/kg) in order to reduce the number of positives cases and the emergence of resistances.

Another possibility that could justify the positive cases is the ingestion of infected earthworms (intermediate/paratenic hosts) from the environment although this hypothesis is highly improbable, except when gulls are moved to the outdoors enclosures in their last stage of rehabilitation. Most likely, the reinfection occurs due to persistence of eggs in the facilities and contamination of food and water (despite the daily cleaning protocols) which combined with the high animal density and the stress of captivity can lead to perpetuate the faecal-oral transmission. In the case of *Contracaecum* sp., the transmission could also occur by ingestion of infected fish, but the provided fish is stored at -20ºC generally for more than 7 days, which according to Fagerholm & Overstreet (2008) prevents its development, making this hypothesis also unlikely.

As for the necropsy findings, approximately 72% of the gulls did not present any external alterations, although 20% showed severe signs of pododermatitis. The internal exam of the cavities revealed a series of vascular, inflammatory and structural alterations that were carefully examined taking into account that the carcasses had been frozen for 1-2.5 months.

Just like previously mentioned, the freezing process causes disruption of tissue architecture and cell lysis by formation of ice crystals which have consequences on microscopic, but also macroscopic evaluation of the organs (Latimer & Rakich, 1994).

Some of the most prevalent alterations were lung congestion (23%) and lungs densification (30%). In these cases, the lungs did not float on the float test, which is suggestive of acute lung inflammation. However, there are no specific studies regarding the accuracy of the float-test on lungs exposed to long freezing periods. Therefore, these results could only be confirmed by histopathology analysis and consequently, must be considered with some level of uncertainty.

Another common alterations were the presence of parasites in the infraorbital area of the nasal cavity (18.46%) and presence of foreign bodies (17%), such as pieces of plastic, which have been frequently found in other studies and in many other species of marine birds (Lindborg et. al, 2012;Provencher et al., 2017). In 5% of the cases, there were signs of chronic inflammation with typical whitish caseous material in the lungs which were highly compatible with Aspergillosis lesions. This result is much lower than the 22% prevalence in Friend & Trainer (1969) studies on Herring gulls exposed to cumulative stress in confinement. However, some gulls were fogged with F10® nebulizer so the number of aspergillosis cases was probably reduced in 2018.

In contrast with previously referred reports on *Larus argentatus*, there were no gross lesions suggestive of amyloidosis, botulism and salmonellosis. Despite being one of the most common descriptions, the intoxication by chemical substances such as organochlorines and organophosphates was not within the objectives of the present study and therefore was not confirmed by toxicological analysis.

One of the objectives of the present study was also to evaluate the relation of some variables with the positive cases. Both Campbell (1935) and Threlfall (1966, 1967, 1968) studies have suggested a higher prevalence of *Syngamus* and *Cyathostoma* in chicks/juvenile rather than in adults. Mortality in the centre also seemed to be higher among the nestlings/fledglings in previous years. At first sight, this tendency contrasts with the results of 66.7% positive adults and 0% nestlings. However, the fact that the sampled population was chosen randomly by a wide number of staff/volunteers resulted in having just two nestlings and three adults and most of the positive cases were detected within fledglings. Therefore, the number of nestlings and adults was not representative and the inexistence of a significant statistical relation between life stage and parasitism should be considered cautiously.

As to the admission months of the necropsied gulls, we can see that despite the detection of two positive cases in November, August was the 2nd month with a highest prevalence of parasites (38.4%). Looking back to the studies of Gethings et al.(2015), one can understand

the importance of temperature and humidity to the infection and larval abundance of syngamids. The Summer of 2018 in the UK was considered the warmest to date with July being the warmest month (MetOffice, 2018) and also the one that registered highest number of deaths/euthanized *Larus argentatus* in the centre (Appendix II). However, July was the summer month with the lowest prevalence of parasites (25%) and statistical analysis showed that parasitism is not significantly influenced by the evaluated admission months.

When comparing the amount of time spent in the centre, in spite of the stress of confinement and higher possibility of reinfection, longer rehabilitation periods do not seem to lead to more cases that are positive. This can be explained by a big portion of these positive cases being euthanized on admission gulls (0 days in captivity) but also by the fact that longer rehabilitation periods are mostly related to gulls that took two doses of fenbendazole and therefore, less likely of being positive to parasites. When analysing the outcome of all the Herring gulls that received the 2nd dose of this substance in 2018, one can see that 73.5% were successfully released and 26.5% were euthanized or died (6% due to respiratory symptoms).

Parasitism is usually related to lower body conditions but that wasn't observed in this study where positive cases actually presented a slightly higher body condition than the whole sampled group. However, birds usually have to be severely affected with high burdens of parasites to become emaciated.

Finally, the presence of post-mortem signs of pneumonia was not substantially associated with presence of parasites and 66.7% of the individuals showed signs of pneumonia that were possibly linked to other causes. When analysing if longer periods of rehabilitation lead to higher number of gulls showing post-mortem signs of pneumonia, it was proven that there was no substantial association between both variables.

5. Conclusions and Recommendations

To the best of the author's knowledge, this study represents the first parasitological study done in Herring gulls admitted in a British rehabilitation centre. This is also the first one on Herring gulls which combines coprological techniques with macroscopic identification of parasites. Considering the declining numbers of the population and the importance of this species for the ecosystem, there is a serious need for more parasitological studies that contribute to this species' conservation. Further studies on the morphological characteristics of *Cyathostoma* sp. eggs are also necessary to a better distinction between species of this genus.

Comparing to the centre's early reports and previous British studies on Herring gulls, it seems that *Cyasthostoma* sp. and *Syngamus* sp. are still common and part of the specific parasitological fauna of this region. On the contrary, these respiratory parasites are rarely

found in other parts of the globe and other gulls' species. As to *Contracaecum* sp. and *Porrocaecum* sp. both were found in low percentages in similarity with previous studies.

Wild gulls euthanized on admission show considerable prevalence of parasites (60%), mainly *Cyathostoma* sp. but the group that was dewormed in the centre still showed 20% of positive cases, 9.2% of them with 50 to 400 EPG in McMaster method. Most probably this was caused by the faecal-oral reinfection due the high stocking density in summer months and by a deworming protocol that proved not totally efficient. Therefore, it is recommended that the centre accesses new protocols with new dosages and extended treatment periods. The administration of a 2nd dose of fenbendazole even several weeks after also showed good efficacy in the rehabilitation of gulls with respiratory symptoms (73.5% releases) and thus, should be continued. If the economic resources permit it and considering the increasing number of admissions, an extension of the facilities is also advised.

The present study also showed that the acute inflammation signs on the lungs are not significantly related to the presence of parasites or the duration of rehabilitation period and consequently other bacterial, fungal and viral infectious agents should be considered and tested in the future. Several contributing factors such as stress, high number of gulls in confined spaces, warm temperatures and consequent immunodepression should be minimized.

Some factors like the admission month of the gulls and their life stage are not substantially correlated with the presence of parasites in the present study although a more representative sample of the age groups is needed for a more accurate analysis. On the other hand, longer rehabilitation times are strongly related with lower prevalence of parasites.

Although none of these parasite species presents a risk for public health, close surveillance should be maintained to prevent dissemination to other captive or domestic birds. Hopefully, this study will give Mallydams Wood centre a better insight on parasitological agents involved and help decrease the number of gulls needing euthanizing due to respiratory signs. In addition, the author hopes it will inspire further studies that contribute to the conservation of Herring gulls and raise awareness on its declining population numbers.

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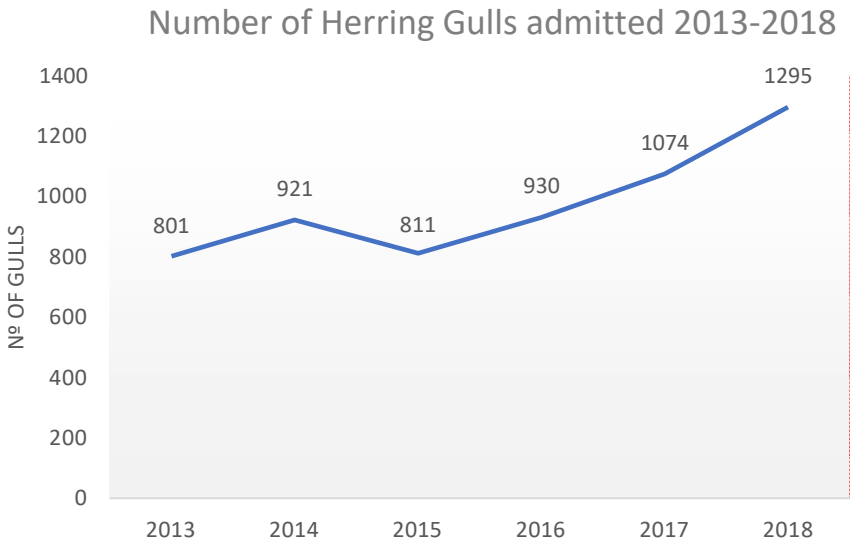
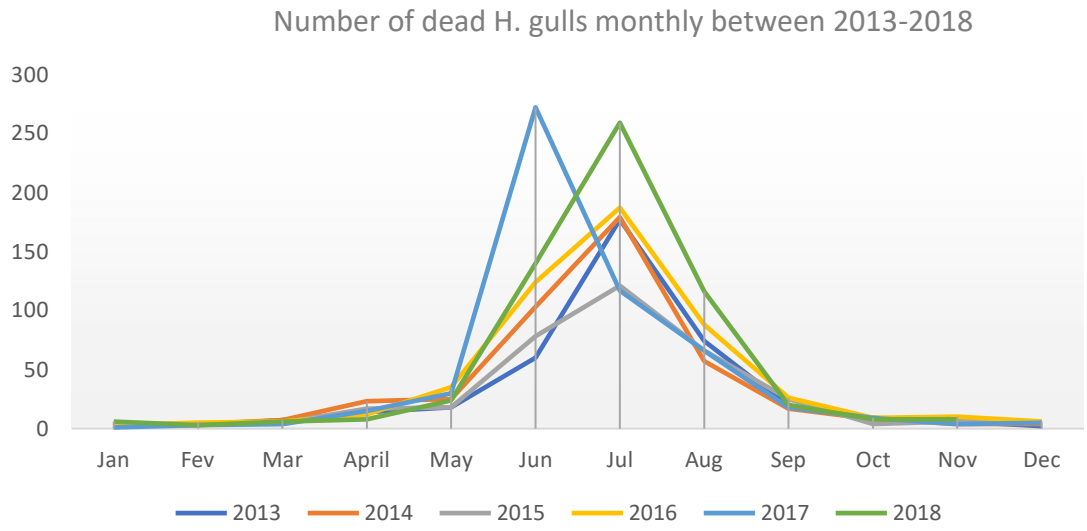
Appendix I – Number of admitted gulls in the last 6 years in the centre

Graphic that shows increasing numbers of admissions of Herring gulls at the centre during the last 6 years with a slight decrease in 2015 followed by exponential numbers in the next years. In 2018, the number of admissions was considered until 10th of December (end of the internship period)



Graphic that shows an increase in euthanized/dead Herring gulls during Summer season, reaching its peak in July each year with exception of 2017, where the maximum of deaths was registered in June.



Appendix II - **Necropsy protocol on wild birds (adapted to the project on Herring gulls)**

1. External examination of the gull looking for any signs of trauma and evaluation of its body condition through the pectoral muscles;

2. Wet the feathers with water in the abdominal area in order to facilitate their removal and prevent dispersion through the room;

3. The carcass should be placed on its back. Cut through the skin between the thigh and the abdomen and disarticulate the coxofemoral joint in order to stabilize the body;

4. Remove the skin from the ventral surface of the gull by cutting across at the caudal edge of the keel and then pulling the skin cranially and caudally;

5. Open the body cavity by incising at the caudal edge of the keel, on each side, through pectoral muscles and ribs and pulling up the pectoral muscles and keel;

5.1 Firstly, observe the liver looking for alterations or any signs of larval migration and also, the air sacs both partially destroyed by the last procedure. After removing the keel and pectoral muscles, observe and incise into the heart and lungs looking for alterations or presence of parasites. To evaluate the presence of acute inflammation signs, the flotation test can be used (by cutting part of the lung and in case it doesn't float that is highly suggestive of pneumonia). If parasites are present, they should be removed and stored in a sample container until further analysis;

6. Proceed by extending the incision laterally in both sides of the abdominal muscle so it can be pulled caudally to expose the abdominal viscera. Perpendicularly to this last incision, another one should be done towards the cloaca;

6.1 Remove the digestive tract by doing two cuts, one cranially to the crop and the other one in the cloaca;

7.

7.1. Using the scissors, start by cutting the commissure of the beak and continue through oesophagus;

7.2. Incise with the scissors in the larynx and continue through the trachea until reaching the bifurcation of the bronchi. Looking for any eventual pathological alterations or signs of worms in the tracheal area.

7.3 Cut transversely through the beak in order to evaluate the presence of worms inside the nasal cavity.

8. Take faecal samples of the different segments of intestine and then, cutting open the digestive tract starting with, proventriculus, gizzard, small intestine, ceca and colon towards the cloaca. Examine the mucosa and surface of these organs.

9. Finally, look at the characteristics of the pancreas, spleen and kidneys looking for pathological alterations.

(Adapted from Wild Birds Necropsy Procedure (1980) by Charles Van Riper III and Sandra G. Van Riper)

Appendix III – Absolute frequency of external and internal alterations found in necropsies

External alterations

			Absolute frequency(n=65)
Vascular alterations	Haemorrhage (coagulated/not coagulated blood)		3
Total (number of specimens with vascular alterations)			3
Inflammation	Chronical inflammatory process	Digits	13
		Tarsometatarsus	1
Total (number of specimens with inflammation)			14
Fractures and joint dislocations			2
Others	Loss of impermeabilization properties		3
	Loss of feathers		1
Total (number of specimens with other type of alterations)			4
No external alterations			47

Appendix III (cont.)

Internal alterations

			Absolute frequency(n=65)
Vascular and haematogenous alterations	Congestion	Lungs	16
		Air sacs	2
		Liver	7
		GIT	1
		Generalized	6
	Haemorrhagic fluid	Respiratory tract	5
		Celomic cavity	2
	Impregnation with haematogenous pigment	Liver	1
Total (number of specimens with vascular and haematogenous alterations)			40
Inflammation	Acute (exudative, erosive)	Lungs	20
		Liver	3
	Chronic (granulomatous, focal)	Lungs	3
		Liver	2
Total (number of specimens with inflammation)			28
Traumatic lesions	Rupture of parenchyma	Liver	1
Presence of Syngamidae family			12
Other alterations	Osseous metaplasia	Lungs	3
	Presence of foreign bodies (pieces of plastic, stones, sticks)	Gizzard	11
Total (number of specimens with other type of alterations)			14
No internal alterations			17