ANA ISABEL VAZ LUCAS

Variability of Harbour porpoise diet with a particular focus on the consumption of whiting and Atlantic herring, in Scottish waters (1992-2014)

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“Declaração de autoria do trabalho”

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referência incluída.

Ana Isabel Vaz Lucas

Acknowledgements

These words go to the ones who were there along the way:

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"*La science, mon garçon, est faite d'erreurs, mais d'erreurs qu'il est bon de commettre, car elles mènent peu à peu à la vérité*."

Jules Verne, 1972

\- Journey to the Center of the Earth -

Resumo

O boto (*Phocoena phocoena*) é um cetáceo comumente observado em zonas de águas pouco profundas (<200m), sendo a sua distribuição sobretudo limitada à plataforma continental. Nas zonas costeiras, são observados frequentes arrojamentos desta espécie provocados pela interação negativa com artes de pesca, por colisões com embarcações e, também por ataques de roazes (*Tursiops truncatus*) e de focas cinzentas (*Halichoerus grypus*). Apesar das ameaças existentes, é nestas zonas que se concentram as suas presas.

O boto apresenta uma alimentação composta por uma variedade de peixes da família Gadidae, Clupeidae, Gobidae, Ammodytidae bem como de cefalópodes. Ao longo dos anos, tem apresentado diferentes preferências alimentares. Na Escócia, são alguns exemplos dessas espécies sobretudo cardumes de peixes pequenos de galeotas (*Ammodytes*), badejos (*Merlangius merlangus*), *Trisopterus* spp. e arenques (*Clupea harengus*).

O objectivo deste trabalho foi investigar a influência de alguns factores (ex. local, comprimento do boto, abundância da presa) respeitantes ao consumo de arenque e badejo, na variabilidade da dieta do boto. Estudo foi realizado para a região da Escócia, focando o período entre 1992 e 2014. Serve o presente documento, também, dar continuidade a um anterior estudo publicado em 2004 pela investigadora Begonã Santos, para a mesma área geográfica.

A observação directa dos períodos de alimentação é uma prática dispendiosa e morosa, dificultada também pelo próprio comportamento tímido dos botos. Assim sendo, a análise de estômagos provenientes de carcaças de cetáceos são uma prática mais recorrente. Este procedimento é duplamente apoiado e criticado por diferentes investigadores. Mesmo inerente a erros, contribui para um estudo mais detalhado da ecologia e biologia de uma espécie.

A equipa escocesa responsável pelo estudo e recolha de animais marinhos arrojados na Escócia (SMASS) foi quem forneceu as amostras analisadas no presente trabalho. Os conteúdos estomacais passaram por uma diversidade de etapas, desde a limpeza, a triagem, até ao seu armazenamento. Depois das diferentes estruturas esqueléticas terem sido separadas do conteúdo estomacal procedeu-se à identificação dos ossos aí

encontrados. Parte desta identificação já tinha sido anteriormente realizada por estudantes e investigadores. Estômagos não antes analisados foram processados pela presente autora.

Durante a identificação foi dada prioridade aos ossos designados por otólitos, e aos ossos da boca do peixe – as premaxilas e as dentárias. Estes ossos permitiram muitas vezes a identificação até a espécie, pois são estruturas com um grau de resistência aos fluídos digestivos. A sua capacidade de conseguirem manter as suas estruturas quase originais, formas e tamanhos permitiram estabelecer uma relação com a espécie ao qual pertenceram. O tamanho e peso dos arenques e dos badejos ingeridos pelos botos foram estimados de forma indirecta através de equações específicas para cada espécie. Quanto ao número de presas presentes no estômago de cada boto, este foi calculado através do número máximo de estruturas ósseas encontradas.

De forma geral, a dieta de um cetáceo pode reflectir a sua condição física. Por exemplo, as habilidades e a experiência na procura de alimento normalmente estão associadas a indivíduos de grande porte e mais velhos. Fêmeas gestantes estão associadas a necessidades energéticas maiores. O boto pode, também, ajustar a sua alimentação em função da abundância e biomassa das suas presas. No caso do badejo e do arenque, os seus stocks têm sofrido grandes variações ao longo do século.

Os dados sobre a abundância e a biomassa das presas nos mares da costa da Escócia foram disponibilizados por relatórios do ICES (International Council for the Exploration of the Sea). Os stocks das áreas que envolvem a costa da Escócia (4a, 4b e 6a) são monitorizados em associação com outros das áreas em redor. Portanto, os dados da costa este, são representativos da combinação dos stocks das áreas 4a, 4b, 3a e 7d, e os da costa oeste são combinados com os das áreas 6a, 7b e 7c.

Na análise estatística os modelos aditivos generalizados (GAMs) demonstraram quais dos factores em estudo (entre as características físicas, espaciais e temporais) foram significativos na variabilidade do consumo de badejo e arenque na dieta do boto, para o período entre 1992-2014. O teste X^2 foi usado para a escolha do modelo cujas variáveis eram mais representativas da realidade. Os dados para a costa oeste da Escócia bem como alguns dos obtidos para o arenque foram passíveis de originar resultados com representatividade discutível, devido ao tamanho reduzido das amostras (<50).

O boto demonstrou ser um predador voraz de badejo quando comparado com o consumo de arenque, sobretudo nas águas costeiras a este da Escócia. O badejo tem apresentado um papel consistente como presa ao longo dos anos, enquanto a importância de arenque se tem mantido reduzida nas tendências alimentares. De forma geral, observou-se que as escolhas alimentares do boto estão relacionadas possivelmente apenas com a abundância das suas presas primárias. Também, o local onde os botos se alimentam pela última vez reflete ser factor de variabilidade na dieta. Assim, como a sazonalidade parece ser determinante na escolha dos tamanhos ingeridos de badejo e arenque. Respeitante ás características físicas do boto, o seu tamanho, também, se destacou sendo um factor de variabilidade. Botos maiores alimentam-se de badejo de maiores tamanhos. Este cetáceo apresentou, também, preferência por badejo e arenque de menores tamanhos que aqueles capturados pelas pescas. O consumo destas duas espécies de peixe observou-se ser independente dos botos serem do género masculino ou feminino.

Importante de referir, que os factores que potenciam a variabilidade da dieta são transversais à relação predador-presa. Considerando os mesmos factores de variabilidade, a dieta parece ser afectada de forma diferente tendo em conta o nível de importância das presas no seu regime alimentar. Continua ainda assim por explicar muitos dos factores que direcionam a dieta dos botos, em especial no consumo de badejo e de arenque, contribuindo o actual trabalho com novos conhecimentos para esta área. Deste modo, contribuiu como estudo ecológico para a monitorização do bem-estar e desenvolvimento da estrutura populacional de botos na Escócia. Para uma abordagem mais próxima da realidade sobre a dieta dos botos nesta região, novas pesquisas envolvendo outras variáveis de estudo e com uma maior representatividade populacional serão necessárias.

Termos chave: Escócia, *Phocoena phocoena*, factores, variabilidade alimentar, *Clupea harengus*, *Merlangius merlangus*.

Abstract

Harbour porpoise (*Phocoena phocoena*) is one of the most abundant cetaceans in Scotland, where it forages for prey such as whiting, sandeel and sepiolids. Currently, whiting is among primary prey, while herring, an important prey historically for porpoises, seemed not to be common in their diet anymore. The present work aimed to study spatial and temporal variability in porpoise diet, and the effects of prey availability and porpoises physical characteristics on the consumption of herring and whiting. Besides presenting new information, this study helps extend the data on porpoise diet beyond a published study from 2004 (by Begoña Santos), for Scotland. The stranded monitoring scheme (SMASS) provided the dietary samples from 1992 to 2014. Regression equations were used to back-calculate the amount of prey eaten based on measurement of fish hard parts (e.g. otoliths) recovered from stomach contents. GAMs and X^2 test analysed the variables that significantly influence the variability of porpoise diet.

Porpoise sex didn't affect the consumption of whiting and herring. On the other hand, geographical location, year and seasonality were significant factors in the presence and size of these prey species in the diet. Porpoises seem to prefer small sizes of herring and whiting than the ones fishery harvested. Over the years, whiting was more important and constant than herring in porpoise diet. Whiting consumption was consistent with trends in whiting abundance (based on ICES stock assessments for the North Sea). Additionally, bigger whiting was eaten by bigger porpoises. The consumption of herring by porpoises did not appear to be affected by the same or equivalent (as in the case of herring abundance) variables.

Although, even with an incomplete knowledge of the variables that affect porpoise diet, the present study provides new insights. Porpoise diet seems to be highly linked to the relationship predator-prey, and changes in the biology or ecology on one or the other, could have consequences for porpoise population well-being and development.

Key words: Scotland, *Phocoena phocoena*, dietary drivers, *Clupea harengus*, *Merlangius merlangus*.

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List of Abbreviations

ICES	International Council for the Exploration of the Sea
UK	United Kingdom
IUCN	International Union for Conservation of Nature
SCANS	Small Cetaceans in the European Atlantic and North Sea
ASCOBANS	Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas
EU	European Union
GPS	Global Positioning System
B_{lim}	Limit reference point for Stock Spawning Biomass
SST	Sea Surface Temperature
SSB	Spawning Stock Biomass
SMASS	Scottish Marine Animal Stranding Scheme
QGIS 2.10	Quantum Geographical Information System version 2.10
IIM	Instituto de Investigaciones Mariñas
OW	Otolith width
OL	Otolith Length
FW	Fish Weight
TL	Total Length
PMXL	Premaxillae length
PMXAH	Premaxillae height of articular process
S-VL	Dentary Ventral Length
DNA	Deoxyribonucleic Acid
TRL/Whit.	*Trisopterus luscus* or Whiting
Whit./BLW	Whiting or Blue Whiting
VPA	Virtual Population Analysis
HAWG	Herring Assessment Working Group
WGNSSK	Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak
WGCSE	Working Group for the Celtic Seas Ecoregion
Q1	1st Quarter (January, February, March)

Q2	2nd Quarter (April, May, June)
Q3	3rd Quarter (July, August, September)
Q4	4th Quarter (October, November, December)
F	Female
M	Male
U	Unidentified sex
NA	Not Available
VIF	Variance Inflation Factor
GVIF	Generalized Variance Inflation Factor
ANOVA	Analysis of Variance
GAMs	Generalised Additive Models
AIC	Akaike Information Criterion
DE	Deviance explained
EDF	Estimated degrees of freedom
CL	Confidence Limits
OSPAR	Oslo/Paris convention (for the Protection of the Marine Environment of the North-East Atlantic)
MSY	Maximum Sustainable Yield
$\mathbf{F_{MSY}}$	Fishing mortality consistent with achieving MSY
F	Instantaneous Rate of Fishing Mortality
$\mathbf{B_{pa}}$	Precautionary reference point for SSB
SACs	Special Areas of Conservation
MAPs	Marine Protected Areas

1. Introduction

1.1. Harbour porpoise (*Phocoena phocoena*), in Scottish waters

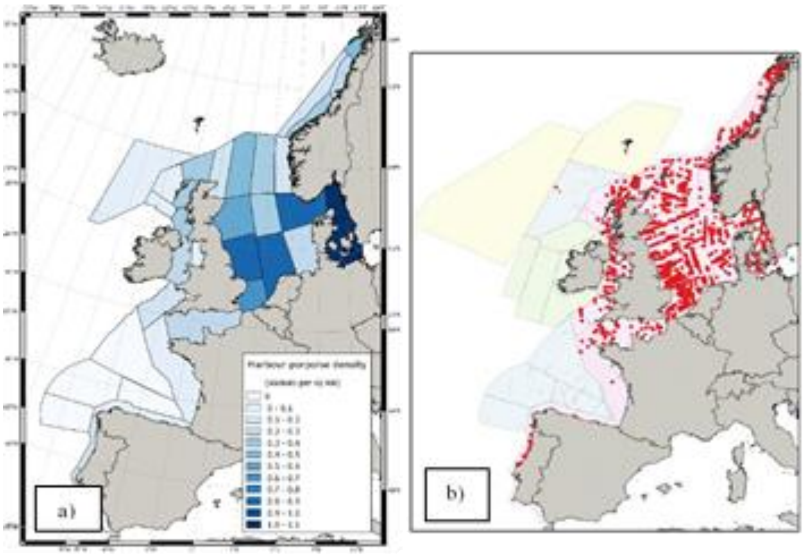


Figure 1.1- Abundance and density of Harbour porpoise, in Scottish waters
a) Estimated density of harbour porpoise for 2016, in Northeast Atlantic shelf waters (Hammond *et al.*, 2017). **b)** Harbour porpoise sightings from the last SCANS-III survey (Hammond *et al.*, 2017).

Harbour porpoise (*Phocoena phocoena*) is one of the most common cetaceans in the North Sea, who is sighted regularly in Scottish coastal waters (Dolman *et al.*, 2014; Hammond *et al.*, 2017). Over the years, ongoing surveys (e.g. SCANS-I/1994, II/2005, III/2016) along the European continental shelfs have provided information on the abundance and distribution of this species (Figure 1.1). Among these waters, porpoise abundance did not change from 1994 to 2016 (SCANS-II, 2008; Hammond *et al.*, 2013, 2017). However, it had a marked decline during the 1980s in the north coast of Scotland (Shetland), but the trend reversed after 1991 (Evans *et al.*, 1996). In 2016, the North Sea and west of Scotland were the area with the highest porpoise abundance estimation, ~369743animals/km^2 (Hammond *et al.*, 2017).

In some Scottish regions, as in the west coast, porpoises distribution is often within 15km from the shore and between 50-150m deep (Marubini *et al*., 2009). But in the eastern side (Aberdeenshire), they occur not in such deep waters, porpoises were detected just around 20m depth (Weir *et al.*, 2007). In a small-scale study carried out in Scotland, porpoises have revealed seasonal migrations. In the west coast, porpoises were observed moving in spring to inshore waters, while on summer they move offshore (Gilles *et al.*, 2016). In the east side (Aberdeenshire), in 1999-2001, an increase number of animals was observed between July and October (Weir *et al.*, 2007). And in the northern waters, in 1990-1995, it was observed in summer, with a peak in August-September (Evans *et al.*, 1996). The availability and distribution of primary prey species and the bycatch of porpoises were, in the case of north Scottish region, described as possible reasons for the changes in their abundance and distribution (Parson *et al.*, 2000; Santos & Pierce, 2003; SCANS-II, 2008; Hammond *et al.*, 2013).

Abundant and widely spread in the Scottish coastal waters (SCANS-II, 2008; Dolman *et al.*, 2014; Hammond *et al.*, 2017), harbour porpoises are an active predator for a wide range of commercial a non-commercial species (Rae, 1973; Santos, 1998; Santos & Pierce, 2003). Porpoises are sight mainly alone or in small groups (e.g. 3-4 individuals) (Jefferson *et al.*, 2008; Dolman *et al.*, 2014), notably single when foraging (Read, 2008). Porpoises have the capacity in moving between marine and freshwater habitats (Leopold, 2015), but they apparently seem to prefer shallow cold seawaters (Rae, 1965; Weir *et al.*, 2007; Read, 2008; Marubini *et al.*, 2009). Occasionally, they also swim to deeper offshore sites (Jefferson *et al.*, 2008; Gilles *et al.*, 2016). Within these areas, porpoise forage all over the water column, during night and day periods (Linnenschmidt *et al.*, 2012; Wisniewska *et al.*, 2016), for both pelagic and demersal species (Corbet & Harris, 1991; Jefferson *et al.*, 2008; Read, 2008). However, they seem to avoid spiny schooling fish (Jefferson *et al.*, 2008) as well as deep fish species (SCANS-II, 2008). Porpoises describe a day-night feeding behaviour (Wisniewska *et al.*, 2016), which could be related with the daily vertical migration of their prey. As an example, cephalopods which are demersal species, approach the surface at night (Read, 2008) being forage by porpoises (Santos, 1998).

Over the years, porpoises dietary studies have been published for Scotland (Southwell, 1881; Rae, 1973; Santos, 1998; Santos *et al.*, 2004) as well as for other areas in the North Sea (e.g. Holland, Denmark, Norway, Sweden, Germany) (Santos, 1998; Lockyer, 2003; Leopold, 2015). Many of these researches are based on the stomach content analysis from individuals found beached, floating at sea, bycaught or killed by other animals (Santos, 1998; Lockyer, 2003; Leopold, 2015). This approach can provide information about the ecology for both prey and predator species (Clarke, 1980; Greenstreet & Tasker, 1996; Gilles *et al.*, 2016). This method is based on the identification of the prey species using the predator stomach remains such as otoliths, beaks, jaw and opercular bones, vertebrae, etc, from the sampled contents (Clarke, 1980, 1987; Pierce & Boyle, 1991; Santos *et al.*, 2004; Bowen & Iverson, 2012).

Around UK waters, including Scotland, porpoise present a wide array of prey species on its diet (Corbet & Harris, 1991; Santos 1998; Santos *et al.*, 2004). Adult porpoises and juveniles share feeding Scottish sites where they forage for different prey species (Santos, 1998; Santos *et al.*, 2004). Some of the Scottish targeting prey are herring, *Trisopterus* spp., cod, sandeel, haddock, whiting, crustaceans and Sepiolidae (Scott, 1903; Rae, 1965; Corbet & Harris, 1991; Santos 1998; Santos *et al.*, 2004). Despite all these species, nowadays, porpoises apparently have preference for sandeels and whiting (Santos *et al.*, 2004). Historically, harbour porpoise preferential prey have been shifting (Scott, 1903; Corbet & Harris, 1991; Santos, 1998). Whiting has been a consistently important prey for porpoises over many decades (Scott, 1903; Rae, 1965; Santos, 1998; Santos *et al*., 2004), while herring was only important in porpoise diet until the beginning of 1990s (Southwell, 1881; Rae, 1965; Corbet & Harris, 1991). Both fish species are, also, of high commercial and economic importance (Whitehead *et al.*, 1986; Simmonds, 2007; Scottish Government, 2017) as well as an important prey in other food webs (e.g. grey gurnard, grey seals) (Leopold, 2015; ICES, 2017).

Ecological studies of harbour porpoises have been focused on describing their diet, as well as potential factors that drive porpoises to adjust their foraging behaviour (Linnenschmidt *et al.*, 2012). Some factors such as porpoise sex and age, prey availability and competition, and season could lead to different food choices (Rae, 1965, 1973; Santos, 1998; Santos *et al.*, 2004; Leopold, 2015). Porpoises have high energy demands (mean energetic densities of the diet>5.5kJ/g) (Spitz *et al.*, 2012), and due to their small body size (Van Beneden, 1889), porpoises have a low energy storage capacity making them more dependent of food (Brodie, 1995). They eat an estimated 1.17kg of daily food (Santos, 1998) which suggests their need of feeding almost continuously, to meet their high metabolic demands (Wisniewska *et al.*, 2016). For example, pregnant females with heavier bodies (Learmonth *et al.*, 2014), or even lactating females (in which body weight increases by 80%) (Yasui & Gaskin, 1986), will require higher energy compared to immature females, could this resulting in differences in the diet. In Scottish waters, porpoises live approximately 11-15yrs (Santos, 1998), ranging from 0.60 to 1.75m in length (Rae, 1973; Learmonth *et al.*, 2014) and weight up to 72.9kg (Learmonth *et al.*, 2014). Although, prey biology and ecology can drive porpoise forage behaviour as well.

This has been supported by the fact that porpoises distribution has been relating to areas in close proximity to the distribution of their prey (Evans *et al.*, 1996; Parson *et al.*, 2000; Santos & Pierce, 2003; Hammond *et al.*, 2013). Also, it seems that if the availability of a common prey declines, porpoises could shift its diet to other prey species (Santos & Pierce, 2003). Climate changes (e.g. Learmonth *et al.*, 2006; Lambert *et al.*, 2014), anthropogenic activities (e.g. bycatch, oil exploration, pollution, shipping, military tests) (Parsons *et al.*, 2000; Weir *et al.*, 2007) and, interspecific relationships (e.g. bottlenose dolphins and grey seals attacks (Santos & Pierce, 2003; Santos *et al.*, 2004; Weir *et al.*, 2007; ASCOBANS, 2015; Leopold, 2015)) seem to dictate porpoises distribution and therefore under the possibility in changing their feeding grounds and main prey.

Currently, harbour porpoises face different threats, fishery has been the most problematic one (SCANS-II, 2008; Marubini *et al.*, 2009). In 2005, he was referred as one of the two main species more affected by fishing around UK waters (Northridge *et al.*, 2016). Due to their interaction with the fishing gears, they may get accidental tangled in nets such as bottom-set gillnets and trawls (Parsons *et al.*, 2000; Jefferson *et al.*, 2008; Heath *et al.*, 2017). In the North Sea, a few mitigation measures had been applied such as the use of pingers in the fishery nets (ICES, 2018). Since 2008, in the IUCN Red List of Threatened Species porpoises are globally classified as species of "Least Concern", although in 1996 they were considered "Vulnerable" (Hammond *et al.*, 2008). This could reflect the current concern and responsibility for the well-being of the harbour porpoise species. The more recent action was, in 2016, with the proposal of most of the west coast of Scotland (Inner Hebrides and the Minches) as Special Areas of Conservation (SACs) for harbour porpoises (Embling *et al.*, 2010; SNH, 2016).

1.2. Historical Harbour porpoise dietary studies

Studies regarding harbour porpoise feeding habits (Treacy & Crawford, 1981; Leopold, 2015), including several in Scottish waters (Southwell, 1881; Van Beneden, 1889; Scott, 1903; Rae, 1965, 1973; Santos, 1998; Santos *et al.*, 2004) have been published in the last decade. Recent studies, for Scottish waters, showed whiting (*Merlangius merlangus*) and sandeel (Ammodytidae) as porpoises Top 2 most important prey, when herring (*Clupea harengus*) represents a small proportion (Santos *et al.*, 2004; Pierce *et al.*, 2007; SCANS-II, 2008; Hammond *et al.*, 2013).

Described as a generalist predator, harbour porpoises have a taste for several species (Leopold, 2015). The identification of different prey in porpoises diets, is not recent. During Rae studies (1965, 1973), he found a variety of species like cephalopods, crustaceans, flatfish, cod (*Gadus morhua*), sprat (*Sprattus sprattus*), mackerel (*Scomber scombrus*), Norway pout (*Trisopterus esmarki*), hake (*Merluccius merluccius*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*). Later on, in 1991 Corbet & Harris discovered new species, including sardine (*Sardina pilchardus*), scad (*Trachurus trachurus*), pollack (*Pollachius pollachius*), gobies, and blue whiting (*Micromesistius poutassou*). Although, for Scotland and the east coast of England, Santos (1998) in her study apart from fish, she found polychaetas at porpoises stomachs. For the west side of European waters, Santos *et al.* (2004) also identified crabs, isopods and amphipods in porpoise diet. However, it is possible that some of those prey might be present because of secondary feeding. Whiting is a piscivorous, that eats many of the prey described above, like crustaceans, annelids, sprat, herring, cod, haddock and other whiting fishes (Hislop, 1991), while herring is eaten by cod and other gadoids (ICES, 2011a). Gadoids can, also, ingest or be hosts for parasitic copepods (Rae, 1973), misleading the real prey species eaten by porpoises. Besides the widely variety of prey species presented on their diet, porpoises showed preferences for sandeel and whiting, along Scottish waters (Santos, 1998).

Old descriptions on porpoise diet around British waters, stated that porpoises mainly feed on herring (Southwell, 1881; Van Beneden, 1889). Years later, at the northeast Scottish waters, porpoises had whiting as primary prey (Scott, 1903). This result was obtained by Scott after stomach content analysis, where he found otoliths belonging to whiting and sandeel. For Scottish waters, Rae carried out studies (1965, 1973), between 1959-1971, showing that almost half of harbour porpoise diet was based on herring (15.6%) and whiting (25%). In a recent study, for Scotland and the east coast of England, Santos (1998) registered a consumption of 14640t of whiting, 13800t of sandeels and 1000t of herring, per year. Besides whiting and sandeels being found as main prey, she even noted cod as a primary prey. At the 21st century, off the east coast of Scotland whiting and sandeels are still the main prey, comprising ~80% of their diet (Santos *et al.*, 2004).

The research from Pierce & Boyle (1991), Santos (1998), Santos & Pierce (2003), Santos *et al.* (2004), Pierce *et al.* (2007) and Leopold (2015) demonstrated that diet composition and food preferences can differ from individual to individual, in relation to age, sex, reproductive status, season, cause of death and reflecting prey abundance and distribution. Therefore, the data collected for diet in a specific region should not be applied uncritically to other geographical locations (Pierce & Boyle, 1991).

Porpoises aged <1yr old demonstrated preferences for gobies and shrimps (Santos *et al.*, 2004). Older individuals show preferences for sandeels, while the youngest animals preferred *Trisopterus* spp. (Santos, 1998). Also, adult porpoise males ate bigger whiting specimens, and feed on a higher variety of prey compared to females (Santos, 1998; Santos & Pierce, 2003). Energy requirements or different skills to feed can be reasons for these trends (Leopold, 2015).

For Scottish waters, during 1992 to 2003, Santos *et al.* (2004) made demonstrated the existence of regional, seasonal, and inter-annual variation in porpoise diets. Santos (1998) noted that sprat was most important in porpoise diet in 1992-1993, while herring started to be important in 1994. Recently, herring and sprat rarely occurred in stomach contents, suggesting that their importance has been changed along the years (Santos, 1998; Santos *et al.*, 2004). Other study carried out by Santos (1998) reported the prevalence of sandeels in spring and summer diets of Scottish harbour porpoises. Santos (1998) and Santos & Pierce (2003) were not certain if it was the increase in their availability and/or the decrease of whiting abundance, that led to this result. Also, the increased amount of whiting during autumn and winter in porpoises diet could be related with the same principle above (Santos & Pierce, 2003). Winter period often means a high need of high-energy prey (e.g. clupeids, sandeels), however as demonstrated by Leopold (2015) and the studies above, porpoises in the winter prefer lean prey, like whiting and gobies, following the opposite trend in spring time. One of the explanations for porpoises change their preferences could be found in the Santos (1998) citation, "(…) *The seasonal movement together with the ecology of some of the prey species could explain some of the seasonal differences found in the diet*".

The cause of those changes remains unclear, however there are analogies than can be made, including if porpoises main prey be depleted by fisheries the possibility of them to switch to prey which are available. In Shetland, when the number of porpoises fluctuated with the status of local sandeel stocks (Evans & Weir, 1996). Or even with species of low preferences, like what occurred in European waters with the decline in herring stocks, the number of harbour porpoises declined too (Smeenk, 1987). However, the above trends could be merely analogies and nothing else, as Weir *et al.* (2007) observed, in

Aberdeenshire, that with the increasing of mackerel the number of porpoises in that area also increased, although mackerel is not commonly recorded in porpoise stomachs.

Rae (1973), Santos (1998) and Santos & Pierce (2003) concluded that harbour porpoise diet consisted mostly in commercial important species, which are the most abundant fishes in Scotland. Santos (1998) found a positive correlation (in 3yr out of 5yr) between the most important species eaten by porpoises and fishery landings in Scotland.

Feeding habits are difficult of being carried out through direct observation in their natural habitat (Hammond *et al*, 2013), for example because of harbour porpoise shy behaviour (Jefferson *et al.*, 2008; Read, 2008). Therefore, the analyses of stomach contents of dead animals will provide the necessary information for whiting and herring. As Casteel (1971) refers the predator stomach contents can help to indicate fish distribution.

1.3. Prey species potential biology/ecology factors of variability

1.3.1. Atlantic Herring (*Clupea harengus*)

Atlantic herring is one of the species from Clupeidae family that occurs all over the North Sea, being widely distributed from the northern Bay of Biscay to Greenland (Whitehead, 1985). The pelagic adult fishes tend to occur in continental shelf seas (to 200m deep) (Whitehead, 1985) while juveniles are found in shallow waters (ICES, 2011a). Maravelias (2001) show for both life stages higher abundances in shallow waters (<150m). Their shallow habitat made them an easy prey for porpoises.

Adults and juveniles, like the carrying capacity of herring’s early life stages (Nash *et al.*, 2009) can be compromised by the availability of food and by ecological conditions (Maravelias, 1997, 2001). Changes in the environmental conditions can shift the traditional spawning grounds and the spawning peaks (Maravelias, 1997). As a demersal spawner, herring moves to shallow waters (15-40m deep) with gravel beds (Whitehead, 1985; Nash *et al.*, 2009; ICES, 2011a), to attach its pelagic eggs (Whitehead, 1985). Herring spawns in autumn and spring, being the first season possible to be extended from September to January (ICES, 2011a). On Scotland, Moray Firth and Firth of Forth are important nursery grounds (ICES, 2011a), the planktonic larvae often move to inshore waters (Whitehead, 1985) and after 2yrs, herring moves into deeper waters to join to the adult population that migrates to the western part of the North Sea (ICES, 2011a).

Maravelias (2001) stated that older and larger fishes occur more offshore. He studied the case of Shetland Islands where on the west coast larger fishes were observed when compared to east side. In North Sea waters the lifespan is less than 7yrs, with a maximum length range of 20-30cm (ICES, 2011a). Herring usual swims in large schools (Whitehead, 1985; Camphuysen, 1990; ICES, 2011a), with diurnal vertical migration patterns, moving to the surface at the sunset and dispersing during the night (ICES, 2011a).

Herring is one of the most important pelagic fishes in several North Atlantic ecosystems, being an important prey for many predators such as other fishes, seabirds and marine mammals (ICES, 2011a). For many hundreds of years, herring has been exploited at the North Sea, being an important source of food and economic value for Europe (Simmonds, 2007). Herring is important for both commercial fisheries and cetaceans lives, in Scotland (Parsons *et al.*, 2000), its distribution and abundance can be a proxy for predators and regulates the fisheries actions.

Herring exploitation goes back several centuries, and due to natural environment changes and human exploitation stocks have shown higher fluctuations (ICES, 2011a). Many years ago (1870s), herring was the most profitable species at Scotland, in the Firth of Clyde, on the west Scottish coast (Thurstan & Roberts, 2010). In 1889-1962, the area was closed to trawlers to protect herring spawning grounds, but after 1962 the rules were adjusted allowing to fish again until the decline of herring after 1973 (Thurstan & Roberts, 2010; Heath *et al.*, 2017). Then, in 1984, even with the decrease on landings, they re-open all the area again to trawling expecting to increase herring catch levels and with the intention to explore new species (e.g. Norway lobster) (Thurstan & Roberts, 2010; Heath *et al.*, 2017). In that region, around 2005, the demersal fishery ceased, and in late 2000s the only demersal fish landings were from bycatches from other fisheries (Thurstan & Roberts, 2010; Heath *et al.*, 2017). Considerable fluctuations in catches were observed before and after the World War II (1939-1945) (ICES, 2011a), but Whitehead (1985) stated that fact could result from the fishing effort, and not from herring availability.

In the North Sea, after 1950s, herring fisheries had a high level of expansion (Simmonds, 2007), to suffer some years later (1964-1978), one of its biggest stock collapses due to human overexploitation (Whitehead, 1985; Simmonds, 2007; ICES, 2011a), possible a consequence of lack of regulation during that time (e.g. no catch limits) (Simmonds, 2007). The herring stock has recovered slowly only after the closure of the fishery and with the application of strict rules (Whitehead, 1985; Simmonds, 2007; ICES, 2011a). Simmonds (2007) reported a moderate to large stock size between late 1980s and early 1990s. However, another period of greater decline was very clear in 1994/95 (Maravelias, 1997; Simmonds, 2007). Simmonds (2007) related this fact with high fishing mortality while the Maravelias (1997) study showed that in 1994/95 environmental changes (e.g. Sea Surface Temperature - SST) were the reason for low herring abundance. In 1997, an EU/Norway management plan was applied, that resulted in the recovery of stock observed in 2003 (Simmonds, 2007; Dickey-Collas, 2016).

Nash *et al.* (2009) agrees that environment changes linked with overexploitation can be the reasons for those herring collapses, and the new EU landings obligations helped to reverse the declines (Dickey-Collas, 2016).

Nowadays, fisheries continue all year with peak catches between October and March with a minimum landing size of 20cm for the North Sea (ICES, 2011a). Its exploitation usually is done by purse-seine, distinct types of trawls and in inshore waters with drift nets (ICES, 2011a). According to Dickey-Collas (2016), presently herring from the North Sea is sustainably fished, with ~400000t catches per year (1995-2013), with the spawning biomass above the limit reference points (B_{lim}=800000t).

1.3.2. Whiting (*Merlangius merlangus*)

Whiting is one of the species from Gadidae family that occurs widely spread along the Scottish coast (ICES, 2011b). It is a demersal species which inhabits shallow waters (10-200m) (Whitehead, 1986; ICES, 2011b), like herring its habitat facilitates to be captured by porpoises.

One of the regular spawning grounds is situated at the north of Dogger Bank, along the east coast of Scotland (Loots, 2010). Even a demersal species, its eggs are pelagic, and the spawning season peak occurs at springtime, between March-June, for the northern waters (Hislop, 1984). High number of immature whiting occurs off the Scottish coast (ICES, 2013). Males grow to a small size than females (ICES, 2011b), and the species can live ~20yrs (ICES, 2011b).

Its migration through north-south are carried out along the coast of Scotland, while in Shetland waters they even move to offshore, in direction to east and south waters (Hislop

& Mackenzie, 1976). On a daily basis, whiting feeds at night near to the bottom and during daylight he moves into the water column and feeds on pelagic prey (Rindorf, 2003). In summer many individuals are observed offshore the Scottish coast (ICES, 2011b). Whiting is a very important prey for many piscivorous fishes and marine mammals (ICES, 2013, 2014, 2017). In cold seasons, the specimens which are not possible to be caught by fishermen due to their small size are the main prey of harbour porpoises (Santos, 1998; Santos & Pierce, 2003).

Over many years, demersal fisheries in the North Sea and adjacent waters have taken whiting as one of the main target species (Whitehead *et al.*, 1986; ICES, 2013), and nowadays it has reached high market values (Scottish Government, 2017). The outburst in 1960s-1970s of gadoids was apparently related with the high productivity of the ecosystem (ICES, 2014a), and could had help to increase whiting abundance. However, catches fell to an historical low in 1998 (ICES, 2011b). Since 2000, whiting has recovered slightly in Scottish waters (Heath *et al.*, 2017), although registering a 2nd historical low level between 2005-2008 (ICES, 2017). Nowadays, for some areas (north, west) of the North Sea whiting abundance has been increasing, while in others (south, east) it has declined (ICES, 2011b, 2014; Napier, 2014).

Environmental factors (e.g. SST) can be linked with the low abundance of whiting in recent years (ICES, 2014a). Since 2008, the spawning stock biomass (SSB) has been well above the biological reference point (B_{lim}=172741t), and for the North Sea and west Scottish whiting has been harvested in a sustainable way (ICES, 2016, 2017d).

With a minimum landing size of 27cm (ICES, 2011b), whiting is caught all year (ICES, 2013) with mixed trawl fishery gears (e.g. demersal, beam, *Nephrops* trawl) along with other gadoids (ICES, 2011b, 2013) but it has been captured as bycatch by industrial fisheries (e.g. Norway pout, *Nephrop*) (ICES, 2013, 2014). Since 2012, more selective gears and a reduced fishery bycatch have decreasing whiting fishing mortality, but even with the reduction on the number of bycatches, the MSY (Maximum Sustainable Yield) remains high (ICES, 2014a). Due to is main role in porpoises diets, his abundance can made lead to changes in porpoises choices.

2. Objectives

Considering the literature (Southwell, 1881; Van Beneden, 1889; Scott, 1903; Rae, 1965, 1973; Santos, 1998; Santos *et al.*, 2004, Pierce *et al.*, 2007; Marubini *et al.*, 2009; Leopold, 2015), it is apparent that herring and whiting have been part of the harbour porpoise diet in Scottish waters over a long period of time. We were interested in investigating the variation in importance of those these two fish species in the diet of porpoises in Scottish waters during 1992-2014. Over this period, fish stock abundance has fluctuated widely and piscivorous predators might be expected to adjust their diet to reflect these changes. In addition, it is likely that energy demands and foraging skills both increase as porpoises grow larger, and that mature females have higher energy needs than mature males, and these differences may be reflected in the diet. We therefore propose the following main hypotheses:

- The importance of whiting in the diet increases when herring are less important and vice versa.
- The consumption of herring and whiting by harbour porpoises increases when these fish are more abundant in the sea.
- The consumption of herring and whiting varies regionally (within Scottish coastal waters), seasonally, over time (1992-2014) and in relation to porpoise physical characteristics.

From these,

- Bigger porpoises will eat higher amount and larger sized herring and whiting.
- Females will eat higher amount and larger sized herring and whiting, compared to males.

3. Material and Methodology

3.1. Source of Harbour porpoise diet samples

Harbour porpoises found stranded on the Scottish coast, and occasionally floating at sea in Scottish waters, entangled in fishing nets or killed by bottlenose dolphins, were used to study the diet of the harbour porpoises in Scottish waters.

The Scottish Marine Animal Stranded Scheme (SMASS) has been collecting samples from dead harbour porpoises from different Scottish locations, during 1989-2016 (Figure 3.1.).

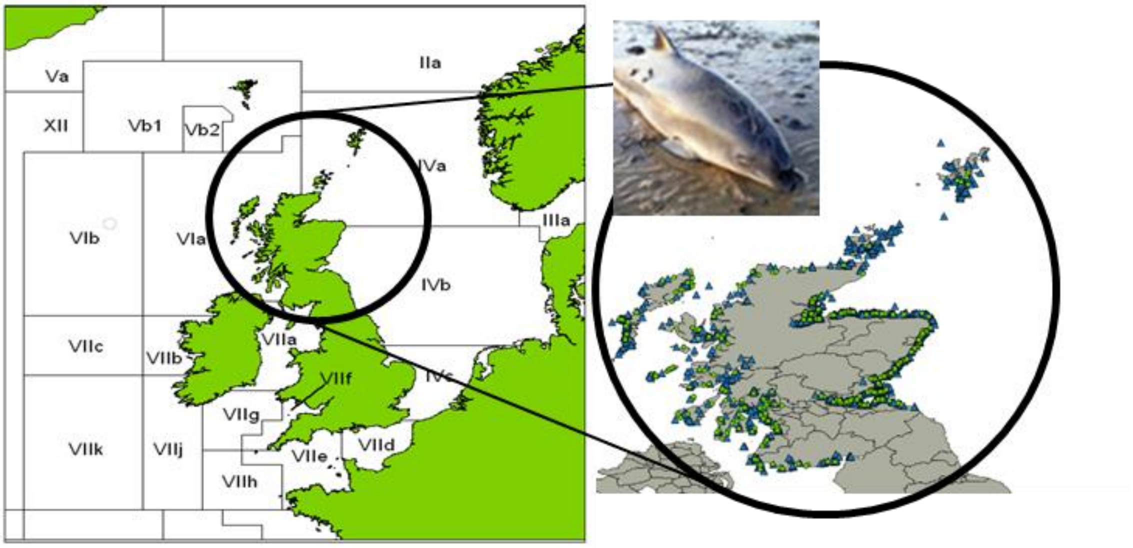


Figure 3.1 - Harbour porpoises stranded in Scotland (1989-2016)
Geographical distribution map of the harbour porpoises found death, around Scottish coast and waters (1989-2012, ▲, n=1450; 2013-2016, ●, n=324). (source SMASS/QGIS 2.10)

Between 1989 and 2016, SMASS recorded 1774 dead harbour porpoises which could be potential sources for a diet study. Unfortunately, from most of them (n=1468) there was no information about their diet, especially from the ones recovered during mid-1989-1991, before the stranding network was fully funded. Of those animals sampled, 8 animals were found with an empty stomach.

The information provided by SMASS was not always complete, probably due to the difficulty of access to some animals or their advanced state of decomposition, but also due to limited funding for necropsies. Dietary data were collected by several researchers and some inconsistencies were found in the way the data were collected. Consequently, the total number of samples used for the analysis during the present study was less than expected.

It’s important to note that the date/location and physical characteristics of porpoises were collected by SMASS’s team during their necropsies. The stomach contents were analysed by researchers at the University of Aberdeen: during 1989-2003, most sampled were analysed by Begoña Santos research while for 2003-2016 several different students collected the data. All this available information was compiled in one single Excel file. Several stomachs not previously examined were processed and the data added to this database as result of the laboratory work of the current author (2004-2014).

In order to study the variability in the diet of harbour porpoises, I analysed the relationships between the consumption of herring and whiting and their stock status, porpoise sex and body length (cm), and the year, season and location where they were found dead.

3.2. Sampling, Processing and Storage diet samples

Firstly, it was mandatory to wear gloves and a lab coat to handle the biological material from the porpoises. Necessary precautions suggested by different authors (e.g. Pierce & Boyle, 1991; Watt *et al.*, 1997). During a necropsy, usually, the stomach samples are frozen, or stored in alcohol or in formalin (Fitch & Brownell, 1968; Treacy & Crawford, 1981; Härkönen, 1986; Watt *et al.*, 1997). The University of Aberdeen study avoided the use of formalin due to its harmful properties and the fact that it dissolves otoliths. The stomach contents were sieved with a mesh of 0.25-0.5mm (Treacy & Crawford, 1981; Prime & Hammond, 1987), and so skeleton bones and otoliths were separated according to their shapes and densities. The contents were placed in an aluminium foil container (the type used for take-away meals), and water was added. Due to the density of otoliths compared with other structures (Treacy & Crawford, 1981; Pierce & Boyle, 1991; Watt *et al.*, 1997) they sunk in the bottom of the container. This gross sorting helped to separate flesh and intact skeletons from the sample, and to avoid the sieve to be clogged (Treacy & Crawford, 1981). Also, floating on the water were the lighter structures that were decantated into the sieve (Treacy & Crawford, 1981). One by one otoliths and bones were removed and kept separately in vials. Residual material from sorting procedure was set aside for future analysis (Treacy & Crawford, 1981). Hard structures, like otoliths and bones were kept dry (Fitch & Brownell, 1968; Härkönen, 1986; Watt *et al.*, 1997; Assis, 2000; Campana, 2004). Otoliths could change their shape if stored in alcohol (Jobling & Breiby, 1986) or formalin (Fitch & Brownell, 1968; Treacy & Crawford, 1981; Campana, 2004). All the storage plastic or glass vial were labelled outside and inside, as recommended by Roper & Sweeney (1983) and Watt *et al.* (1997), using a water-resistant marker and pencil on waterproof paper respectively.

3.3. Skeleton structures used for diet identification

Fish remains from stomach contents were the source to describe the harbour porpoise diet. As the literature suggested the recovery of skeleton structures (Casteel, 1971), and other hard parts of the body (Bowen & Iverson, 2012) helped in the identification of the prey. To identify herring and whiting and to estimate their weight and length, the informative skeletal remains used were mainly *sagittae* otoliths and jaw bones (premaxillae, dentaries)

Along the years, studies have shown how useful and accurate otoliths have been to identify prey species due to their particular shape and size (Adams, 1940; Casteel, 1976; Frost & Lowry, 1981; Härkönen, 1986; Jobling & Breiby, 1986; Pierce & Boyle, 1991; Assis, 2000; Bowen & Iverson, 2012). Their use relies on their resistance to digestion process (Fitch & Brownell, 1968; Treacy & Crawford, 1981; Härkönen, 1986; Bowen & Iverson, 2012), for example, Gadidae otoliths are more resistant than the ones from Clupeidae (Da Silva & Nielson, 1985; Jobling & Breiby, 1986). The sagita seemed to be the most adequated to use not only because of its shape and structure, but also it is the largest earstone among marine fishes (Jobling & Breiby, 1986; Bowen & Iverson, 2012). However, for some researchers the use of the 3 pairs of otoliths (*sagittae, asterisci, lapili*) are a stronger tool to distinguish between species (Adams, 1940). Equally important, it's to have an identification key reference of the different species life stages (Jobling & Breiby, 1986). As an example, it seems that otoliths from adults can be well identified (Casteel, 1976), while, for some families, otoliths from juveniles are hardly identified to the species level (Härkönen, 1986).

As a suggestion procedure, to be visualized under the microscope the otoliths were oriented with the sulcus cavity up, with the posterior margin to the left side and anterior margin right-sided (rostrum), (Härkönen, 1986; Assis, 2000).

Besides otoliths, other skeleton bones were mentioned by the literature as useful for diet analysis, too (Fitch & Brownell, 1968; Casteel, 1976; Watt *et al.*, 1997; Granadeiro & Silva, 2000; Bowen & Iverson, 2012). To help in the identification and when otoliths weren't representative of the sample, jaw bones offered an excellent way due to their variety of forms (Watt *et al.*, 1997; Bowen & Iverson, 2012), specially the premaxillae (Watt *et al.*, 1997). However, even with the use of premaxilla, between species from the same family the differences were not always obvious. Vertebrae were useful when the head of the prey wasn't eaten, and because they can be found in high amounts in the stomach (Casteel, 1976; Watt *et al.*, 1997). Due to their hard structure and low variation along the vertebral column (Watt *et al.*, 1997), the caudal vertebrae were used just only to help clarifying the identification of the species. Bowen & Iverson (2012) said that vertebrae are useful for identification, but they don't provide prey quantities, and therefore in this study they were not used for estimations. Beyond jaw bones and vertebrae, as recommend by researchers, other structures were used in case of identification doubts, such as opercular series (Watt *et al.*, 1997), cleithrum (Hansel *et al.*, 1988; Wheeler & Jones, 1989), or posttemporal bones (Wheeler & Jones, 1989). According Watt *et al.* (1997) and Bowen & Iverson (2012), the importance of other bones relies on how difficult is to distinguish otoliths between species, and an example is inside the Gadidae family (e.g. whiting vs blue whiting) (Pierce & Boyle, 1991).

In the case of some families, the right and left structure of a premaxillae and otoliths (e.g. Gadidae, Clupeidae) did not have any significant asymmetry and so did not require a separately analysis (Messieh, 1972; Frost & Lowry, 1981; Watt *et al.*, 1997). All them have distinctive features that facilitated the identification process (Härkönen, 1986; Watt *et al.*, 1997; Granadeiro & Silva, 2000; Campana, 2004).

The prey remains analysis was very time consuming and almost inexpensive but for a reliable identification it requires experience "eyes" (Pierce *et al.*, 2004), and so prof. Graham Pierce was consulted several times. Also, to an inexperienced worker, as the present author, the recommendation was to make use of a reference collection and identification guides (Pierce & Boyle, 1991; Assis, 2000; Pierce *et al.*, 2004; Bowen & Iverson, 2012). The reference collection, from Scotland region, it's held in the IIM (Instituto de Investigaciones Mariñas de Vigo). The publish guides for bones were from North Sea species (Watt *et al.*, 1997), and the otolith guides were from Northeast Atlantic and Artic species (Härkönen, 1986). Casteel (1976) and Watt *et al.* (1997) refer both tools, guides and reference collections, as of high value for identification of prey species.

Fitch & Brownell (1968) demonstrated the importance of knowing the resident fishes of the study region, for after to be compared with the collection material. Casteel (1971) even refers that this fact offers reliability to the information acquired from the bones identification process. Therefore, the knowledge of the Scottish fauna and previous information about harbour porpoise diet, for a specific region, helped to give an idea of the possible species that can be found on their stomach contents.

3.4. From skeleton to quantification of diet composition

The next step after the sorting of the bones, it was to measure otoliths and jaw bones which helped to predict the length and weight of herring and whiting that was eaten by porpoises. Also, it was counted the number of otoliths, premaxilla and dentaries in each stomach.

The number of individuals of herring and whiting was estimated firstly from the number of otoliths. In the absence of otoliths other remains were used, and when the otolith was broken, but it still had more than half of its size, it was counted as one otolith. Per stomach, when resulted to have ≤50 otoliths of one fish species, all of them were measured; when >50, a random sample of 30-50 of those otoliths were measured. As suggested by many authors, to obtain meaningful values is necessary to use randomly samples with a moderate-large size (n>30) and evenly distributed (Härkönen, 1986; Pierce *et al.*, 2004; Pierce *et al.*, 2007; Bowen & Iverson, 2012).

During counting, some samples had enormous amounts of otoliths and so they weren’t counted one by one, instead it was weighted a sub-sample (±0.01mg), and from that it was calculated the approximately real number of otoliths presented in the main sample.

After counting the total number of otoliths, premaxilla and dentary, it was calculated the number of herring and whiting, per stomach. For each species, the highest number of structures identified it was considered the minimal number of individuals eaten from that same species. It was taken into account that some skeleton structures like maxillae, premaxillae, dentaries, otoliths and eyes exist in pairs (Pierce & Boyle, 1991; Santos, 1998), hence during the estimation of the number of prey, each structure was considered to be 0.5 of a fish. This step was made very cautiously because of the high probability of overestimation, when using different bone structures. For that reason, it was considered only the number of otoliths to predict the final number of individuals, unless otoliths weren't present. An example, (M007/06), it was the case of whiting that was identified with 7 otoliths and 4 premaxillae. Because they are both paired bones their number was divided by two and because the highest number came from otoliths, so it resulted in 3,5≈4 whiting (0.5 means 1 more otolith and so it contributes with 1 more individual) as the minimum number eaten by that porpoise. However, for the same sample, an unknown Gadidae was identified with 3 otoliths, 33 maxillae, 15 dentaries and 1 premaxillae. The highest structure's number was 33 maxillae and so it should had been 16.5≈17 unk. Gadidae that had been eaten, but no. Some of that 33 maxillae can had belonged to the whiting referred above or other species from the same family, and at the end it was consider only 2 unknown individuals Gadidae (3/2=1.5) present in the stomach. Other situation, it was when there was a sample with bones that were identified with more than one species and bones from only one species. After divided the total number of structures by 2, the total minimum number of individuals was calculated like: 1.5whiting ind.+2.5 Whit./Trisop. ind.= 4ind., rather than 1.5whiting ind.≈2+2.5 Whit./Trisop. ind.≈3 = 5 individuals.

For measurements it was used a calliper (0.05mm) for bigger structures (centimetres size at the naked eye). And a binocular microscope for smaller ones, as advised by Fitch & Brownell (1968). This last instrument had an eyepiece graticule (0.1mm/10mm) and 4 lens magnifications (x6, x12, x25, x50). To get the real size of the bones the final measurement values were calibrated using the correction factors (per unit): x6 - 0.26mm; x12 – 0.125mm; x25 – 0.06mm; x50 – 0.029mm.

Fish length and weight were calculated from regressions on otolith size, based on Härkönen (1986), Brown (unpublished) and Hislop (unpublished) work (Appendix). Although, due to the otoliths shape there are standard measurements, for Clupeidae and Gadidae family, is the otolith width (OW) and the length (OL), respectively (Härkönen, 1986) (Figure 3.2, top images).

For measuring skeleton bones, Watt *et al.* (1997) referred that "*all the dimensions should be taken in straight lines, without correcting the curvature of the bone*". For jaw bones it was essential to measure the specific dimensions for each species, as happened to otoliths (Watt *et al.*, 1997; Hernandez-Milian, unpublish). The standard dimensions for premaxillae and dentaries (Figure 3.2) which were measured were the ones mention in their respectively regression formulas (Appendix). Jaw bones were described as good estimators of fish length and weight when the right measures are selected (Hansel *et al.*, 1988).

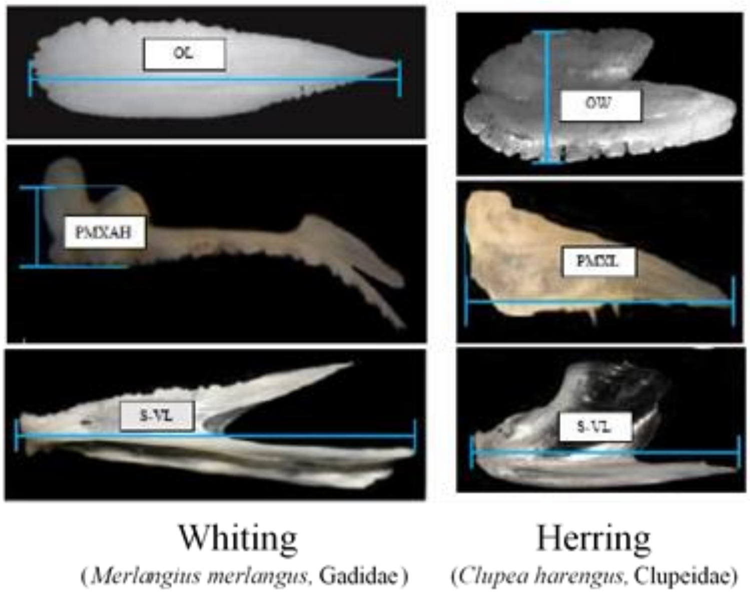


Figure 3.2 - Standard measures for otoliths and fish jaw bones
Standard measurements for whiting and Atlantic herring, from otoliths (**top images**), premaxillae (**central images**) and dentaries (**bottom images**). (adapt. Hernandez-Milian, unpublish)

It was supposed to use standard measurements, due to their consistency with the real dimensions (Casteel, 1976; Härkönen, 1986; Watt *et al.*, 1997; Brown, unpublish; Hernandez-Milian, unpublish). However, in some occasions, it was necessary to use dimensions beyond the ones stablished at the beginning. The dimensions suggested are rated regarding their precision with the real fish size and so when it wasn't possible to measure the standard dimensions, the next in the scale rate it was taken in its place. Sometimes it was even measured more than one dimension for a single bone, especially with broken structures and ambiguous identification among the species (e.g. Whiting/*Trisopterus* spp., Whiting/Blue whiting, unknown Clupeidae).

The regression formulas were chosen regarding their Pearson's correlation coefficient (r) and sample size (n). It wasn't always possible to calculate length and weight through the bone size regressions. Regressions for herring from otolith length to fish weight and length weren't available. Also, when the final identification of an otolith or a jaw bone was more than one species, the regression formula used was a combination of those species (e.g. otolith identified as Whit./BLW (Appendix). However, not all the combined samples had combined regressions to be applied, like the case of Whit./*Trisopterus* spp.. The jaw bones were the ones with less access to regression equations. Although, broken and eroded otoliths or bones were not submitted to any type of calibration. The lack of bone regressions had limited their contribution to estimate fish length and weight.

At the beginning the two main species that had been referred along the text, herring and whiting, became a combination of other fish species. During the sorting, some samples were identified with more than just only herring or whiting, as mentioned before. For this reason, the data coming from the bone structures identified as Whit./BLW (*Merlangius merlangus/Micromesistius poutassou*), Whit./*Trisopterus* spp. (*Merlangius merlangus/Trisopterus* spp.) and whiting (*Merlangius merlangus*) were used as information of whiting. And Herring/Sprat (*Clupea harengus/Sprattus sprattu*), unknown Clupeidae and herring (*Clupea harengus*) as data of herring.

Each value resultant from the bone regression was representative of the length and weight of each fish, in the porpoise stomach. However, because the bones used were paired, in the case of the fish weight, that value was multiply by 0.5. In a general way, each porpoise had more than one individual from the same species on its stomach, so it was calculated an average value for weight (after x0.5) and length, too. Also, when it was measured less bones (≈40) that the ones that were really found in the stomach, the rest of the fish weight were estimated through a rule of three (e.g. mean whiting weight of all sample=8.45g, no. of whiting otoliths measured=30, no. of whiting otoliths counted=115, total mean weight=8.45gx115/30). In the case of length this step was not necessary. Therefore, the length (mm) and weight (g) of whiting and herring found in each porpoise stomach was estimated.

The diet composition of harbour porpoises included the number or the presence/absence of whiting and herring in the stomach, as much as herring and whiting length and weight. Later on, to predict the variability of the diet, the response of those variables were study in relation to porpoises physical characteristics, geographical area, year, seasonality and the stock status of herring and whiting.

3.5. ICES stock assessments of herring and whiting

Every year, as a commercial species, herring and whiting are subjects of an analytical stock assessment (VPA) which means that estimations of their stock in the North Sea are available. The recruitment data and the spawning stock biomass (SSB) were undertaken by the respective ICES assessment working groups (e.g. ICES Herring Assessment Working Group), during research trawls surveys (Santos *et al*., 2004). The ICES areas 6a, 4a, 4b, where these assessments are carried out, include the surrounding Scottish waters. Survey grids are based on ICES statistical rectangles (30x30 nautical miles), each rectangle is often fished per ships of two different countries, and usually twice a year they do a survey (January-February; August-September) (ICES, 2012). During the tow the standard fishing speed should be between 3.5-4.5knots, for 30min., with an average of 4/5 hauls per day and with a maximum fishing deep of 200m for the North Sea (ICES, 2012).

Herring stock data was available for ICES area 6a (West of Scotland) (ICES, 2014), but since 2015 it has been an assessment combined with the area 7b-c (ICES, 2017a). ICES still assume that separate stocks exist (ICES, 2017a), but because a mixture of fish from the surrounding areas were identified, ICES began to combine the assessments advise (ICES, 2014). Despite data just for area 6a seemed more specific, the combined report had updated estimations and assumed to be more complete. In this report the data recruitment data was for the age of 1, and the SSB had the followed note "For this autumn-spawning stock, the SSB is determined at spawning time and is influenced by fisheries between 1 January and spawning (September)." (ICES, 2017a). Also, area 4 (North Sea) advice was combined with 3a and 7d divisions (ICES, 2017c), and was the one used in the present study of ICES areas 4a, 4b. The report area 4 had recruitment data for the age of 0 and SSB was estimated at spawning time (September). Both combined reports have been collected by the ICES Herring Assessment Working Group for the Area South of 62ºN – HAWG (ICES, 2017a, c).

Likewise, in the case of whiting, the advice ICES report was a combination of 4 area with division 7d (North Sea and eastern English Channel) (ICES, 2017d). The report for 6a area was single (ICES, 2016). Whiting recruitment (age 1) and SSB data for 4 and 6a ICES area were collected by the Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak (WGNSSK) and the Working Group for the Celtic Seas Ecoregion (WGCSE), respectively (ICES, 2016, 2017d).

For the stock status, the recruitment and SSB data of whiting and herring were used as variables which may predict the variability of whiting and herring that was eaten by porpoises. The data reports, due to some of their particularities, required the division of the waters around Scotland in east coastal waters (4a, 4b) and west coast (6a).

From the historical GPS coordinates registered by SMASS's team, it was possible to link an ICES area to each harbour porpoise found (Figure 3.1). It's important to take in mind that after died the floating body of the animal was possible affected by currents and winds and so the location where the porpoise was found is a source of bias (Peltier *et al*., 2012).

3.6. Statistical analysis of diet data

Excel, R software and Brodgar programme were the main tools to get further with the data analysis and the statistical models.

Brodgar version 2.7.5 was the statistical software chosen for most of the data analysis. It's a user-friendly programme suitable for the analysis of ecological data (Zuur *et al*., 2007). The software package had an interface to R version 3.5.0, which makes possible data exploration, univariate and multivariate analysis and other techniques (Brodgar's manual, 2000; Zuur *et al*., 2007). The programme and its manual were available at www.brodgar.com (Brodgar's manual, 2000). R Studio version 3.5.0 (2018-04-23) helped get through the analysis of Negative Binomial, Binomial models, and model comparison (Chi-squared test).

3.6.1. Data Sorting

Porpoises diet contents and their body characteristics, from 1992-2014, were provided by previous published and unpublished works. All this information was compiled in only one Excel file. From those prior databases some information needed to be reviewed and rectified (e.g. going back to the original notebooks, when they were available). Other needed to be completed and updated, and even new calculations were done with previous data. When there were cases of reviewed identification, the highest number of structures was considered (it was possible that some bones and otoliths had been lost or broken during the procedures). When possible, the data from the present author was used instead of the other available data. Not all the databases had the same information, most of the researchers counted the number of otoliths, but only some had measured them. Other prey remains can be considered but otoliths were the more often ones. Therefore, the use of equations to estimate prey length and weight were limited to be used in all the data.

After organizing the excel database, some samples were left out from the final database such as the empty stomachs, samples with missing diet information or mislabelled, stomachs with unidentified content and samples with imprecise location. Although, from the more recent years 2015 (n=60) and 2016 (n=38) the data that has been collected was insufficient to included here. Samples that belonged to the study ICES area but the animals where found stranded in English coast, or the opposite, samples which were collected in Scottish territory but are already out of the borders of the study ICES area (e.g. 7a) were excluded.

3.6.2. Data Exploration and Modelling

The data was analysed over a series of figures and tables. To study the diet variability in response to porpoise physical characteristics, time, season, geographical location and prey stock status, linear regressions and generalised additive models (GAMs) were used for the statistical analysis.

The data exploration was done using Brodgar software (Brodgar's manual, 2000; Zuur *et al.* 2007, 2010). The response variables were whiting and herring number, presence, length and weight. And the explanatory variables were porpoise length, weight and sex, year, season and Scottish coast (ICES area) where they have been found. The variables ICES area, sex and season needed to be transformed in numerical characters. These categorical variables in the end were ICES area:1=4a, 2=4b, 3=6a, sex:1=Female, 2=Male, 3=Unidentified, and season:1=Jan., Feb., Mar.; 2= Apr., May, Jun.; 3=Jul., Aug., Sept.; 4=Oct., Nov., Dec.. The explanatory variables were explored for outliers, correlation and variance inflation factors (VIF). Porpoise weight variable was excluded due to its high correlation with length (correlation=0.88).

Generalised Additive Models (GAMs) were then used to explore the patterns in the data. The response variables showed different data distributions, Normal distribution, Binomial and Negative Binomial distribution. For modelling a Negative Binomial distribution, it was used R Studio instead of Brodgar, with the guidance of Dalgaard (2002) work, and previous commands not published. For a Normal distribution a logarithmic transformation of the response variable values was applied, when an improvement of the model was verified. Considering the distribution of the data, different combination of models were tested, variables not having a significant effect at the 5% level (F-statistic) were eliminated one by one from the models. Also, for the smoothers, an amount of smoothing can be applied to the variable using a base dimension (k) when it justifies. This will improve the model, but should be used carefully. For example, it’s expected that the abundance of herring will vary over the years, so the resulted trend could have many shapes sometimes even difficult to interpret, so this variable should not be smooth. In case of length, for instance, it’s expected not to show to much variation, so to a clear analysis a base dimension can be applied.

Analysis of Variance (ANOVA) was used to test for herring and whiting differences in the physical characteristics of porpoise (sex, length), time, season, geographical location and fish stock status. A 95% confidence limit (CL) for the response variable and the best fitted line have designed the best model for the selected data. The selection was based on the Akaike Information Criterion (AIC), and/or in deviance/degrees of freedom residual, and/or in the estimated degrees of freedom (edf) and/or on the resultant graphics of the model (e.g. histogram, Q-Q plot, etc). In case of doubts choosing a model, a Chi-squared test was applied.

To study the relation between the number of prey eaten with the number (recruitment) of prey in the surrounding waters, the frequency of occurrence of whiting and herring was used. Frequency of occurrence: the number of stomachs containing remains of herring/whiting was expressed as a percentage of the number of stomachs examined in each year for the west and east coast, separately. The sample size for the data related with the west coast was small (n=47) for both prey, so the results needed precaution on their interpretation.

For the relationship between amount of prey (g) eaten and the amount (SSB) of prey existent in the waters of the study area, it was calculated indirectly the total mean weight (g) – biomass - of whiting and herring. Biomass: the total mean weight of the prey bone structures was expressed as a proportion of all stomachs weight, examined in each year for the west and east coast, separately. Also, for this study of the west coast, the sample size was small, whiting (n=47) and herring (n=42), and again the resultant predictions might be quite unrealistic.

Not all the years, between 1992-2014, were possible to cover with diet information, and so the blank years shown in the figures (Figure 4.2, Figure 4.4, Figure 4.10, Figure 4.12) cannot be compared against the years with provided information. The blank years did not mean the absence of whiting or herring in the sampled stomachs, they were years that any reference was registered, neither its presence or absence.

In the case of herring, due to such a small sample size for the west Scottish coast, in the two studies referred above, it was not possible to fit a model. Also, either to study its length or weight in relation to the other variables, the sample size was quite small (n=17), despite the bigger sample size (n=313) used for analysis of the presence/absence of herring in the stomachs. In the analysis of its importance in the porpoise diet, a preferential Binomial model was tested, due to the high number of stomachs without herring present (n=282 without, n=24 with). For whiting, both a Binomial and a Negative Binomial model were applied, especially because the presence of a similar number of stomachs with (n=154) and without whiting (n=160).

3.7. Sources of potential bias

Each stage, since the sampling to the final data were potential sources of bias in assessing harbour porpoises diet information from their prey remains.

The use of stomach contents to predict cetacean diet is a questionable procedure. Some authors agree its use can bring problems of identification and quantification of marine mammal diets, but it stills the most adopted method (Pierce & Boyle, 1991; Pierce *et al.*, 2004; Santos *et al.*, 2004). However, it is important to keep in mind, that the diet information coming from stranding or bycaught animals can be biased due to different factors. The porpoises that were sampled can have a different diet due to their group age (Santos, 1998), a sick cetacean can have an atypical diet (Pierce & Boyle, 1991; Learmonth *et al.*, 2014), a bycaught animal could be feeding near fishing areas where a certain species its more abundant, porpoises feeding near the coast can have a high representation of coastal fish species on their stomach (Santos, 1998).

Sampling error and the loss of material during sorting can be minimized by increasing sample size when possible (Bowen & Iverson, 2012), and by collecting the whole digestive tract from the animal (Pierce & Boyle, 1991). Every handling procedure can break the otoliths and as solution Härkönen (1986) referred the possibility of them to be reconstructed.

To decrease the identification error, different body structures were analysed, as suggested by different authors (Casteel, 1976; Da Silva & Nielson, 1985; Pierce & Boyle, 1991; Watt *et al.*, 1997; Santos, 1998; Granadeiro & Silva, 2000).

Both otoliths and bones are exposed to a certain digestive degradation, and so their original shape and size could be modified, special the more fragile ones (Härkönen, 1986; Pierce & Boyle, 1991; Granadeiro & Silva, 2000; Campana, 2004; Pierce *et al.*, 2004; Bowen & Iverson, 2012). So, to solve this problem, Härkönen (1986) recommended to measure the maximum otolith width, or to know the digestion time and how much the structure shortened in size (Pierce & Boyle, 1991; Bowen & Iverson, 2012). However, those authors stated that applying a correction factor to calculate fish weights could be difficult and not always available. Although, a single correction factor may be not enough, and both regressions for eroded and uneroded structure sizes should be calculated. As more practical solution, Pierce & Boyle (1991) suggested only the use of undigested bone parts. Even though, small progresses have been done regarding correction factors (Bowen & Iverson, 2012). Also, the size of the hard parts can differ with the body weight of the individual (Pierce *et al.*, 2007). Such sources of error took Jobling & Breiby (1986) and Granadeiro & Silva (2000) referring the limitations of the use of otoliths to analyse marine mammal diets.

Sometimes, the presence of fish can be both under and overestimated. One of the reasons is when the fish head is not ingested (Härkönen, 1986; Jobling & Breiby, 1986; Bowen & Iverson, 2012). Some authors suggested the use of other structures when they are available (Casteel, 1976; Da Silva & Nielson, 1985; Watt *et al.*, 1997; Granadeiro & Silva, 2000), or the use of DNA, fatty acids, stable isotopes or protein identification techniques (Pierce *et al.*, 2004). Regurgitation of indigestible remains (Pierce & Boyle, 1991) or the existence of secondary prey inside the eaten fishes (Härkönen, 1986; Pierce & Boyle, 1991; Pierce *et al.*, 2007) were other scenarios that can be minimized if the distribution of the prey species is known (Pierce *et al.*, 2004).

The use of regression equations to estimate length and fish weigh was advised by Pierce & Boyle (1991) because it’s a quick method and the fact of having just a few variables it reduces the potential error. Relationships estimated from the standard dimensions and

from small samples can provide a general application fish length-fish weight (Pierce & Boyle, 1991). On this project, all otoliths and jaw bones were used for identification and quantification of the prey number, while the estimation of fish weight and length was limited by the bones with regression equations.
Still Frost & Lowry (1981), Granadeiro & Silva (2000) and Pierce *et al.* (2007) advised a carefully interpretation of the results derived from regressions of a different region from the one of the study regions.

The use of frequency occurrence, in the study of prey number in the diet vs its recruitment data, was referred by Pierce & Boyle (1991) a source of bias due the higher importance that could be given to incidental prey found in the stomach contents. In the estimation of amount (g) of prey – biomass-, a single heavy prey can have a higher representativity in the diet.

The estimation significance and the prediction could be biased because of the small sample sizes for certain regions and prey (sample sizes have depended on the response variable).

The diet results from the stomach remains and their predictions can be seen as more representative of the variability of diet of death harbour porpoises than from the living population (Santos, 1998).

4. Results

4.1. Harbour porpoises sampled data-series

In Scotland, between 1989-2016, SMASS reported 1774 harbour porpoises that had been mostly found stranded on the coast. Besides the registration of local and date of their dead, some physical characteristics and biological samples were taken by the SMASS's team.

4.1.1. Spatial and temporal distribution

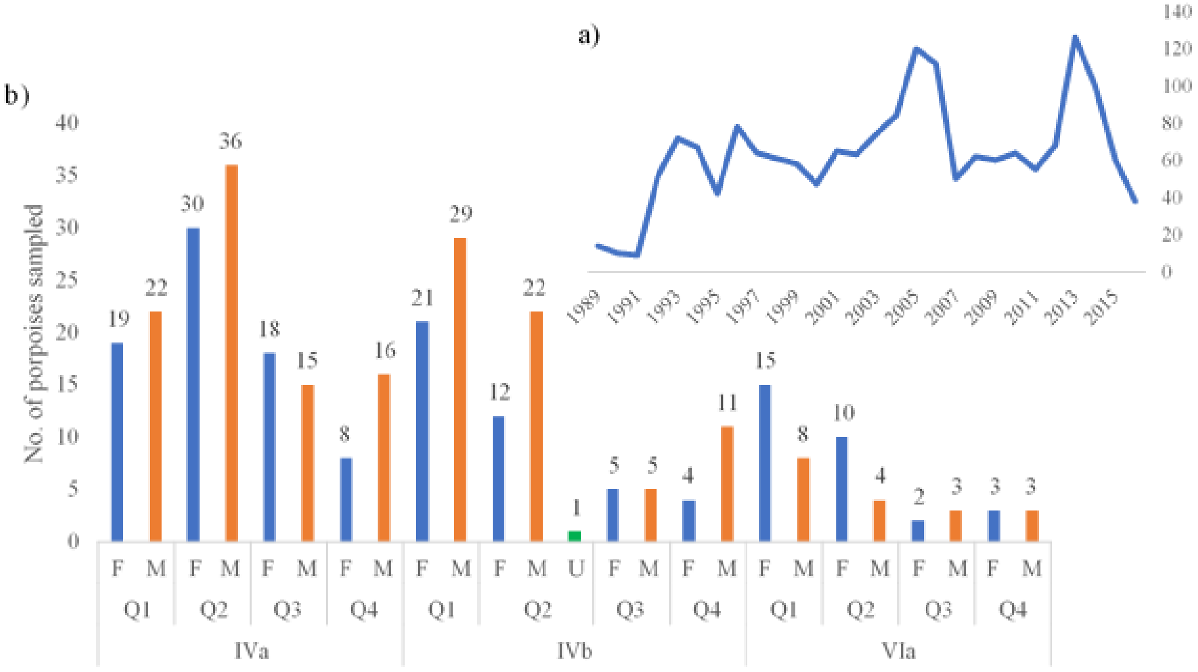


Figure 4.1- Spatial and temporal distribution of sampled Harbour porpoise
a) Annual total number of the registered death harbour porpoises (n=1774) from the Scottish coast, 1989-2016. **b)** Total number of harbour porpoise's stomachs sampled (n=322), by sex (F=Female, M=Male, U=Unidentified), by Scottish coast (east: area 4a, 4b, west: area 6a), and season (Q1=Jan., Fev., March; Q2=Apr., May, Jun.; Q3=July, Aug., Sept.; Q4=Oct., Nov., Dec.), between 1992-2014.

The spatial distribution of all the registered death porpoises (n=1774) can be seen in Figure 3.1. (Material and Methodology section) and the temporal-series in Figure 4.1a), for the Scottish coast, between 1989-2016. The annual total number of death porpoises, found by SMASS, had been showing fluctuations along the last 27 years. Along those years, a mean of ≈66 animals, per year, had been reported.

From now on, the data just will stand on to the period between 1992-2014, because just 18% (n=322) of the sampled harbour porpoises had provided relevant diet information for the present study, Figure 4.1b).

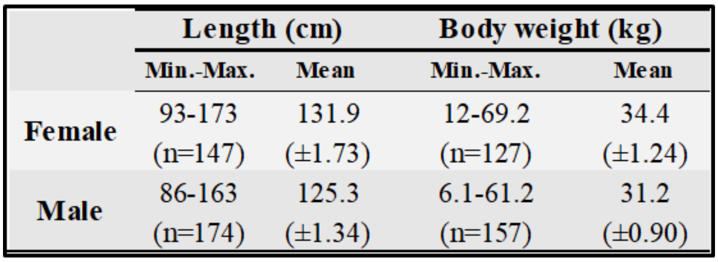
Among those 18%, a higher number of porpoises had been found on the first 6 months (71%) comparatively to the rest of the year (28.9%). There were more on the east Scottish coast (4a=50.9%; 4b=34.2%) than on the western side (6a=14.9%). In general, males had been reported in higher number (M=54%) than females (F=45.7%). The exception was on the west coast with 62.5% of females registered. Both sexes were found mostly in the east coast (85.1%) and between January and June (Q1=35.4%, Q2=35.7%).

From the 322 harbour porpoises sampled, 152 (47%) of both sexes did not have on their stomachs herring or whiting. Likewise, the individual with the unknown sex (U=1) had on its stomach other fish contents but not whiting or herring. Empty stomachs were found in 8 (2.48%) animals, 7 of them were males that stranded between 2003-2011.

4.1.2. Physical characteristics

Sex, length (cm) and body weight (kg) were some of the physical characteristics collected during porpoise's necropsies. Those measurements corresponded mostly to the animals found on the Scottish coast, between 1992 to 2014, (Table 4.1).

Table 4.1- Sizes of Harbour porpoise dead population
Length (cm) and weight (kg) of porpoises found death on the Scottish coast, between 1992-2014 (n=321). Minimum (Min.) and maximum (Max.) total length (cm) and weight (kg), with the sample size in parentheses. The mean sizes with their standard error in parentheses. *One harbour porpoise which the sex wasn't possible to identified, neither its length and weight isn't describe here.



	Length (cm)		Body weight (kg)	
	Min.-Max.	Mean	Min.-Max.	Mean
Female	93-173 (n=147)	131.9 (±1.73)	12-69.2 (n=127)	34.4 (±1.24)
Male	86-163 (n=174)	125.3 (±1.34)	6.1-61.2 (n=157)	31.2 (±0.90)

For the study region, females were found to be larger and fatter than males. The smallest male had 6.1kg, however it was an animal in moderate decomposition.

4.2. Herring and whiting variability in the diet of Harbour porpoises

In the last years, for the Scottish waters, whiting has been one of the main prey species of harbour porpoises diet. Other fish species such as herring make part of their diet, too. Both fishes are important commercial species in that region.

Their size and amount eaten by porpoises can be affected by year, seasonality, geographical location of the feeding area, porpoise body length or sex, and by their stock status reported for the region.

Linear regressions and GAMs were used to explore the response of herring and whiting to those variables.

4.2.1. Importance of herring in the diet considering its stock recruitment

ICES reports, from 1992-2014, were available with herring recruitment data. The quantity of herring that was eaten was obtained through the number of harbour porpoises stomachs with that species on them.

In order to explore if the occurrence of herring eaten was related with their present recruitment in the surrounding waters, recruitment data from ICES reports and the proportion of stomachs with herring, found in the sampled porpoises, were used as proxies.

For the Scottish coast, the occurrence of herring in all the stomachs examined was limited to some years, as shown in Figure 4.2.

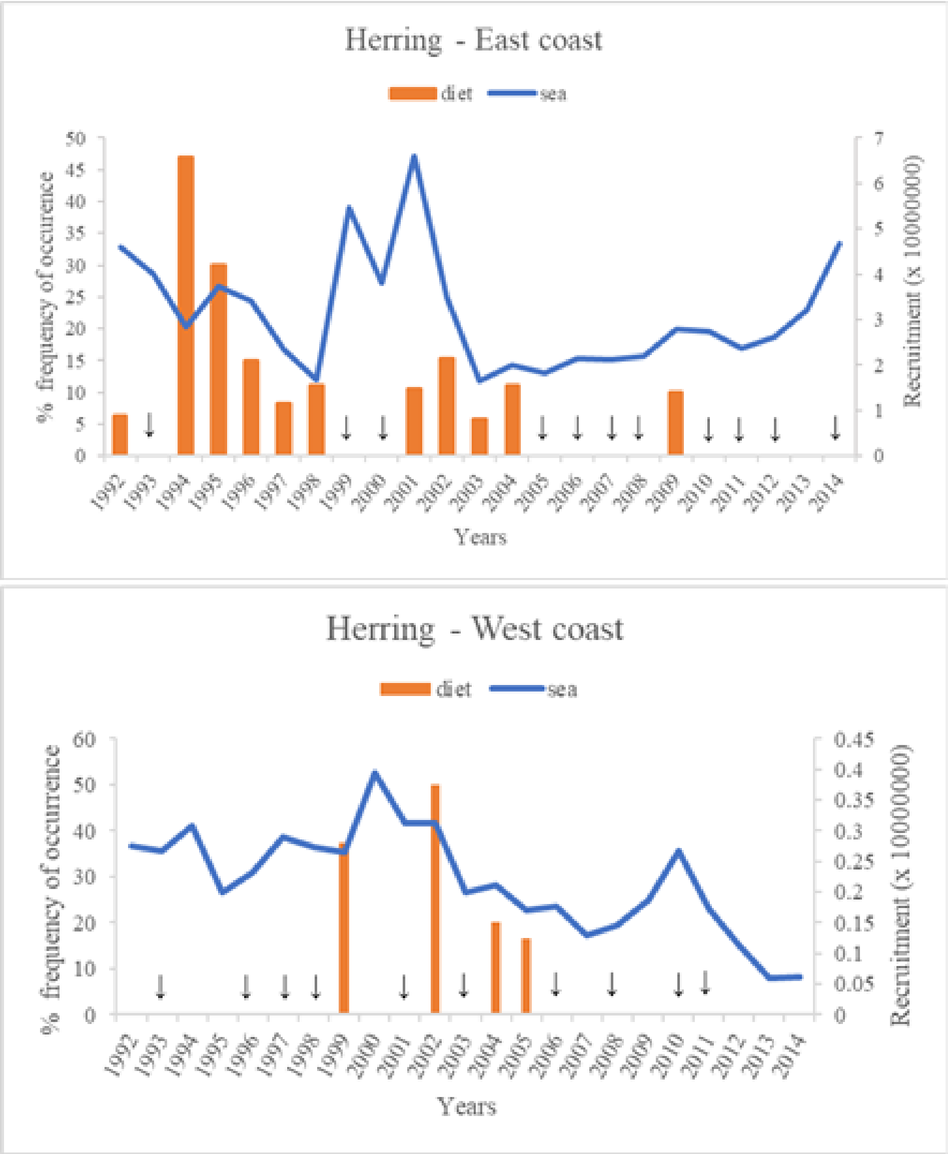


Figure 4.2- Annual occurrence of herring in diet and sea (recruitment), in Scotland
Frequency of occurrence (%) of herring in *Phocoena phocoena*'s stomachs (total number of stomachs with herring per the total number of stomachs recovered, in each Scottish coast, in each year), between 1992-2014. Herring recruitment data (thousands), for each ICES Scottish area, between 1992-2014. **Upper**: ICES area 4 (4a+4b) combined with 3a and 7d divisions data (recruitment age 0) – east coast (n=267) (ICES, 2017c). **Lower:** ICES area 6a combined with 7b-c data (recruitment age 1)– west coast (n=47) (ICES, 2017a). Empty stomachs weren't considered. The blank years do not indicate absence of herring in the stomachs (↓ indicates absent years). *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

For the east coast, only the year 2013 did not register stomachs with the reference of occurrence or non-occurrence of herring. Considering all the time-series for the east coast, in the middle of the 90's, herring had occurred in higher numbers in the stomachs, specially in 1994. In that same year, the reports had registered a decreasing in the abundance of this species, in the sea.

For the western coast, a total of 40 stomachs were sampled without the existence or non-existence of herring in their contents. On the western coast, in 1999 and in 2002 a high frequency of herring was identified in the diet.

The annual herring recruitment had experienced fluctuations, from year to year, for both coasts. Generally, after 2001 there was a collapse on herring recruitment stock, more gradually in the case of west coast.

Although there was no clear trend between the frequency of herring in the porpoise diet and its availability in the sea over the time-series, for the Scottish coast.

To confirm the unclear pattern resultant from the graphical analysis (Figure 4.2), GAMs were used for a second exploration. For the east coast of Scotland, a Poisson model explained 40% of the variation of herring in the diet, also the mean number of herring in the stomachs had not a statistically effect (p-value=0.765). The full model formula was: herring mean number ~ 1+s(herring recruitment), with AIC=44.2 (n=265). In this case, even without an effect, a possible slightly relation could be present for the east coast (Figure 4.3). For the west side of the coast, a Poisson model was plotted due to the type of distribution of the data. An AIC=4 and the formula: herring mean number ~ 1+s(herring recruitment), the model explained deviance resulted in -Inf.% (n=46), meaning the impossibility of fitting a model to the data of the west coast.

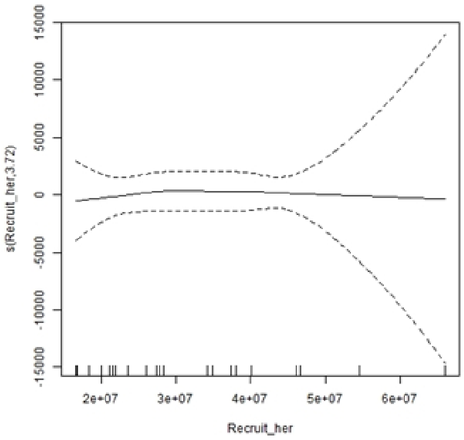


Figure 4.3- Number of herring eaten in response to Recruitment stock
Smoothing function of the best fitted Poisson model applied on the mean number of herring found in the *Phocoena phocoena* stomachs from the Scottish east coast, in response to its recruitment (thousands), between 1992-2014. DE=40% (n=265). The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

4.2.2. Importance of herring in the diet considering its stock biomass (SSB)

The amount (grams) of herring that was eaten was obtained through the quantification of their weight found in the stomach contents of harbour porpoises.

In order to explore if the amount of herring eaten was related with their biomass in the surrounding waters, SSB data from ICES reports and the total mean weight of herring, found in the sampled stomachs, were used as proxies.

Considering the Scottish coast, it seemed that during many years porpoises hadn't any herring on their stomachs when they died (Figure 4.4).

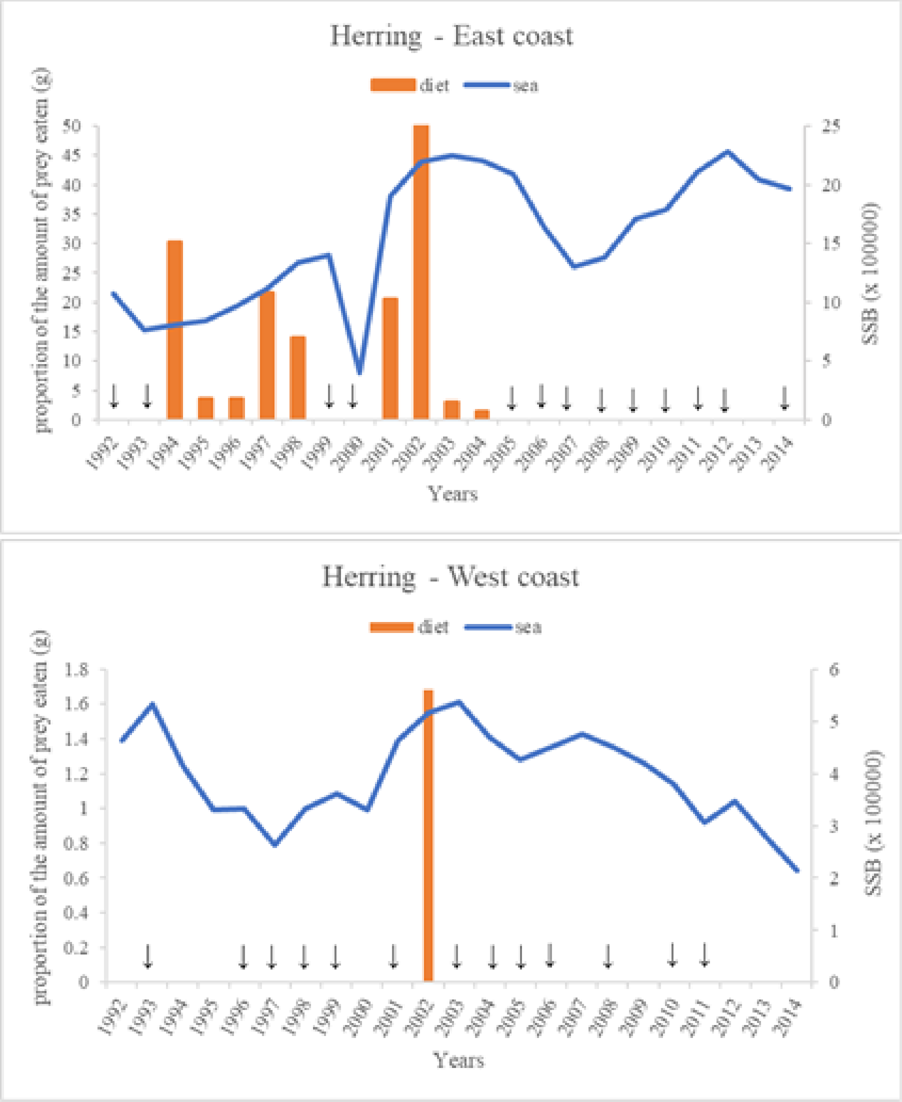


Figure 4.4- Annual occurrence of herring in diet and sea (SSB), in Scotland
Total mean weight (g) of herring eaten by harbour porpoises (*Phocoena phocoena*) (proportion of the total mean weight of herring per number of total stomachs with herring, in each Scottish coast, in each year), between 1992-2014. Herring spawning stock biomass (tonnes) for each ICES Scottish area, between 1992-2014. **Upper:** ICES area 4 (4a+4b) combined with 3a and 7d divisions data– east coast (n=257) (ICES, 2017c). **Lower:** ICES area 6a combined with 7b-c data (SSB was determined at spawning time)– west coast (n=42) (ICES, 2017a). Empty stomachs and stomachs with herring but not weighted weren't considered.

The blank years do not indicate the absence of herring in the stomachs (↓ indicates absent years). *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

In the east coast, for the year 1994 and 2002, the mass of herring found in the stomachs of sampled porpoises was higher, and corresponded to periods of increase herring biomass in that area, too. The herring biomass showed an abrupted decline in 2000, which reverted after.

For the west coast, only the porpoises stranded in 2002 had some herring weight on their stomach contents. The high amount eaten that year corresponded to a period when herring biomass stock had increased, too.

In the last few years, the herring SSB had been decreasing in Scottish east and west waters. Also, during the period here represented any clear pattern related with the mass of herring eaten by porpoises and herring biomass in the sea was observed.

To recheck the results from the Figure 4.4, GAMs were used to confirm. For the east coast of Scotland, a Poisson model explained 47.6% of the mass (g) variation of herring in the diet. Although, the average mass (g) of herring in the stomachs hadn’t statistically effect (p-value=0.169). The full model formula was: herring mean mass ~ 1+s(herring SSB), with AIC=108.14 (n=248). Even without an effect, a possible small relation could be present for the east coast (Figure 4.5). For the west side of the coast, a Poisson model was tested due to the distribution of the data of this region. The formula was: herring mass ~ 1+s(herring SSB), with AIC=4. The DE was -Inf.% (n=42) which meant the unfitted data of herring mass (g) to create a model.

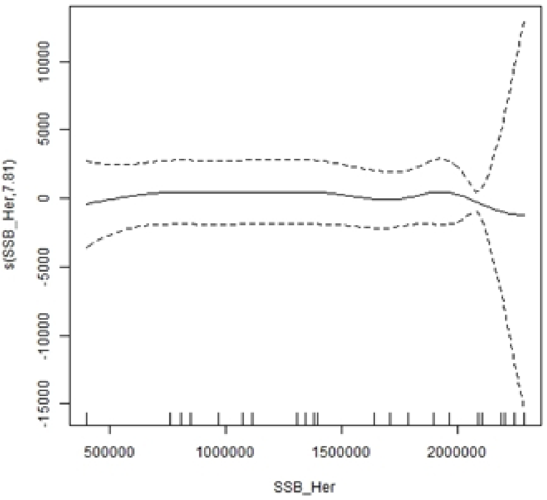


Figure 4.5- Mass (g) of herring eaten in response to SSB
Smoothing function of the best fitted Poisson model applied on the average mass (g) of herring found in *Phocoena phocoena* stomachs from the Scottish east coast, in response to its SSB (tonnes), between 1992-2014. DE= 47.6% (n=248). The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *herring is referred here as a combination of data of *Clupea harengus, Clupea harengus/Sprattus sprattus,* and unknown Clupeidae.

4.2.3. Variability in the presence/absence of herring in the diet

Among the 314 (8 had empty stomachs) sampled porpoises, 282 did not have herring on their stomach contents. A GAM for presence/absence of herring in the harbour porpoises diet was constructed to explore patterns which resulted in a Binomial model (0-1) has the best fitted model. Here it was considered the presence/absence of herring in the stomachs instead of the total number of herring ingested. The Binomial model with a logit link, had as full model: herring presence/absence ~ year + ICES area + porpoise length. A backward selection in which the variable with the highest p-value was sequentially dropped from the model, resulted with the variables year, and geographical location to have statistically effect. On the other hand, porpoise’s sex and body size (cm), and even season had not statistically effect. Although, the model had an AIC=191.62 and a deviance explained=19.3%.

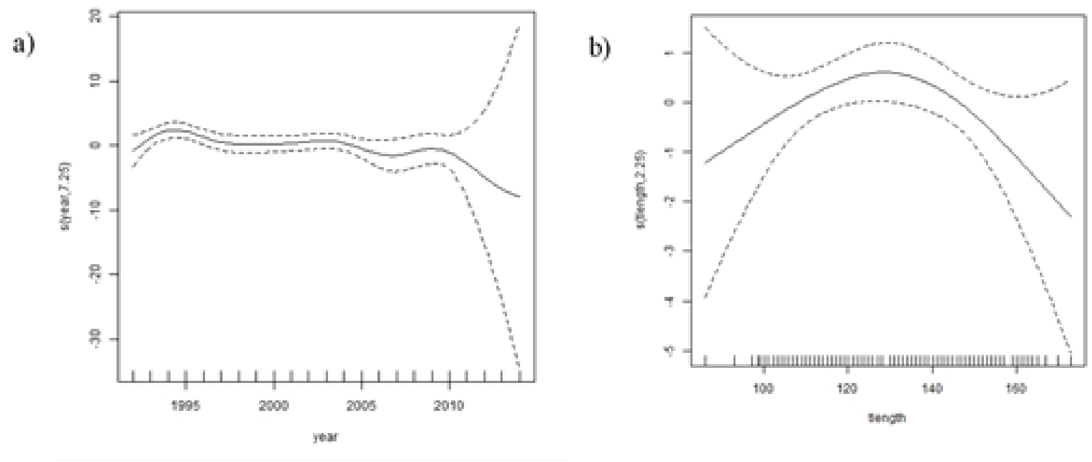


Figure 4.6- Presence/absence of herring eaten in response to year and porpoise length
Smoothing function of the best fitted Binomial model applied on the presence/absence of herring in the sampled *Phocoena phocoena* stomachs, between 1992-2014, from the Scottish coast. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained=19.3% (n=313). **a)** Smoother for year. **b)** Smoother for porpoise total length (cm). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

The ANOVA function suggested that the presence/absence of herring in the diet slightly changes with the geographical location where porpoises had their last meal (ICES area, p-value=0.0196). Similarly, the presence/absence of herring experienced small changes along the years (p-value=0.019),Figure 4.6 a). This trend it was not so precisely, as it was in the years after 2010.

As shown by ANOVA, porpoise's body length had not statistically effect (tlength, p-value=0.140), however, the Figure 4.6b) above suggested that a possible trend could existed, and even its presence in the formula improved the final model. The presence/absence of herring in the diet possible increased until the porpoises reach around 130cm length and decreased after. This case of non-statistically effect limited the interpretation of the Figure 4.6b).

4.2.4. Variability in the length of herring in the diet

For Scottish waters, from the examined samples the size of herring eaten by harbour porpoises ranged from 4.8 to 29.6cm.

The herring length (cm) relation regarding the length of porpoise (cm) is demonstrated in Figure 4.7.

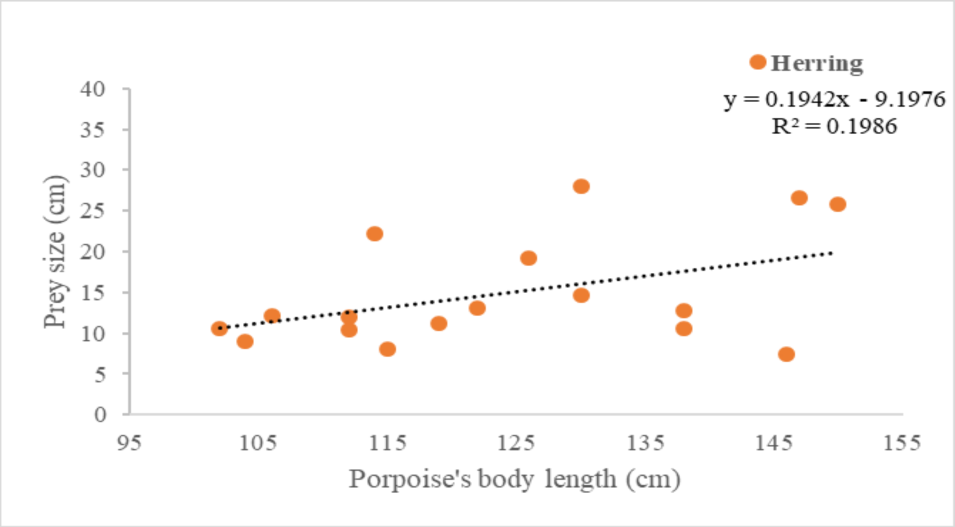


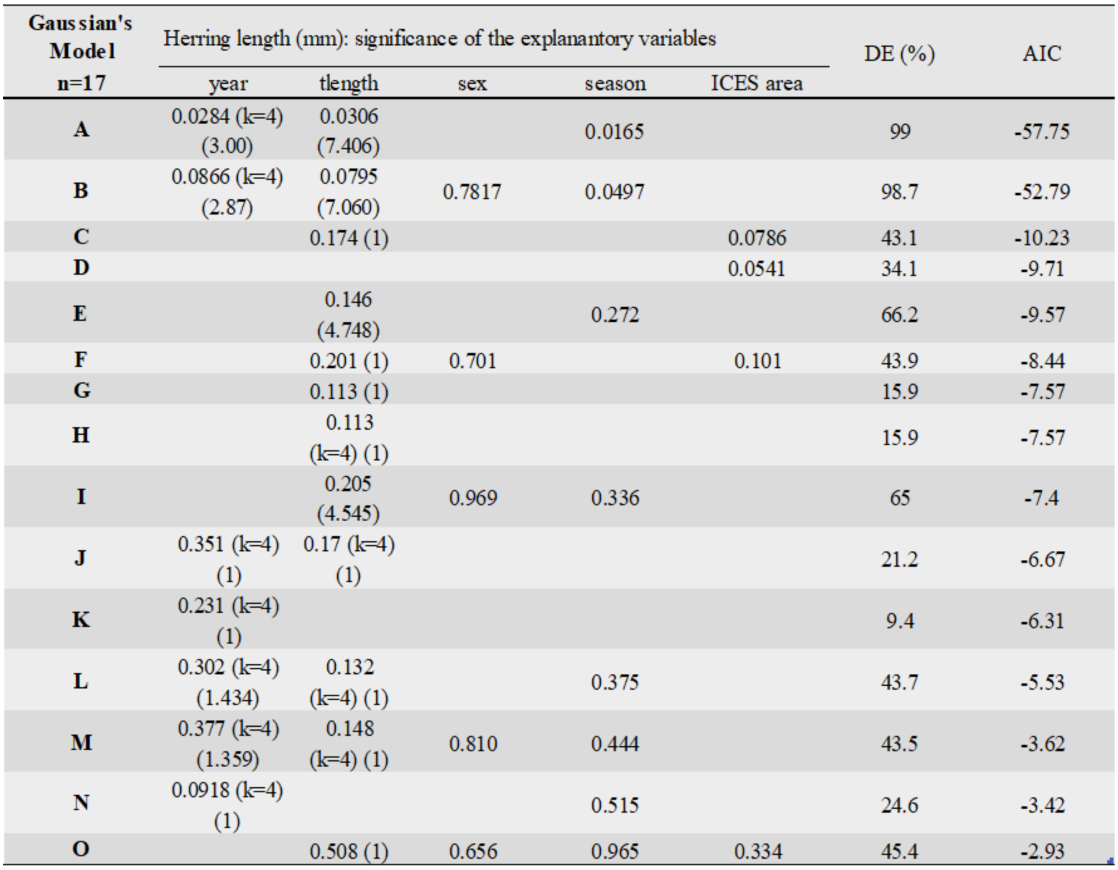
Figure 4.7- Herring length (cm) vs Porpoise length (cm)
Mean length (cm) of herring in the diet regardless porpoise's body length (cm), between 1992-2014, for Scottish waters. R-squared and regression equation are given. *herring as a combination of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

The small slopes from the equation given in Figure 4.7 suggested that herring size would not change with the length of the porpoise (herring, b=0.1942). Although, the small R-squared value indicates a small variability of the herring length around its mean.

The GAM exploration of the variation of herring size (mm) in the diet of harbour porpoises regarding different explanatory variables (porpoise length, sex, geographical location, time period) required the analysis of many models with different combinations as shown in the next Table 4.2.

Table 4.2 - Tested GAMs for herring length (mm)
Summary of generalised additive models for mean length (mm) of herring. Sample size (n) =17. The response variable was log-transformed. For each model, the table shows deviance explained (DE) and Akaike Information Criterion (AIC) value. Each explanatory variable used in the model is indicated with a p-value for significance. For smoother variables, the estimated degrees of freedom (edf) and, when useful, the bases dimension (k) are in parentheses. When the variable wasn't considered in the formulation of the

model, the cell in the table was left in blank. Any combination formula with year without a bases dimensions transformation did not fit any model. *herring is referred here as a combination of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.



Gaussian's Model n=17	Herring length (mm): significance of the explanantory variables					DE (%)	AIC
	year	tlength	sex	season	ICES area		
A	0.0284 (k=4) (3.00)	0.0306 (7.406)		0.0165		99	-57.75
B	0.0866 (k=4) (2.87)	0.0795 (7.060)	0.7817	0.0497		98.7	-52.79
C		0.174 (1)			0.0786	43.1	-10.23
D					0.0541	34.1	-9.71
E		0.146 (4.748)		0.272		66.2	-9.57
F		0.201 (1)	0.701		0.101	43.9	-8.44
G		0.113 (1)				15.9	-7.57
H		0.113 (k=4) (1)				15.9	-7.57
I		0.205 (4.545)	0.969	0.336		65	-7.4
J	0.351 (k=4) (1)	0.17 (k=4) (1)				21.2	-6.67
K	0.231 (k=4) (1)					9.4	-6.31
L	0.302 (k=4) (1.434)	0.132 (k=4) (1)		0.375		43.7	-5.53
M	0.377 (k=4) (1.359)	0.148 (k=4) (1)	0.810	0.444		43.5	-3.62
N	0.0918 (k=4) (1)			0.515		24.6	-3.42
O		0.508 (1)	0.656	0.965	0.334	45.4	-2.93

The apparently best fitted Normal model was the model A which had the lowest AIC value (-57.75), gather with variables with statistically effect. However, looking to the edf of the smoother variables it was possible to identify an overfitted model. So, in this situation the best fitted model chosen was the Normal model with a log transformation with an identity link, the model C of the Table 4.2. Considering the edf values, it was the next in line with the lowest AIC value (-10.23), and it had only 2 variables which made it simple, too. The full model: herring length~1 + ICES area + porpoise length. None of the remaining variables (location, porpoise length) had statistically effect. Even without effect, it was observed different length sizes of herring in the stomachs of the porpoises stranded in the different coasts of the Scotland. In the eastern coast, porpoises ate herring with a length up to 22.2cm (mean 10.8cm), in the north side, up to 29.6cm (mean 17.8cm) and in the west coast a length up to 8.9cm (mean 8.2cm).

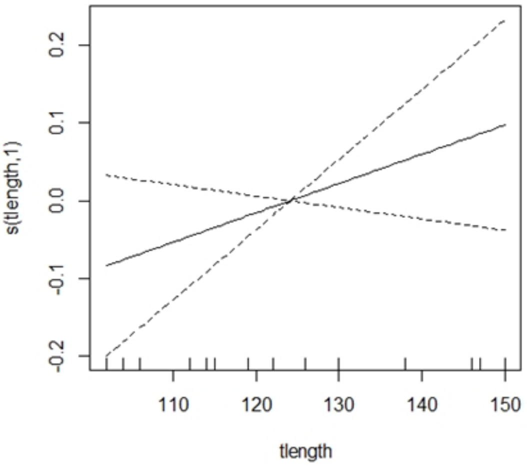


Figure 4.8- Length (mm) of herring eaten in response to porpoise length (cm)
Smoothing function of the best fitted Normal model applied on the length of herring (mm) found in *Phocoena phocoena* stomachs from the Scottish coast, in response to the porpoise total length (cm), between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained= 43.1% (n=17). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

As shown by ANOVA, porpoise's body size had not statistically effect (tlength, p-value=0.174), however, a possible trend that big porpoises ate big herring could existed (correlation=0.58). The prediction of porpoise length seemed not to be precise for smaller and bigger sizes (Figure 4.8). Also, porpoise's sex, year and season had not significant effect, either. The variables composing the best model, even without a significant effect they improved the final model.

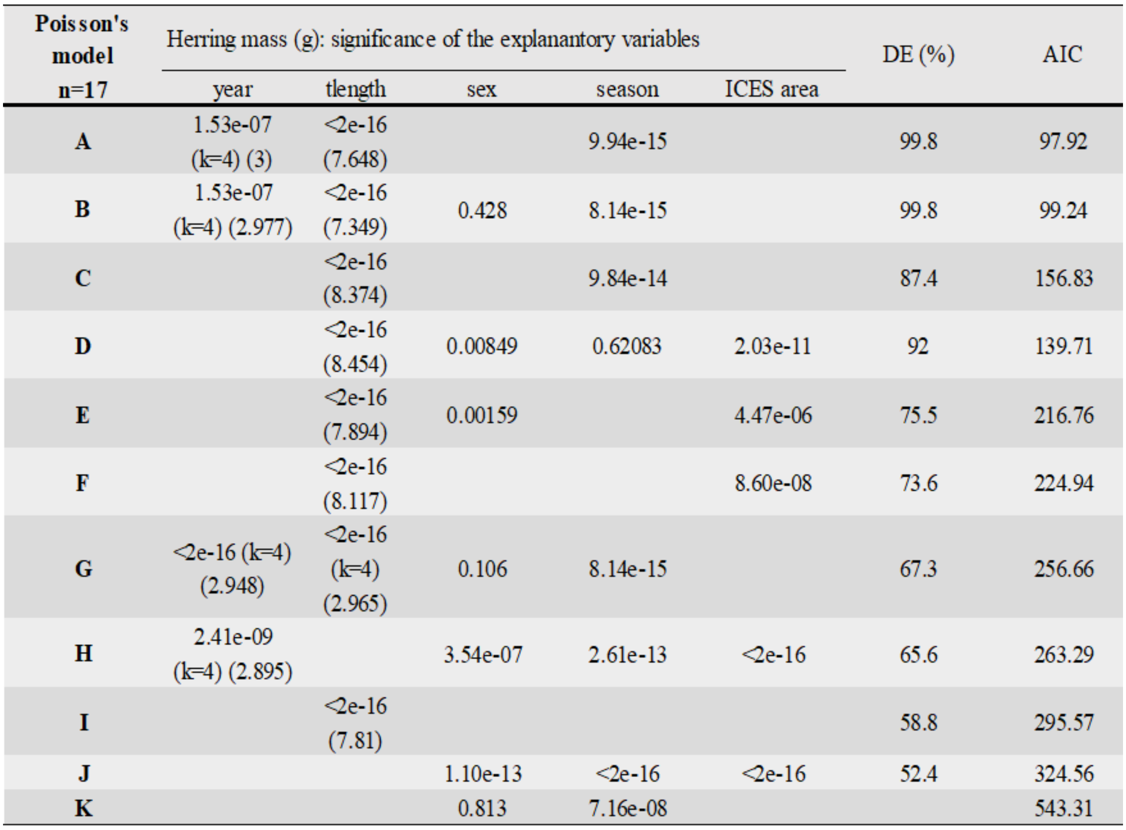
4.2.5. Variability in the mass (g) of herring in the diet

From the examined stomachs, the heaviest herring eaten by harbour porpoises had a maximum of 110g.

For the GAM exploration of the variation of the herring weight (g) in the diet of harbour porpoises with various categorical variables, there were a few possible combinations of models, Table 4.3.

Table 4.3 - Tested GAMs for herring mass (g)

Summary of generalised additive models for total mean weight (g) of herring. Sample size (n)=17. For each model, the table shows deviance explained (DE) and Akaike Information Criterion (AIC) value. Each explanatory variable used in the model is indicated with a p-value for significance. For smoother variables, the estimated degrees of freedom (edf) and, when useful, the bases dimension (k) are in parentheses. When the variable wasn't considered in the formulation of the model, the cell in the table was left in blank. Any combination formula with year without a bases dimensions transformation did not fit any model. *herring is referred here as a combination of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.



Poisson's model n=17	Herring mass (g): significance of the explanantory variables					DE (%)	AIC
	year	tlength	sex	season	ICES area		
A	1.53e-07 (k=4) (3)	<2e-16 (7.648)		9.94e-15		99.8	97.92
B	1.53e-07 (k=4) (2.977)	<2e-16 (7.349)	0.428	8.14e-15		99.8	99.24
C		<2e-16 (8.374)		9.84e-14		87.4	156.83
D		<2e-16 (8.454)	0.00849	0.62083	2.03e-11	92	139.71
E		<2e-16 (7.894)	0.00159		4.47e-06	75.5	216.76
F		<2e-16 (8.117)			8.60e-08	73.6	224.94
G	<2e-16 (k=4) (2.948)	<2e-16 (k=4) (2.965)	0.106	8.14e-15		67.3	256.66
H	2.41e-09 (k=4) (2.895)		3.54e-07	2.61e-13	<2e-16	65.6	263.29
I		<2e-16 (7.81)				58.8	295.57
J			1.10e-13	<2e-16	<2e-16	52.4	324.56
K			0.813	7.16e-08			543.31

The apparently best fitted Poisson model was the model A which had the lowest AIC= 97.92 and a 99.8% of DE (expected when small sample sizes), gather with variables with high statistical significant effect. However, looking to the edf of the smoother variables (year, tlength) it was possible to identify an overfitted model. So, in this situation the best fitted model chosen was the Poisson model with a log link, the model G of the Table 4.3. Considering lower edf values, it was the next in line with the lowest AIC value (256.66) with a deviance explained= 67.3%. The full model: herring weight~1 + year + porpoise length + sex + season. The remaining variables (year, porpoise length, season) had statistically significant effect while porpoise's sex and geographical location had not.

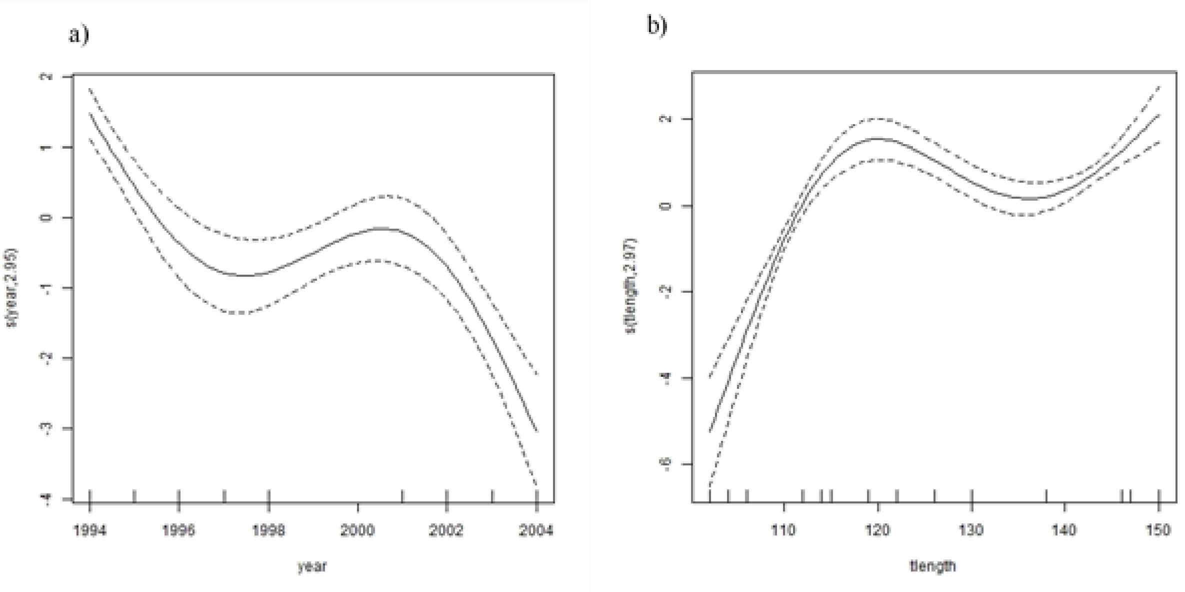


Figure 4.9- Mass (g) of herring eaten in response to year and porpoise length (cm)
Smoothing function of the best fitted Poisson model applied on the total mean weight of herring (g) found in *Phocoena phocoena* stomachs from the Scottish coast, between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained= 67.3% (n=17). **a)** Smoother for year. **b)** Smoother for porpoise total length (cm). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *herring is referred here as a combination of data of *Clupea harengus*, *Clupea harengus/Sprattus sprattus*, and unknown Clupeidae.

Year and the porpoise length variables appeared to have equally significant effect (p-value=<2x10^{-16}). Also, season (p-value=8.14x10^{-15}) has very significant effect, too. The mass (g) of herring eaten apparently changes with the feeding season. Year and porpoise length predictions are possible to see in the Figure 4.9. The mass (g) of herring eaten by porpoises seemed to had a general decline along the years. However, the mass (g) of herring ingested would tend to increase with the size of the porpoise (correlation=0.49).

Even without a significant effect with the geographical location, the mass of herring eaten showed general differences in the diet of porpoises. In the east coast, porpoises ate herring with a weight up to 36.3g (mean 10.8g), for the north coast, up to 110g (mean 30.8g), and in the western side fish up to 1.98g (mean 1.68g).

4.2.6. Importance of whiting in the diet considering its stock recruitment

In the same way as for herring, whiting recruitment data was accessed through ICES reports, and the ICES areas surrounding Scotland were analysed as east and west waters, for the period between 1992 to 2014.

Likewise, the quantity of whiting that was eaten was obtained through the number of harbour porpoises stomachs with whiting present.

To explore if the occurrence of whiting eaten was related with their present recruitment in the surrounding waters, recruitment data from ICES reports and the proportion of stomachs with whiting, found in the sampled porpoises, were used as proxies.

Harbour porpoise's stomachs showed that whiting was still eaten along the years, since 1992, as shown in the next Figure 4.10.

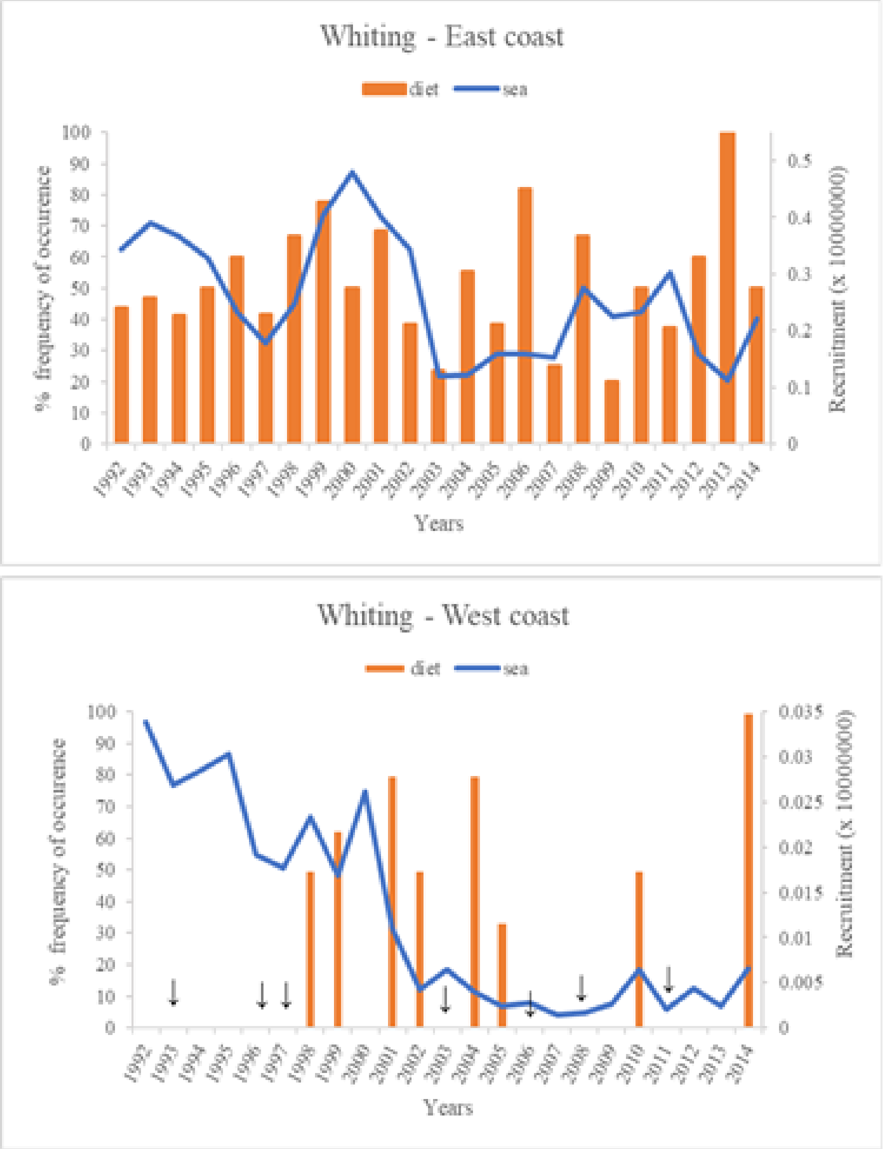


Figure 4.10- Annual occurrence of whiting in diet and sea (recruitment), in Scotland
Frequency of occurrence (%) of whiting in *Phocoena phocoena*'s stomachs (total number of stomachs with whiting per the total number of stomachs recovered, in each Scottish coast, in each year), between 1992-2014. Whiting recruitment data (thousands), for each ICES Scottish area, between 1992-2014. **Upper:** ICES area 4 (4a+4b) combined with 7d divisions data (recruitment age 1) – east coast (n=267) (ICES, 2017d). **Lower:** ICES area 6a (recruitment age 1)– west coast (n=47) (ICES, 2016). Empty stomachs weren't considered. The blank years do not indicate absence of whiting in the stomachs (↓ indicates absent

years). *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

The frequency of whiting in porpoises diet was constant for the east coast, from 1992 to 2014. In 1992-1993, 1997-1999 and 2007-2008 the number of whiting recruitments in the sea and in the diet had both increased. However, for 1996-1997, 2001-2003 and 2008-2009 the opposite trend was observed. So generally, the peaks of whiting recruitment not always coincided with higher or lower frequency of whiting in the diet.

On the west coast, the whiting recruitment has been decreasing since 1992, and after 2000 it collapsed. There were years when the occurrence of whiting was null in the stomachs. In 1998 and 1999, high and low peaks of whiting recruitment in the sea did not coincide with a higher or lower frequency in the diet, respectively. The year of 2014 was the one with high frequency of whiting in the diet, despite the low recruitment in the same region.

Therefore, a general trend wasn't clear between the whiting availability and the frequency of whiting in porpoise diet, for both Scottish coasts, over the time-series.

For an additional exploration of the unclear pattern resultant from the histogram (Figure 4.10), GAMs were used for a statistical analysis. For the east coast of Scotland, a Negative Binomial model (Theta=0.296) explained 0.09% of the variation of the number of whiting eaten. The full model formula was: whiting mean number ~ s(whiting recruitment), with AIC=640.2071 (n=267). The mean number of whiting in the stomachs hadn't statistically significant effect (p-value=0.635), but, a slightly positive response of the number of whiting in the diet could had existed (Figure 4.11a)). For the west side of the coast, a Negative Binomial model (Theta=0.285) was the best one fitted, too, with AIC=125.115, and the formula was: whiting mean number ~ s(whiting recruitment). The model explained 22.3% of the variation number of whiting in the diet, and it resulted in a statistically significant effect p-value (0.0115) for the recruitment number of whiting in the sea. The model suggested a positive trend (Figure 4.11b)), meaning that higher the number of whiting in the sea had increased the number of whiting eaten by porpoises. However, due to the small sample size (n=47), those values and trends might not illustrate the reality and need to be carefully interpreted.

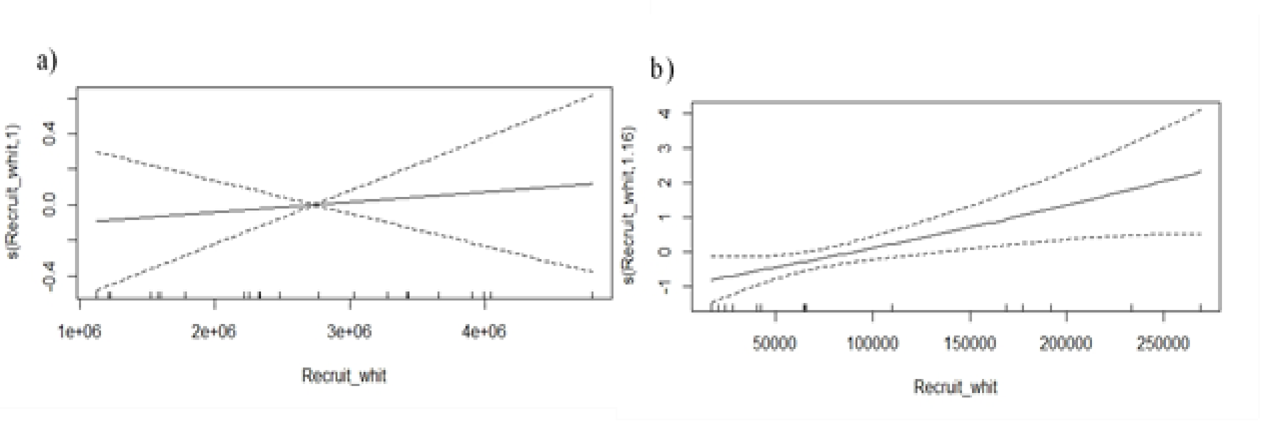


Figure 4.11- Number of whiting eaten in response to Recruitment stock
Smoothing function of the best fitted Negative Binomial model applied on the mean number of whiting found in *Phocoena phocoena* stomachs from the Scottish coast, in response to its recruitment (thousands), between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. **a)** East coast, DE= 0.09% (n=267). **b)** West coast, DE=22.3% (n=47). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

4.2.7. Importance of whiting in the diet considering its stock biomass (SSB)

The whiting amount (grams) that was eaten was obtained through the quantification of their weight found in the stomach contents of harbour porpoises.

To explore if the amount (g) of whiting eaten was related with their biomass in the surrounding waters, SSB data from ICES reports and the total mean weight of whiting, found in the sampled stomachs, were used as proxies.

In Scottish waters, whiting seemed to be part of harbour porpoise diet along the years, between 1992 to 2014 (Figure 4.12).

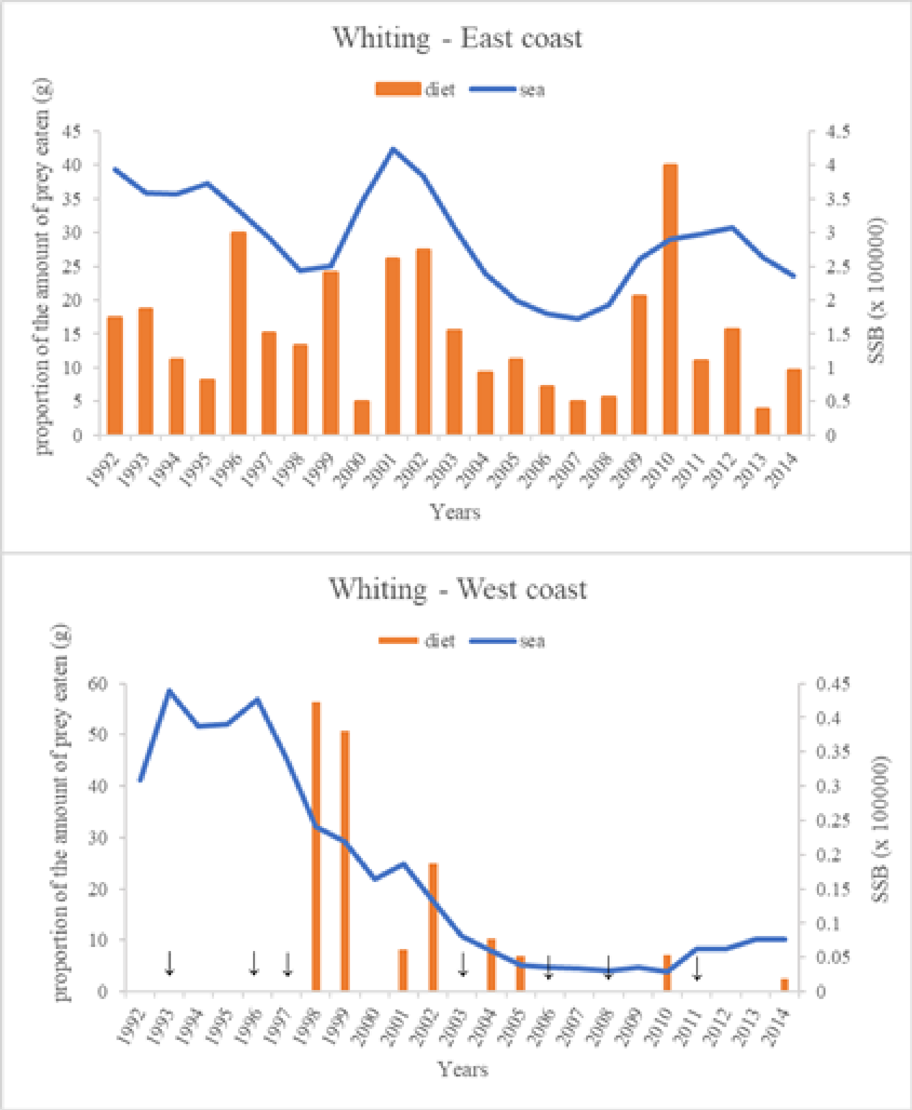


Figure 4.12- Annual occurrence of whiting in diet and sea (SSB), in Scotland
Total mean weight (g) of whiting eaten by porpoises (*Phocoena phocoena*) (proportion of the total mean weight of whiting per number of total stomachs with whiting, in each Scottish coast, in each year), between 1992-2014. Whiting spawning stock biomass (tonnes) for each ICES Scottish area, between 1992-2014. **Upper:** ICES area 4 (4a+4b) combined with 7d – east coast (n=265) (ICES, 2017d). **Lower:** ICES area 6a – west coast (n=47) (ICES, 2016). Empty stomachs and stomachs with whiting but not weighted weren't considered. The blank years do not indicate the absence of whiting in the stomachs (↓ indicates absent years). *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

For the east coast of Scotland, for all the years between 1992-2014, whiting had been identified in porpoises stomach contents. During the same period of time, the SSB of whiting in the east waters of Scotland, had suffer two main declines, first in 1995 to 1998, and then between 2001-2007. Generally, those years were related with periods of low whiting mass (g) in the stomachs contents, too. Also, in 2001, whiting SSB reached a high peak which it was coincident with a high mass (g) of this fish in the porpoise's stomachs.

However, not all the high peaks of whiting biomass were indicators of a high mass of fish in the stomachs and vice-versa.

In the west Scottish waters, whiting SSB had showed higher values in the beginnings of 90's, and since then had decreased (Figure 4.12 Lower). The existence of higher SSB values of whiting in the waters it was not always linked with a higher mass (g) of whiting ingested by porpoises. However, it's possible to see years, after 2003, when whiting SSB was decreasing and the mass (g) of whiting found in porpoise's stomachs in that same years was lower, and sometimes even none. In the case of the year 2001, SSB of whiting had a higher peak but the mass (g) found in porpoise's stomachs was lower than the year after, when the SSB was decreasing.

A general trend was not clear between the weight of whiting eaten by porpoises and the whiting SSB in Scottish waters, for both coasts.

For a statistical analysis of the results of the Figure 4.12, GAMs were used. For the east coast of Scotland, a Negative Binomial model (Theta=17.718) explained 99.2% of the mass (g) variation of whiting in the diet. The full model formula was: whiting mean mass ~ s(whiting SSB), with AIC=3674.049 (n=265). Also, the average mass (g) of whiting in the stomachs had statistically significant effect (p-value=$<2x10^{-16}$). The fitted GAM suggested a negative relation, meaning that higher the biomass of whiting in the sea less mass (g) of whiting was found in porpoises diet, for the east Scottish coast (Figure 4.13a)). For the west side of the coast, the best fitted model was a Negative Binomial (Theta=0.98), with AIC=626.4964, with the formula: whiting mass ~ s(whiting SSB). The model explained 85.9% of the variation of the whiting mass (g) in the diet (p-value=$<2x10^{-16}$), with a significant relation with the whiting SSB in the sea (Figure 4.13b)). However, because of the small sample size (n=47) those results for the west Scottish coast need to be interpreted very carefully and might not even be representative of the reality.

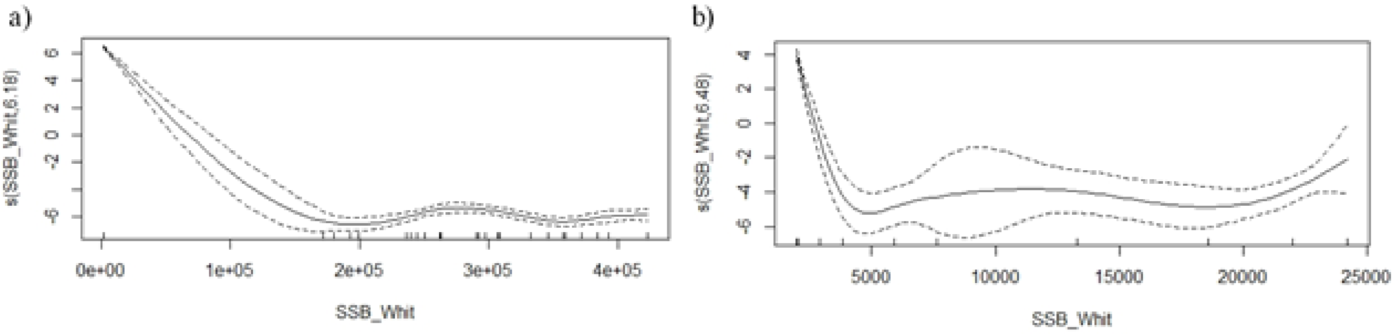


Figure 4.13- Mass (g) of whiting eaten in response to SSB
Smoothing function of the best fitted Negative Binomial model applied on the average mass (g) of whiting found in *Phocoena phocoena* stomachs from the Scottish coast, in response to its SSB (tonnes), between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. **a)** East coast, DE= 21.9% (n=265). **b)** West coast, DE=51.6% (n=47). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

4.2.8. Variability of the presence/absence and number of whiting in the diet

The amount of whiting eaten resulted in two different and fitted statistical models: a Negative Binomial which considered the number of whiting found in each sampled stomach and the Binomial model (0-1) which deal with the presence/absence of whiting in the stomach contents.

After tested a combination of models, the Negative Binomial with a log link function that resulted had as full model: whiting abundance~ year + ICES area + season. It had an AIC=1538.174, a Theta value=0.181 and a deviance explained=12.7%. This model showed that the explanatory variables such as sex and porpoise body length hadn’t statistically significant effect. Meaning that no matter which sex, or the size of the porpoises the number of whiting eaten will not change. However, the ANOVA function suggested that the number of whiting eaten by porpoises its highly affected by the geographical location and seasonality. Season showed the highest significant effect (p-value=2.08×10^{-05}), followed by ICES area (p-value=0.00108) and lastly the years (p-value=0.0037). The prediction trend along the years can been observed in the next Figure 4.14.

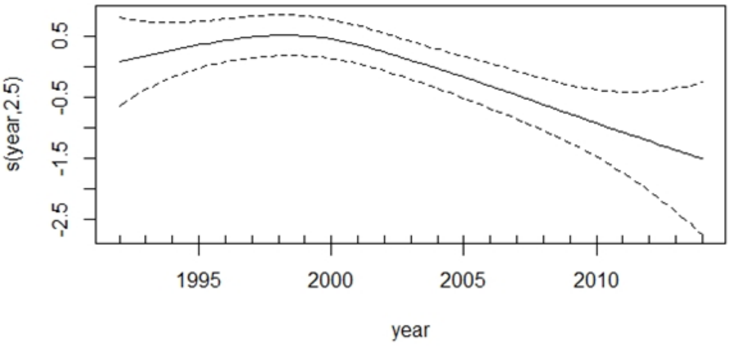


Figure 4.14- Number of whiting eaten in response to year
Smoothing function of the best fitted Negative Binomial model applied on the total number of whiting found in *Phocoena phocoena* stomachs from the Scottish coast, in response to years, 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained= 12.77% (n=314). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

From 1992-1998, porpoises showed an increased number of whiting on their stomachs. After that period, they had decreased the consumption of that same fish species. Also, for the earlier years and more recent ones, the model was less precise about the observed trends.

In a succinct way for the Negative Binomial model, the physical characteristics of porpoise seem not to predict the number of whiting ingested while the geographical area and temporal (year, season) variables do it.

Equally important was the Binomial model (0-1), because there were 154 stomachs with the presence of whiting and 160 without. Different model combinations were design, after a Chi-squared test was applied and the final chosen Binomial model with a logit link had as full model: whiting abundance~ ICES area + season, with an AIC=424.2414 and a deviance explained= 4.98% (n=313). From the model, season hadn't statistically significant effect (p-value=0.07614). Likewise, sex, porpoise body length and years had not significant effect, either. On the contrary ICES area p-value suggested that the geographical area was associated with the presence/absence of whiting in the diet (ICES area, p-value=0.00205).

For the two models, despite both AIC values cannot be compared, the Negative Binomial kept year, season and geographical location as variables with significant effect while Binomial model only considered with a significant effect the location where the porpoise feed for the last time. In the Binomial model, the presence/absence of whiting was limited to the simplest act of fish consumption (for each year during 27 years and 4 seasons the differences between the samples were not so greater than the ones for west and east coast). Instead, the Negative Binomial model (total number of prey) considered the high and low numbers of whiting ingested per year, season and area, reflecting this the time and place when and where porpoises had eaten more or less whiting.

4.2.9. Variability in the length of whiting in the diet

For Scottish waters, from the examined samples the length of whiting found in harbour porpoises stomachs ranged from 1.2 to 35.6cm.

The correlation between whiting length (cm) and the length of porpoise (cm) is in Figure 4.15.

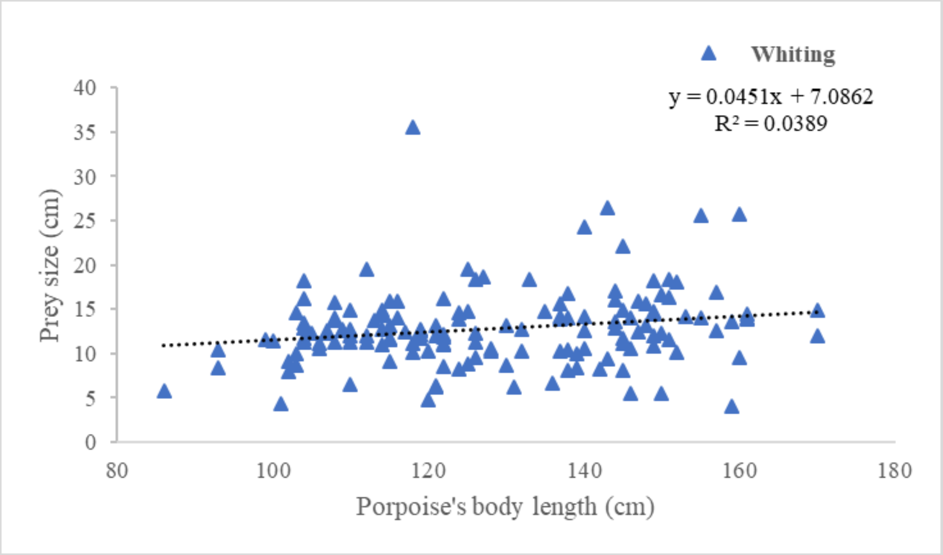


Figure 4.15- Whiting length (cm) vs Porpoise length (cm)
Mean length (cm) of whiting in the diet regardless the porpoise's body length (cm), between 1992-2014, for Scottish waters. R-squared and regression equation are given. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp.

The small slopes from the equation given in Figure 4.15 suggested that the size of whiting would not change with the length of the porpoise (whiting, b=0.0451). Also, the small R-squared value indicates that the model explains a small variability of whiting length around its mean.

A GAM for the length of whiting in the harbour porpoises diet was constructed to explore patterns with different variables (porpoise length, sex, geographical location, time period). It resulted in a Normal model has the best fitted one. The Normal model with a log transformation and an identity logit link, had as full model: whiting length~1 + season + porpoise length, with an AIC= -161.44. The resulted remaining variables season and porpoise length had statistically significant effect. ANOVA showed a slightly higher variability in the size of the whiting that was consumed with the porpoise length (p-value=0.011) than to season (p-value=0.0128). The response of the size of the whiting eaten to the porpoise length is shown in the Figure 4.16.

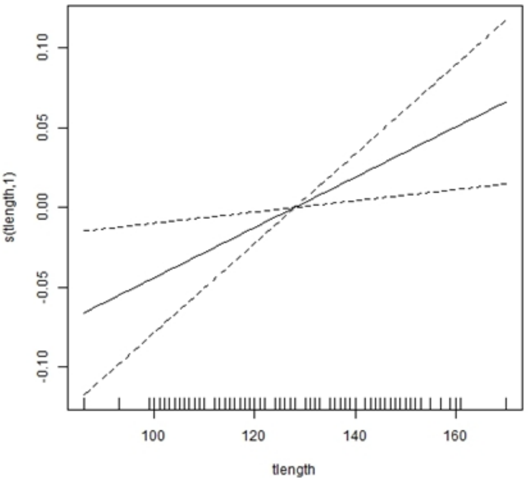


Figure 4.16- Length (mm) of whiting eaten in response to porpoise length (cm)
Smoothing function of the best fitted Normal model applied on the whiting mean length (mm) found in *Phocoena phocoena* stomachs from the Scottish coast, in response to the total length of porpoises (cm), between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained= 9.99% (n=152). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

The Figure 4.16 suggested that the size of whiting eaten increased with porpoise's body length. The position of the fitted line and the confidence limit's lines show that the previous trend referred it was always present even if slightly (never the fitted line was horizontal).

Porpoise's sex, geographical location and year weren't considered significantly predictors for the size of whiting eaten by porpoises. Even without significant effect, it was observed differences in the length of the whiting found in the porpoises stomachs that stranded in the different coasts of the Scotland. For east and north coast, whiting was found within a length up to 35.5cm (mean 12.7cm and 12.5cm respectively), and for the western coast with a length up to 32.8cm (mean 14.2cm).

4.2.10. Variability in the mass (g) of whiting in the diet

The whiting found in harbour porpoises stomach contents had a maximum of 227g of weight.

After the analysis of a combination of models the Normal distribution model with an identity link and a log transformation was selected as it produced the best values. It has as a full model: whiting weight ~ year+ season+ porpoise length, with an AIC=242.63. Season and porpoise length were variables with statistically significant effect. Year, geographical location and porpoise's sex did not appear to change the mass (g) of whiting ingested by porpoises. Even without significant effect with the geographical location, the mass of whiting eaten showed general differences in the diet of porpoises. In the east coast, porpoises ate whiting with a weight up to 177.7g (mean 14.05g), for the north coast, up to 227.2g (mean 20.08g), and in the western side fish up to 142g (mean 23.12g).

The response trend for year and porpoise body length is shown in the next Figure 4.17.

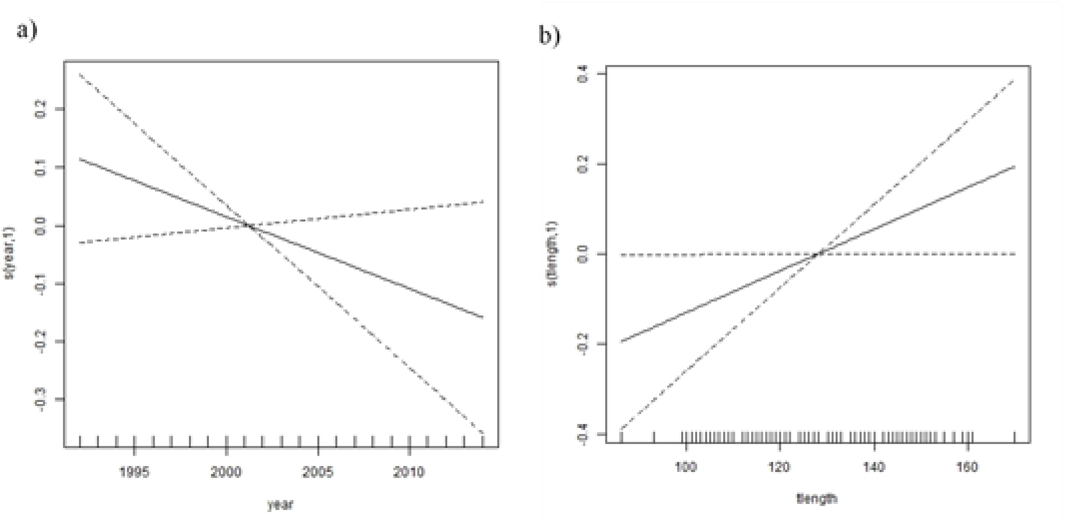


Figure 4.17- Mass (g) of whiting eaten in response to year and porpoise length (cm)
Smoothing function of the best fitted Normal model applied on the total mean weight of whiting (g) found in *Phocoena phocoena* stomachs from the Scottish coast, between 1992-2014. The s() is a smooth spline smoother and the number in the smooth spline smoother is the degrees of freedom for the smoother. Deviance explained= 10.7% (n=152). **a)** Smoother for year. **b)** Smoother for porpoise total length (cm). The solid line is the estimated smoother and the dashed lines are the 95% confidence limits. *whiting is referred here as a combination of data of *Merlangius merlangus, Merlangius merlangus/Micromesistius poutassou* and *Merlangius merlangus/Trisopterus* spp..

Year even with a p-value=0.1134, it had improved the model. Also, the Figure 4.17a) showed that a possible trend could existed, like the decreasing in the mass (g) consumption of whiting or the possibility of no changes at all, along the years. This case of variable without significant effect limited the interpretation of the Figure 4.17a).

The mass (g) of whiting consumed seemed to change slightly with the length of the porpoise (p-value=0.0458) and more with the season (p-value=0.00369). The Figure 4.17b) suggested that the mass (g) of whiting ingested increased with the porpoise length. For porpoise between 120-140cm of body length that pattern was more precise than to the smaller and bigger porpoises.

5. Discussion

The use of stranded animals has been increasingly considered a useful indicator to approach the biology and ecology of small cetaceans (Meager & Sumpton, 2016). The next chapter will mainly discuss significantly aspects of the variability of harbour porpoise diet who feed in Scottish waters, between 1992-2014.

Firstly, a brief description of the observed characteristics of dead porpoises. Then the second part, it will be about the factors predicted to affect the consumption of herring and whiting by harbour porpoise. Geographical location, seasonality, the biology of predators and the availability of prey are some examples of those factors.

5.1. Characteristics of Harbour porpoises strandings in Scotland

Most of the dietary studies that use stranded animals can give a general idea of the mortality patterns and of the characteristics of the dead animals for a certain region. The present study analysed samples from strandings between 1992-2014 for the Scottish area, and noticed that most of the porpoises stranded between January and June (Figure 4.1b). Many of the causes of those deaths were unclear, but the ones identified included attacks by bottlenose dolphins, physical trauma, bycatch, starvation and pathological conditions. Other researchers, also, showed that exact same period as the one with the highest number of strandings (Santos, 1998), with a peak value in June (Ross & Wilson, 1996). Learmonth *et al.* (2014) observed in more detail that mortality periods varied with porpoise length (e.g. February-June, for individuals 90-130cm; May-August, for individuals ≤90cm).

Porpoise characteristics such as body length, age, maturity can also be associated with the seasonal mortality period of the present study. Considering the study carried out by Learmonth *et al.* (2014) for Scottish waters, porpoise reproduction season occur in November-June with a gestation period of approximately 10-11months, and so it's expected to observe a higher number of neonates from May to August. For example, in the region of Aberdeenshire, a high peak of calves sights was observed in June (Weir *et al.*, 2007). Also the lactation period takes approximately 8 months (Lockyer, 2003), however, some small calves start to eat solid food before ending the weaning (Learmonth *et al.*, 2014). Gannon *et al.* (1998) suggested this as evidence of calves initial forage behaviour. The inexperience of the young animals (e.g. became entrapped in nets (Heath *et al.*, 2017)), and their vulnerability (be killed by other animals (Patterson *et al.*, 1998; Leopold, 2015)) are factors that apparently can increase their early mortality. For example, between March-August, Santos (1998) demonstrated that 41.7% of her sample were juveniles attacked by bottlenose dolphins. Considering all those facts, it's likely that those very small porpoises become vulnerable when they start to forage for the first times, and therefore the first 6 months of the year seem to be very prone to a higher number of deaths. The present study did not have enough information about the age or the age group of the necropsied animals, but it is possible to use body length as a proxy for age as was done by Santos *et al.* (2004), and so further work can be carried out.

Body sizes of the stranded porpoises differ by sex: females had reached bigger sizes than males. The mean body sizes estimated were F=132cm, 34kg and M=125cm, 31kg, sizes similar to the ones observed in other studies (Santos in 1998, F=128cm, 33kg, M=128cm, 34kg; Learmonth *et al.* in 2014, F=128cm, 31kg, M=124cm, 30kg). Comparing the three studies for the Scottish coast, in the end, females not always have a higher body size compared to males. Also, very small individuals were included in the study (e.g. 6.1kg; 86cm), and such small body sizes could be associated with natural dimensions of neonates (77.6-80.5cm, 6.7-8kg, Learmonth *et al.*, 2014), bad health conditions, or an advanced state of decomposition.

The body weight and length were analysed without considering the maturity state of porpoises (or pregnant females). For example, the body length for mature males can range from 135-157cm while immature individuals are smaller (84-130cm), as well as mature females and males are heavier due to the increased mass of the gonads (Lockyer, 2003; Learmonth *et al.*, 2004). However, when the effect of maturity is removed, Santos (1998) concluded that females were bigger than males.

Regarding porpoise sexes, it was registered that the stranded males (n=174) and females (n=147) was not so different. Similarly, other authors detected also small differences between the animal sexes who stranded in Scotland (Santos, 1998; Santos *et al*., 2004; Learmonth *et al*., 2014). In fact, Santos *et al.* (2004) noticed differences in the causes of death between sexes, with males being more vulnerable to bycatch. Bycatch has been considered to be a relevant human threat to harbour porpoise populations, particularly the bottom set gillnets (Parsons *et al.*, 2000; Davies *et al*., 2004; Jefferson *et al.*, 2008; Heath *et al.*, 2017), a type of fishery common for herring and cod which are species forage by porpoises (Davies *et al*., 2004; ICES, 2017).

Geographical differences were also observed with the type of death by other authors. Porpoises apparently have less risk of being bycaught on the west coast of Scotland (OSPAR, 2017). Dolphins attacks have been subject of several studies, and it seems that a higher number of porpoises were dying in the eastern coast of Scotland (Ross & Wilson, 1996; Santos & Pierce, 2003). As a matter of fact, this study also analysed a higher number of porpoise deaths from the east coast compared to the number of carcasses recovered from west of Scotland.

Looking an ordinary map is possible to see that the west of Scotland has a rugged coastline (Hebrides islands) (Scottish Executive Resources, 2003), and so not so accessible to beach patrolling (Sveegaard, 2011). On the other hand, the east coast was considered to be a more favourable feeding ground (Davies *et al*., 2004), which could attract a higher number of animals to that same area. Hammond *et al.* (2017) sighted a higher abundance and density of individuals on the east side of Scottish coast (Figure 1.1).

The availability of prey is also considered to be related with the distribution of porpoises (Smeenk, 1987; Camphuysen, 2004), additionally, there are authors (Silva & Sequeira, 2003) that even defend the probability of oceanographic factors affect the number of strandings, too. The relationship between stranding trends and population dynamics can be often unclear. The use of stranding data as representation of trends or demographic parameters for cetacean populations or communities is still controversial, some don’t trust

(Siebert *et al.,* 2006) while others believe in their ecological significance in case of species monitoring and conservation (Sveegaard, 2011; Learmonth *et al*., 2014; Meager & Sumpton, 2016).

5.2. Variability in the diet of Harbour porpoise

Considering the importance that the predator-prey interactions have in determining the structure and dynamics of species communities (Greenstreet & Tasker, 1996), the diet of the harbour porpoise was studied with a particular focus on the consumption of herring and whiting.

For instance, herring is a fatty fish and porpoises fish them near to the bottom, while whiting is a lean. So, the presence of those two prey in the porpoise diet could be affected individually by different factors. In addition, each prey is distinguished by own characteristics, together they also share common places over their distribution. In fact, the distribution and abundance of one species can affect other species who share the same resources (Greenstreet & Tasker, 1996). So, here it will be discussed the factors that affected herring and whiting as a food choice of harbour porpoises, in Scottish waters.

5.2.1. Interference of prey availability in the diet

Porpoises are predators of many fish species such as whiting, herring, sandeels and gobies (Santos, 1998) but usually the diet consists mainly of a small number of prey (Santos & Pierce, 2003). Therefore, they are considered opportunistic by some authors (Davies *et al.*, 2004; Leopold, 2015) and generally, this means that the prey are ingested as encountered, and this way prey availability can affect the diet (Santos & Pierce, 2003). Previous studies already demonstrated the existence of a relationship between porpoise diet and the abundance a specific prey (e.g. haddock (Santos *et al*., 2005a), sandeel (Evans & Weir, 1996)). With this in mind, it was determined whether the fluctuations in herring and whiting stocks influenced porpoise diet. Surveys taken in the North Sea, that included Scottish waters of the east and western side, showed different trends for whiting and herring within porpoise diet, between 1992-2014.

Any general conclusion resulted from the analysis of the west coast as potential factor affecting diet variability due the lack of an appropriate sample size.

Regarding the east coast, and despite what was expected, the consumption of herring and whiting seemed not to be affected by their abundance in the sea. However, porpoise seemed to eat amounts (g) of whiting coincident with the whiting trend biomass.

Porpoises did not seem to consume herring, given their abundance or biomass at sea, for the period between 1992-2014. Reviewing old studies, porpoise was a predator of herring (Southwell, 1881; Van Beneden, 1889; Rae, 1965, 1973) but nowadays herring seem not to be such a regular choice in the meal (Santos, 1998; Santos *et al*., 2004). Herring stocks have experienced some collapses in the North Sea, between 1964-1978 and in 1994, and at the end of the 20th century (Whitehead, 1985; Maravelias, 1997). SSB had fluctuations from 1997 to 2016, but always above the full reproductive capacity ($SSB>B_{pa}$) (ICES, 2017c). Also, since 2003, its recruitment has been low despite a sustainable fishing mortality ($F<F_{MSY}$) and the large size of the stock (ICES, 2017c). Therefore, it hasn't contributed to the increase in the spawning stock, at least until 2014 (ICES, 2017c). All those local reductions and shifts in herring stock that occur in the east part of Scotland could contribute to the more recent low presence of herring in diet. For example, Santos *et al*. (2004) noticed herring in low proportions in porpoise diet in the 1990s, but even after the recovery of the North Sea herring stock, herring dietary trend did not change. Although, there is a case for Dutch waters, where the authors associate the presence of porpoises with the abundance of herring. Smeenk (1987) said that even with species of low preference such as herring, the number of porpoises decrease simultaneously with the decline of herring stocks. Identically, for the same region, Camphuysen (2004) affirms that with the return of herring an increased number of porpoises was sighted.

The decline of herring in porpoise diet can also reflect the shift interest by porpoises to other species. Hughes (1993) and Spitz *et al*. (2012) said that the diet should be adjusted according to the quality and availability of food. According to Brodie (1995), porpoise small size doesn't let them to store much energy making them more dependent of food. Although, Spitz *et al.* (2012) and Wisniewska *et al.* (2016) estimated high energy needs for porpoises. So, and regarding the optimal foraging theory (Hughes, 1993) it's expected that porpoises will prefer spend their energy with food that fulfill their energetic demands. Herring is a fish with a high calorific value (Leopold, 2015), is fished in big schools (Whitehead, 1985; Camphuysen, 1990; ICES, 2011a) of small individuals (Santos, 1998; Read, 1999; Santos & Pierce, 2003). It's possible that even with those characteristics, herring had been shifted for other species. Possibly, the energy spent by porpoises to forage for herring its less cost effective rather than for lean but bigger fish, such as whiting. Sandeels, for example, also with a small size and being a rich source of energy living in the bottom (Leopold, 2015) could be one of those alternative species. In addition, sandeel was identified as one of the main prey eaten by porpoises, in Scottish waters (Santos *et al*., 2004; Pierce *et al*., 2007; Hammond *et al*., 2013). In Shetland islands (north of Scotland), for instance, Evans & Weir (1996) observed fluctuations in the number of porpoises with the status of local sandeel stocks. In other regions, for example in the eastern North Sea, Skagerrak and Kattegat, where herring is a primary prey for porpoises, their distribution was positively correlated with herring abundance (Sveegaard, 2011).

Similar to herring consumption, the whiting abundance trend wasn't coincident with the whiting diet patterns. Different from herring, it was observed that the consumption of whiting had a negative trend with the biomass of whiting available in the Scottish eastern sea. According to more recent studies, whiting represents a big proportion of porpoise diet in the Scottish waters (Santos *et al*., 2004; Pierce *et al*., 2007; Learmonth *et al*., 2014). For the period between 1992-2014, the present study detected porpoises with preference for whiting rather than for herring, based on a higher consumption (weight and number) of whiting, and a higher number of porpoises stomachs with the presence of whiting compared to herring. Besides whiting often occurrence in porpoise diet, whiting have experienced historical low stock levels (ICES, 2011b, 2017), as similar with what happen to herring. That situation was observed for the east coast in 1998 (ICES, 2011b) and between 2005-2008 (ICES, 2017). The low values of SSB and recruitment could resulted in those low stock levels in the follow years (Figure 4.10, Figure 4.12). Also, SSB has fluctuated between 1992-2014 (ICES, 2017d), and the whiting mortality has been above its MSY (was unsustainable harvested until 2000). As demonstrated by Nash *et al*. (2009) when a higher number of juveniles is fished it's expected to see a low recruitment in the follow years. ICES surveys carried out in the east coast perceived a decrease of recruitment after 2003 (ICES, 2017d). Therefore, it seems that the stock has been exploited in a way that is compromising the ability of the stock to replace itself, implying low spawning sizes in the later years. Under this whiting stock perspective, the study also showed porpoises ate less amount (g) of whiting when whiting biomass increased. An increased amount on the weight of whiting in the east Scottish coast, can also attract other predators competing with porpoises for whiting. In the North Sea, other marine animals and piscivorous fish are potential predators (ICES, 2011a). For example, grey gurnard can cause up to 50% of the predation mortality (whiting age-0) in the north and east side of Scotland (ICES, 2017), exhibiting this way a high repercussion in the whiting population of this area.

Despite the optimal foraging theory (Hughes, 1993), whiting is a low-fat fish (Leopold, 2015), and it's apparently an important prey for porpoises, in Scottish waters. Some authors related whiting availability with periods when other energetic-rich prey are not accessible (Zheng *et al*., 2001; Santos & Pierce, 2003; Santos *et al*., 2004). This could, also, suggest that besides whiting, porpoises could adjust their diet to the abundance of other species (e.g. cod or haddock were, also, important for porpoises in Scotland, Santos, 1998; Santos *et al*., 2004).

5.2.2. Interannual variation in diet

Evidence of interannual variation in the consumption of herring and whiting by porpoises was observed for the period from 1992-2014. The annual diet variation includes the importance of prey and the prey size, which showed different results for herring and whiting.

Herring importance in porpoise diet showed variation with years, but because of a low number of samples after 2005, that trend was not so clear after that year. Also, importance of Clupeidae (sprat, herring) in porpoise diet varied in a study carried out by Santos (1998) and Santos *et al.* (2004), for Scotland. In all three studies, unlike herring, whiting didn't show any annual variation in importance in the diet. Dietary studies for porpoises, in Scottish waters, corroborated with these results as they show that nowadays whiting is still one of the primary prey but herring is uncommon in porpoise stomachs (Rae, 1965, 1973; Santos, 1998; Santos *et al*., 2004). Santos (1998) was more precise in the case of herring variation, and described herring as more important in 1994 than in 1992 or 1993 (in a study from 1992-1996). Herring ICES surveys also showed the variation of herring abundance with the years (ICES, 2017a). Santos *et al.* (2004) mentioned that the differences in the porpoise diet can be associated to different prey abundances in Scotland. Herring had suffering some stock fluctuations over the years (Figure 4.4). For example, on the west coast of Scotland (Firth of Clyde) at the end of the 20th century the stock of herring had collapsed (Whitehead, 1985; Maravelias, 1997; Thurstan & Roberts, 2010), explained by the overexploitation and the destruction of herring habitats by the fishing gears (Thurstan & Roberts, 2010). For instance, the landings in Clyde ports of roundfish (e.g. herring, cod, haddock) declined from 35% in 1993, to 2% in 2008 (Thurstan & Roberts, 2010). The same authors, also, emphasized the fact that long before the 20th century (in the 1870s) herring was the most profitable species in the Firth of Clyde. However, after 2 centuries herring landings begun to reflect its enormous stock decline in the region which might also be reflected in the decreasing importance of herring in porpoises diet. And, if the availability of a common prey declines, this could lead porpoises to shift to other prey species (Santos & Pierce, 2003).

Despite lack of evidence of between-year variation in the presence of whiting in porpoises diet, the number of whiting in the diet varied, over the period 1992-2014. Also, this change, approximately after the year 2000, started to describe a decline in the number of whiting ingested. Additionally, as was seen before, the number of whiting in the diet did not change with whiting stock abundance. With all this in consideration, it's possible to suggest that the consumption in number of whiting by porpoises depends more on porpoise choice than whiting availability, at least for porpoises feeding in the east Scottish coast. So even with low stock levels in Scottish waters (ICES, 2011b, 2017), those seem apparently irrelevant to porpoise diet.

In relation to prey size there wasn't any evidence that whiting size varied in the diet. A similar result was shown by Santos *et al.* (2004) but not by Santos (1998). This latter author described porpoises eating smaller whiting in 1995 compared to the other years. On the contrary, in the present study, the herring mass eaten showed to vary with the years. Unfortunately, due to the small sample size (n=17) this apparently variation of herring is inconclusive, and so any conclusion needs to be carefully done. Although, ICES surveys showed annual variation in herring biomass in Scottish waters (ICES, 2017c). Also, with the age, the mean weight of herring changes (Dickey-Collas, 2004) being biological supported by annual growth variations (Heath *et al.*, 1997). Also, it seems that herring length change with spawning season within the years (Dickey-Collas *et al.*, 2005). Moreover, herring length differences could be associated also to temperature and prey food availability (Fiksen & Folkvord, 1999; Johannessen *et al.*, 2000). In other studies, herring size different results were observed. Santos *et al.* (2004) did not see interannual variation in the size of the Clupeidae eaten by porpoises, in Scottish waters.

5.2.3. Seasonal variation in diet

The importance of herring and whiting in the porpoise diet didn’t vary with the season. However, many studies had showed the opposite for porpoises feeding in Scotland grounds. Santos (1998) showed whiting to be more important in the diet in autumn than in spring. Also, and being sandeels other primary prey for porpoises in the Scottish waters, the higher importance of whiting in autumn (Santos, 1998) could be related to the lower availability of sandeels (Santos & Pierce, 2003). However, this is also supported by the study of Zheng *et al.* (2001). Through surveys, the authors found a decline in whiting during the summer, which was also consistent with whiting abundance trends. Although, the importance of whiting was not evident seasonally, in the present study, the number of whiting in porpoises stomachs had varied, which possibly reflect the seasonally different abundances observed by Zheng *et al.* (2001).

Additionally, Santos (1998) saw that the size of whiting in the diet also changed with season. Porpoises ate smaller whiting in autumn and bigger in spring and summer. A similar result was found in the present study. Here both length and mass of whiting had change in the diet with season. These can be related with the migration pattern observed by different age-classes of whiting, older whiting seem to prefer offshore waters and whiting <1yr live inshore (Zheng *et al.*, 2001). As expected, also the mass of herring eaten varied in porpoise diet with season. Unfortunately, due to the small sample size (n=17) the observed trend is inconclusive. However, this trend could be related with the body changes during herring life cycle (Winters & Wheeler, 1996; Heath *et al.*, 1997). For instance, previous studies showed that herring grow faster in spring than in autumn (Fiksen & Folkvord, 1999). It could also be related with herring seasonal inshore-offshore migrations (Maravelias, 2001; ICES, 2011a). Herring is a spring and autumn spawner (ICES, 2011a), so, at least, two times a year herring body size changes due to the increase of gonads weight (Winters & Wheeler, 1996). Spawning occurs in coastal waters (Nash *et al.*, 2009; ICES, 2011a), and as a nursery area offsprings stay approximately 2 years in shallow waters before they migrate as juveniles (ICES, 2011a, 2017e). As porpoises often harvest in shallow waters (SCANS-II, 2008), the consumption of herring in different maturity stages (immature=25.2g, mature=151.3g, ICES, 2017b) can possibly contribute to diet variation with season. This also can reflect that even an analysis with a small sample size, a seasonal small variation in the prey size in the diet could be detected.

5.2.4. Geographical variation in diet

Univariate analysis described the variability in the diet for Scottish coast. For the period between 1992 to 2014, porpoises, feeding in Scottish coastal waters, showed that the presence of the herring and whiting in their stomachs varied with the feeding location.

Besides whiting and herring, other previous dietary studies had described other fish species and cephalopods in porpoises diet (Santos, 1998; Santos *et al.*, 2004). For Scotland, the research carried out between 1992-2003 described porpoises with high preference for whiting (51.1%), sandeels (48.9%) and Sepiolidae (33.5%) while herring (9%) was not so often on their diet (Santos *et al.*, 2004). They even found different species preferences between places, in the north (Shetland) haddock/saithe/pollock were more numerous than in the diet of east porpoises. In the east and north coast sandeels were a more important prey than in the western coast. For the west coast, they noted that it was whiting and *Trisopterus* spp. that were more common in porpoise diet. Those evidences suggest that porpoises prey will change with the feeding ground. So, even if a species is a primary prey, as was observed for whiting in the west, it's possible that whiting rank in porpoise diet it will change due to the presence of another important species in other location. Also, herring could be less important in one feeding ground than in another, despite the fact that, in general, it is a species of low importance for porpoises, as was supported by the high number of stomachs without herring that was observed for both coasts. Although, the presence of herring change with geographical location, which suggests, that variations in the diet could be detectable even for relatively less often prey in the diet.

The models also predicted that porpoise ate different amounts (in number) of whiting considering porpoises feeding sites. It was already described that the abundance of whiting did not contribute to the variation in the number of whiting observed in the diet. So, it suggests it was not the different amounts of whiting in the different feeding places that reflected the significant relationship observed between number of whiting in the diet and geographical location.

The consumption of a high or low number of whiting could be related with many factors such as porpoise energetic demands, prey-catching abilities or feeding ground characteristics.

For example, if a place is a nursery/breeding area, so it’s likely that pregnant females and the ones in lactating period will eat more, as it was confirmed by Gannon *et al*. (1998). Also, this author suggested that calves learn to forage for food usually targeting small sizes even before the weaning. This learning process can also happen in more protected area, and this way contributes, probably in small amounts, to the number of whiting predated in a specific area. The identification of feeding and nursery/breeding grounds were recognised to be important in the conservation (SACs) and protection of a species (Marine Protected Areas - MAPs), for porpoises this has been implemented under the EU Habitats Directive (92/43/EEC) (Weir *et al*., 2007; Embling *et al*., 2010). Recently, in 2016 the Inner Hebrides and the Minches (west of Scotland) were submitted to the European Commission as a candidate for Special Area of Conservation (SAC) for harbour porpoise (Embling *et al*., 2010; SNH, 2016).

Differences in the topographic Scottish coast can, also, provide easier food for porpoises. Knowing that whiting, considered a demersal fish (ICES, 2017), migrates in the water column to feed (Rindorf, 2003), shallow waters and more closed areas can facilitate whiting to be fished by porpoises. For example, Marubini *et al.* (2009) studied habitat preferences, for the northwest of Scotland, a region with a coastline with a series of sea fjordic sea lochs. The authors predicted a significant relationship between topographical variables (e.g. bottom topography, sea state, tide height) and the distribution of porpoises, who prefer water that extends for 15km from the shore and depth between 50m-150m.

Additionally, the quality of whiting can be different from place to place which could lead a certain area to be a favourable feeding ground. For instance, older whiting was observed offshore while younger juveniles where sited near Scottish coast (Zheng *et al.*, 2001), and as well-known porpoises prefer to forage in shallow waters (Rae, 1965). However, migrations to specific regions just for feeding are well known among other marine mammals (e.g. whales, dolphins) (Spitz *et al.*, 2012). In the west of Scotland (Hebrides), porpoises were sighted travelling between feeding grounds (Embling *et al.*, 2010). Porpoises forage strategies are mainly determined by the quality of prey rather than its quantity (Spitz *et al.*, 2012). For example, for Scottish coastal waters, Santos *et al*. (2004) estimated size of whiting eaten by porpoises, for three different geographical places, and they showed that bigger whiting was eaten in the north than in the west.

The present study did not show evidence that porpoises feed on different sizes (mass or length) of herring and whiting for the Scottish coasts. However, even not statistically evident, porpoises ate whiting and herring with different size ranges for each geographical location. The porpoises sampled here ate slightly bigger whiting in north and east coast, and bigger herring in the north compared to east side. In a general view, those porpoises ate whiting bigger (1.22-35.6cm, up to 227.2g) than herring (4.82-29.6cm, up to 110g). For example, larger herring seems to occur more offshore (Maravelias, 2001) while porpoises feed more often in coastal waters (Rae, 1965; Santos *et al.*, 2004). So, porpoises seem not to follow the big fish, as they apparently prefer small fish sizes (Santos, 1998; Read, 1999; Santos & Pierce, 2003). Santos *et al.* (2004) also estimated that porpoises ate smaller herring (29cm) than whiting (35.5cm). This was expectable for those two species as surveys carried out in Scottish waters showed that whiting (15-53cm, 54-419g) was larger and bigger than herring (8.4-30cm, 4.6-236.4g) (Wheeler, 1969; ICES, 2017b). From this, it's possible to see that porpoises feed in herring and whiting smaller/lighter than the ones caught by fishermen.

In addition, the differences in prey size are not so simple as describing them as for east, west or north of Scotland. Oceanographic conditions (e.g. SST) and the availability of food were demonstrated to be also determinant in the distribution of fish species as this could affect their development (Heath *et al.*, 1997; Zheng *et al.*, 2001).

As the relation predator-prey is linked (Greenstreet & Tasker, 1996), changes in prey distribution or abundance could also be reflected in the diet of harbour porpoise.

5.2.5. Ontogenic variation in diet

For Scottish waters, evidence of variation in the diet related to body size but not with porpoise sex. As a general result, bigger porpoises ate bigger whiting. For herring a small variability with porpoise length was suggested by dietary data, but not quite statistically significant, reflecting the small sample size (<50) of the estimated herring length eaten by porpoises.

Although the statistical analysis was inconclusive, it's likely that bigger porpoises eat large (mm) herring as well as bigger (g) herring. For whiting, bigger porpoises prefer to eat big (g) and large (mm) individuals, too. For both prey species, the previous trend did not describe the smallest and the biggest porpoises due to the lack of precision of the 95% CL, reflecting small sample sizes for very small and very large porpoise individuals.

Knowing that energy requirements and foraging experience differ between ages (Leopold, 2015), and in general age can be related to the length of the body, it was expected to see variations in the diet with porpoises body length. Such an age-length relationship was described by Learmonth *et al*. (2014), for porpoises from Scottish waters. Newborns (0-2mo. old) had a body length between 77.6-80.5cm, young porpoises (1-7yr) ranged from 119-153cm and adults (6-20yr) body length was from 135 and 173cm. A more detailed study developed by Santos (1998) and Santos *et al.* (2004) demonstrated those differences in the diet of adults and young individuals, in Scotland. Juvenile harbour porpoises ate smaller whiting than did adult (Santos, 1998), gobies were more important for porpoises with ≤ 118cm and medium size porpoises preferred more clupeids than large and small porpoises individuals (Santos *et al*., 2004). Also, young porpoises preferred *Trisopterus* spp., while older individuals showed preference for sandeels (Santos, 1998). Santos (1998) and Santos *et al*. (2005) referred to similar observations for Dutch waters, where adults ate bigger sandeels and gobies than juveniles while smaller porpoises ate more gobies than did bigger porpoises, and for Danish waters where adults ate smaller blenny and whiting than juveniles. A similar result was observed for calves in the Northwest of Atlantic (Gulf of Maine), where the proportion of prey types and sizes of prey differed also from those of adults (Gannon *et al*., 1998). Some authors considered those differences to possibly be related with feeding grounds (Santos, 1998), or experience in prey-catching (Gannon *et al*., 1998). For example, calves (<1yr) seemed to prefer gobies and shrimps (Santos *et al*., 2004); these small prey captured could reflect the first steps in foraging before calves shift to big prey (Smith & Read, 1992; Gannon *et al*., 1998). In addition, the digestive tract structure of toothed cetaceans has been suggested to limit the size of prey they can ingest (MacLeod *et al*., 2007). So, the small body size of porpoise can also be other explanation to the preference for small size prey and additionally for diet prey length variation with porpoises body size.

For the Scottish waters, this study did not detect variation in the diet related to porpoise sex. But as a matter of fact, females ingested the biggest sizes of herring and whiting, while males ate the high amount for those two species.

Variability in the diet with sexes was observed by other authors for Scotland, despite the models from the present study did not show it. Adult males prefer bigger whiting, and feed on a higher variety of prey compared to females (Santos, 1998; Santos & Pierce, 2003). This can reflect different feeding grounds or less diet selection by male porpoises

(Santos & Pierce, 2003). Additionally, it has been suggested that the daily food consumption is calculated in function of the body weight (3.5% by Yasui & Gaskin, 1986; individuals ≤50kg, 8% of the body weight by Santos *et al.*, 2005). Considering that, pregnant and lactating females will require higher energy (Yasui & Gaskin, 1986; Learmonth *et al.*, 2014) and so diet differences will be expectable. In fact, it's possible that females nursing will adapt their food choices when forage with calves (Smith & Read, 1992). Also, males body weight change during maturity stages (e.g. active mature males, testis mass range between 1.3% to 6.8% of body mass, immature, 0.25%, and, pubescent and resting mature males, from 0.5%-1.1%) (Learmonth *et al.*, 2014). In other regions, like Denmark and Holland, also, a few differences were found between sexes (Santos, 1998). In the Northwest of Atlantic, in Gulf of Maine (Gannon *et al.*, 1998) and Gulf of St. Lawrence (Fontaine *et al.*, 1994) no variability in the diet was found between the diets of male and female porpoises.

6. Conclusion

The general conclusions here suggested refer to harbour porpoises feeding in Scottish waters during 1992-2014.

- Stranded porpoises can be a good source of diet information, when the sample size is representative of the population in the region. In a general way, stranded porpoises physical characteristics (e.g. body size) but not porpoise sex, seem to be similar among stranded animals in space and time.
- Porpoises are an important predator of whiting in the east coast of Scotland.
- Consumption of herring or whiting was independent of porpoise sex, but not on porpoise length. Bigger porpoises preferred to eat bigger prey.
- Whiting was a consistent important prey for harbour porpoises over the years, with a decline in the amount ingested after, approximately, the year 2000.
- Herring importance has still remained low in the diet of these cetaceans.
- Porpoises ingest different amounts of whiting and herring when they fed in different places, likely relating to characteristics of the feeding ground.
- Variation in the diet of porpoise may follow the abundance of preferred prey in the sea but does not follow the abundance of other prey.

- Season had an important role determining the quantity and quality (size of prey) of herring and whiting, likely relating to prey biology and to fishery exploitation.

In conclusion, our current knowledge of the drivers of harbour porpoise diet, with particular reference to consumption of Atlantic herring and whiting is still incomplete but the present study provides some new insights. Important to point out, that some factors in the diet variation were seen to be transversal to the prey species while others did not. As a prey-predator relationship is always present, porpoise diet can shift not only due to its food choices but also due to factors directly related with prey.

7. Future work

Several topics for future work are suggested here:

1. Noting that the number of stomachs available was very low in some years, it may be useful to search for additional stomachs held in storage by SMASS, especially for porpoises stranded in the more recent years (2015-2017). It is important to collect enough samples to make future results more reliable and representative of the population as a whole.
2. To estimate the fish length and weight, if available in the future, to use regressions derived from fish caught in the area of study (Santos, 1998), and also regressions for each month of the year, because their body weight changes during their life cycle (especially during spawning time) (Eggers *et al*., 2014).
3. New statistical analysis, using GAMs to predict single variable effects. As an example, it would be useful to study the spatial variability in the diet in more detail (for east, north and west coasts). Biological and environmental variables should be considered within each area and period of time.
4. To expand this study to other harbour porpoise prey (e.g. cod, cephalopods, etc), and to test other variables (e.g. porpoise weight, age, maturity state, four seasons, etc) which can affect the variability of the diet. Also, a new study can include not only the analysis of stomach contents but also the whole digestive tract. The analysis of compound specific stable isotopes will additionally provide information due to the relationship between the isotopic composition of predator and their prey (isotopes of $^{13}C/^{12}C$, $^{15}N/^{14}N$), of a dietary history of individuals

over different time periods (Pierce *et al.*, 2004; Mendes *et al.*, 2007; EMD, 2011). Therefore, with these technical approaches will be possible to have a dietary history of porpoises over different time periods for a more complete diet knowledge.

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APPENDIX

REGRESSIONS FOR ESTIMATION OF FISH SIZE

Regression equations used to predict fish lengths (mm) and weights (g) from otolith, premaxilla and dentary measurements (Figure 3.2). Data from Härkönen (1986), Brown (unpbl. data), Hislop (unpbl. data), Watt *et al.* (1997) and Hernandez-Milian (unpublish). Combine regressions use data from more than one species (*).

<u>Otolith</u> - Härkönen (1986), Brown (unpbl. data), Hislop (unpbl. data)

(a) $FW= 4.910 * OW^{5.193}$ $r^2=0.845$ (Herring) Härkönen (1986)

(b) $FL=-87.490+184.390*OW^{1}$ $r^2=0.790$ (Herring) Härkönen (1986)

*(c) $FW=7.509*OW^{4.437}$ (Herring/Sprat) Härkönen (1986)

*(d) $FL=-40.271+152.071*OW$ (Herring/Sprat) Härkönen (1986)

*(e) $FL=-40.271+152.071*OW^{1}$ (Clupeidae) Härkönen (1986)

*(f) $Fw=7.50981*OW^{4.4371}$ (Clupeidae) Härkönen (1986)

(g) $\log FW= -1.89907 + 3.5375*\log OL$ n=55 (Whiting) Härkönen (1986)

(h) $FL = -4.87+19.621*OL$ (Whiting) Hislop (unpbl.)

(i) $\log FW=-0.11556+3.72252*\log OW$ (Whiting) Brown (unpbl.)

(j) $FL=-88.550+85.390*OW^{1}$ (Whiting) Hislop (unpbl.)

*(k) $FL=0.5+20.448*OL^{1}$ (Whit./BLW) Härkönen (1986)

*(l) $FW=0.011983*OL^{3.61130}$ (Whit./BLW) Härkönen (1986)

From above: FL= fish length, FW= fish weight, OW= otolith width, OL= otolith length, n=sample size, r^2= Pearson correlation coefficient.

<u>Premaxillae</u> - Watt *et al.* (1997)

(m) $\ln TL= 3.4183+1.1664*\ln PMXL$ $r^2=0.969$ (Herring)

(n) $\ln TL= 4.2107+0.9822*\ln PMXAH$ $r^2=0.990$ (Whiting)

(o) $\ln TL= 2.6165+0.9954*\ln PMXL$ $r^2=0.984$ (Whiting)

From the above equations: TL= total length, r^2=Pearson correlation coefficient; PMXAH – height of articular process, “*from the highest point of the articular process to the ventral surface of the ramus*”; PMXL – total length of premaxilla, “*from the most anterior point of the bone to the most posterior*”.

<u>Dentary</u> - Hernandez-Milian (unpublish)

(p) TL= -32.3+12.4*S-VL $r^2=0.989$ (Herring)

(q) TL= 12.34+9.02*S-VL $r^2=0.986$ (Whiting)

From the previous regressions: TL= total length, r^2=Pearson correlation coefficient, S-VL – total length of dentary, of the ventral side, from the most anterior point of the symphysis to the most posterior one.

ANNEX

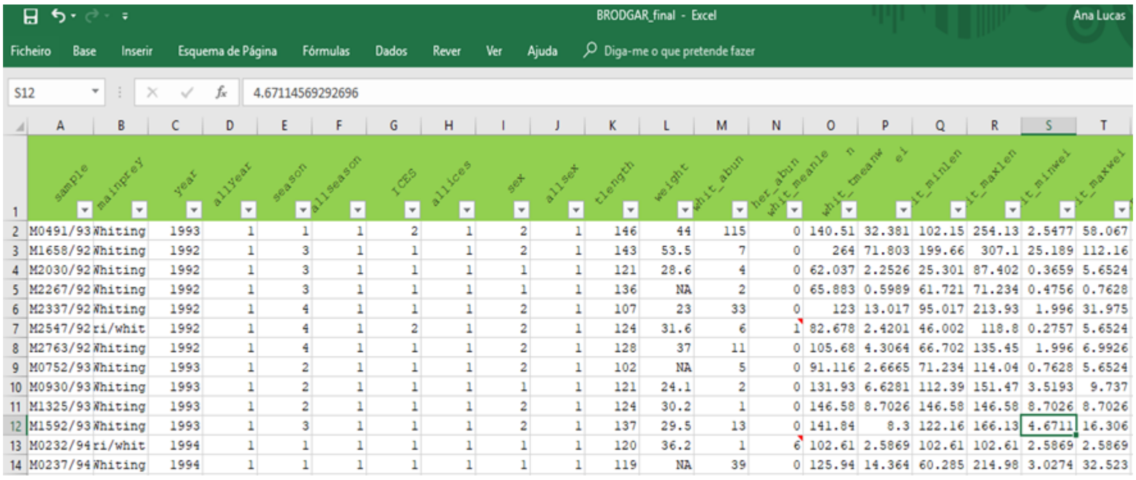
EXAMPLES OF STATISTICAL ANALYSIS USING WHITING DATA

Index of Figures

Before to start importing the data to the statistical analysis programmes and to do the data exploration, some data changes aspects and previous work has been done in the Excel file.

The construction of the Excel spreadsheet had its specifications in order to import data to Brodgar and R Studio. Each head column and row were labelled shortly, differently and without spaces with `Courier` letter (Figure I 1- Partial data imported for statistical analysis

No empty cells were left behind and missing values were replaced by NA (NA cells are automatically transformed in the value 999 by the Brodgar software).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	sample	mainprey	year	allyear	season	allseason	ICES	allices	sex	allsex	tlength	weight	whit_abun	her_abun	whit_meanlen	whit_tmeanwei	it_minlen	it_maxlen	it_minwei	it_maxwei
2	M0491/93	Whiting	1993	1	1	1	2	1	2	1	146	44	115	0	140.51	32.381	102.15	254.13	2.5477	58.067
3	M1658/92	Whiting	1992	1	3	1	1	1	2	1	143	53.5	7	0	264	71.803	199.66	307.1	25.189	112.16
4	M2030/92	Whiting	1992	1	3	1	1	1	1	1	121	28.6	4	0	62.037	2.2526	25.301	87.402	0.3659	5.6524
5	M2267/92	Whiting	1992	1	3	1	1	1	1	1	136	NA	2	0	65.883	0.5989	61.721	71.234	0.4756	0.7628
6	M2337/92	Whiting	1992	1	4	1	1	1	2	1	107	23	33	0	123	13.017	95.017	213.93	1.996	31.975
7	M2547/92	ri/whit	1992	1	4	1	2	1	2	1	124	31.6	6	1	82.678	2.4201	46.002	118.8	0.2757	5.6524
8	M2763/92	Whiting	1992	1	4	1	1	1	2	1	128	37	11	0	105.68	4.3064	66.702	135.45	1.996	6.9926
9	M0752/93	Whiting	1993	1	2	1	1	1	2	1	102	NA	5	0	91.116	2.6665	71.234	114.04	0.7628	5.6524
10	M0930/93	Whiting	1993	1	2	1	1	1	1	1	121	24.1	2	0	131.93	6.6281	112.39	151.47	3.5193	9.737
11	M1325/93	Whiting	1993	1	2	1	1	1	2	1	124	30.2	1	0	146.58	8.7026	146.58	146.58	8.7026	8.7026
12	M1592/93	Whiting	1993	1	3	1	1	1	2	1	137	29.5	13	0	141.84	8.3	122.16	166.13	4.6711	16.306
13	M0232/94	ri/whit	1994	1	1	1	1	1	1	1	120	36.2	1	6	102.61	2.5869	102.61	102.61	2.5869	2.5869
14	M0237/94	Whiting	1994	1	1	1	1	1	1	1	119	NA	39	0	125.94	14.364	60.285	214.98	3.0274	32.523

Figure I 1- Partial data imported for statistical analysis
Illustration of part of the abundance and biomass spreadsheet in Excel, that was imported to Brodgar.

Brodgar only recognizes numbers and not letters, and so the variables ICES area, sex and season needed to be transformed. The categorical variables in the end were ICES area:1=4a, 2=4b, 3=6a, sex:1=Female, 2=Male, 3=Unidentified, and season:1=Jan., Feb., Mar.; 2= Apr., May, Jun.; 3=Jul., Aug., Sept.; 4=Oct., Nov., Dec.. Only, for the exploration of the type of distribution, those same variables were even compiled if they were only one variable (e.g. all years=1, all seasons=1), as it will be explained later on text.

- Brodgar software

To show step by step, how was done the statistical analysis using Brodgar, it was chosen the whiting data-series.

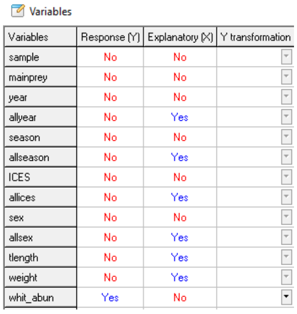
Initially, it was followed the instructions in http://www.brodgar.com/index.php/download and it ended with the download of the Brogdar software version 2.7.5 (www.brodgar.com/setup275.exe).

Figure I 2- Brodgar version 2.7.5. interface

Brodgar's manual (2000) helped to manage the tools to do the statistical analysis, gather with Zuur *et al* (2007, 2010) publications.

To start, the box for decimal separator was set as a dot (12345.12), then data was imported from an Excel spreadsheet, and using Brodgar data viewer the data was checked if it has been imported correctly. In this case, all numbers were checked if they had "." instead of ",", if there were any empty cells, and if the values and headers were in their right column and row. To correct this step, for the whiting data analysis it was taken seven attempts, going back and forward to the Excel spreadsheet to solve the problems (e.g. a completely empty row that was in the middle of the data, a sample with the values in the wrong columns).

After a cleaning data table, the variables were divided as response (Y) or explanatory (X), selecting YES or No for each one (Figure I 3), and the file was saved as "whitingabundance7.brd", and the data imported process was finished.



Variables

Variables	Response (Y)	Explanatory (X)	Y transformation
sample	No	No	
mainprey	No	No	
year	No	No	
allyear	No	Yes	
season	No	No	
allseason	No	Yes	
ICES	No	No	
allices	No	Yes	
sex	No	No	
allsex	No	Yes	
tlength	No	Yes	
weight	No	Yes	
whit_abun	Yes	No	

Figure I 3- Variables and Transformations window, in Brodgar.

In the exploration phase, it was observed the way as the response variable, “whit_abund”, behave with the explanatory variables “allyear”, “allseason”, “allsex”, “allices”, “tlength” and “weight”.

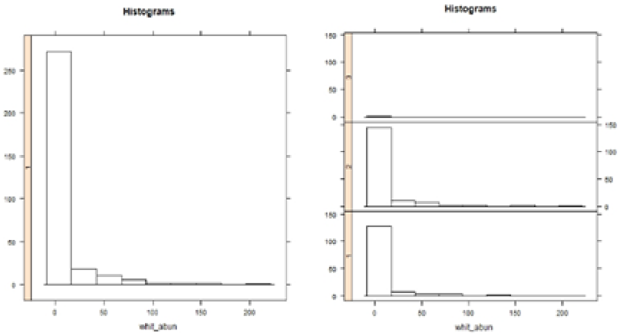


Figure I 4- Distribution data histogram.
Left: Distribution of the number of whiting eaten considering harbour porpoise sex as one variable (females+males+unidentified=1). Right: Distribution of the number of whiting eaten considering harbour porpoise sexes as separately variables, (females=1, males=2, unidentified=3).

The explanatory variables were used first as they were a single variable, e.g. “allsex” (all sexes were compiled in only one sex), as shown in the left Figure I 4. This provided an easier graph to interpret the type of whiting distribution when compared to the many graphics from Figure I 4 at the right. This was the only reason to compile the explanatory variables, “allyear”, “allseason”, “allsex” and “allices”. After this analyse, the original explanatory variables, “sex”, “ICES”, “season” and “year” were selected with YES, and the others with NO, at the Brodgar data viewer in “Variables and Transformations”. Every time the data was changed, it was needed to save the changes and finish the data import process.

Go forward in the exploration process, different graphics were plotted to search for outliers, normality, homogeneity of variances, correlation and variance inflation factors (VIF).

Through the previous histograms of all the explanatory variables a Poisson distribution for the number of whiting found in the stomachs was identified (left Figure I 4, example for the sex variable), and a Q-Q plot confirmed the inexistence of a Normal distribution (the points do not lie on a straight line) (Figure I 5).

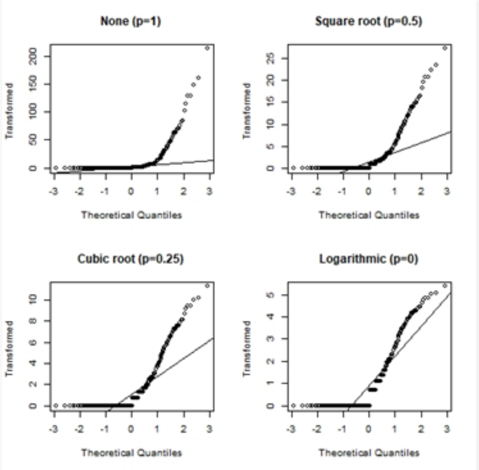


Figure I 5- Distribution data Q-Q plot.
Q-Q plot with a non-Normal distribution for the whiting abundance in harbour porpoise's stomachs.

For the search of outliers, it was preferentially plotted a Dotplot graph instead of a Boxplot, especially because of the high number of variables.

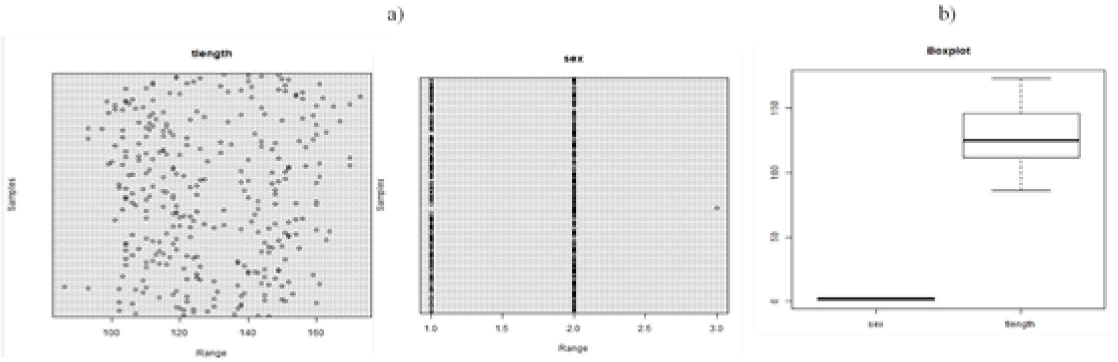
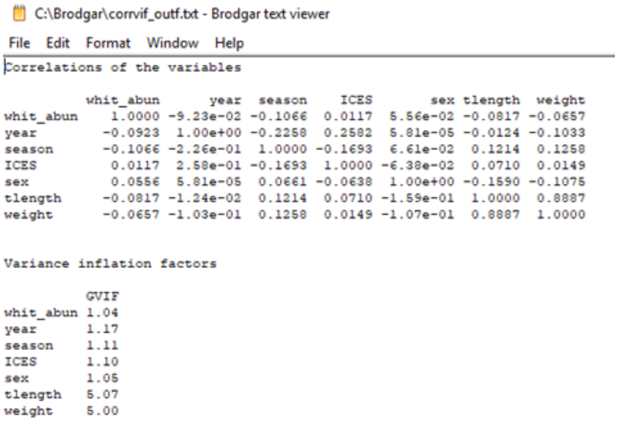


Figure I 6- Distribution data Dotplot and Boxplot.
a) Explanatory variables "tlength" and "sex" given in separately windows by Dotplot. b) Explanatory variables "tlength" and "sex" whiskers-boxes given together by Boxplot.

The Dotplot made the interpretation easier than to plot one variable each time to have readable whiskers-boxes, as is possible to see for the same two variables in Figure I 6. Selecting all the variables at once, the Dotplot resulted in separately windows. The same technique with Boxplot resulted in only one window, making it very hard to interpret, as in Figure I 6b).

The variables “tlenght” (Figure I 6a)), “weight”, “whit_abund” showed outliers, and those low and high extreme values were checked in the Excel file, and at the end they were all biological acceptable.

After, it was tested the existence of correlation between covariates and their VIF. It was chosen the tool “Correlation and VIF” which resulted in the followed values (Figure I 7).



```
Correlations of the variables

          whit_abun      year  season    ICES       sex tlength  weight
whit_abun    1.0000 -9.23e-02 -0.1066  0.0117  5.56e-02 -0.0817 -0.0657
year        -0.0923  1.00e+00 -0.2258  0.2582  5.81e-05 -0.0124 -0.1033
season      -0.1066 -2.26e-01  1.0000 -0.1693  6.61e-02  0.1214  0.1258
ICES         0.0117  2.58e-01 -0.1693  1.0000 -6.38e-02  0.0710  0.0149
sex          0.0556  5.81e-05  0.0661 -0.0638  1.00e+00 -0.1590 -0.1075
tlength     -0.0817 -1.24e-02  0.1214  0.0710 -1.59e-01  1.0000  0.8887
weight      -0.0657 -1.03e-01  0.1258  0.0149 -1.07e-01  0.8887  1.0000


Variance inflation factors

          GVIF
whit_abun 1.04
year      1.17
season    1.11
ICES      1.10
sex       1.05
tlength   5.07
weight    5.00
```

Figure I 7- Output for correlation and variance inflation factors
Output of the correlation and GVIF for whiting abundance.

As the Figure I 7 showed the GVIF (Generalized Variance Inflation Factor) for length and porpoise’s weight were >3 and for the other variables was lower than 3. With a GVIF>3 and a correlation=0.8887 (≈1), between length and porpoise’s weight it was demonstrated a high correlation (collinearity). The other variables showed a r<0.3, so low correlation between them. A Pairplot was even created to confirm the same aspects in a different view (Figure I 8).

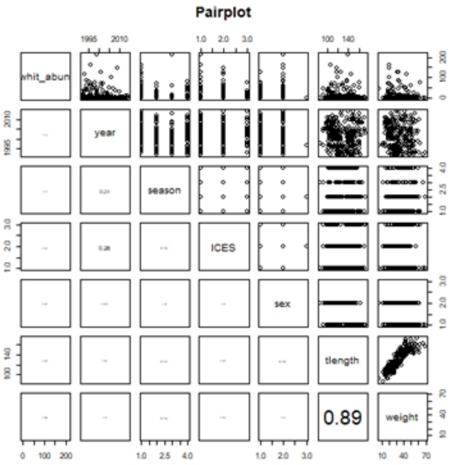
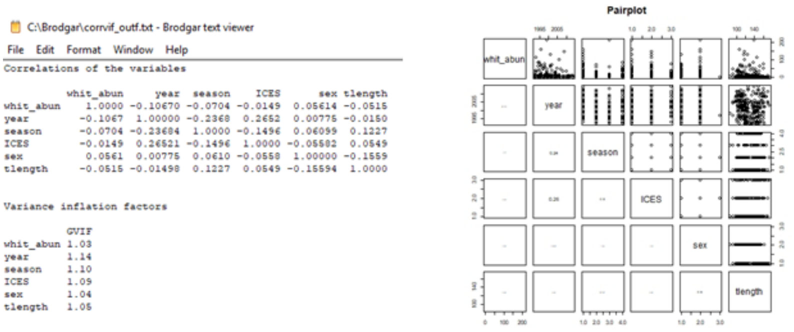


Figure I 8- Pairplot and Scatterplot for the data.
Pairplot for the number of whiting in the diet vs all the explanatory variables (year, season, ICES area, porpoise sex, porpoise length, porpoise weight). The lower diagonal part of the pairplot shows the correlation coefficient in which the font size is proportional to the value of the correlation. The upper diagonal shows the scatterplots.

In the Pairplot, bigger the font size of the number so higher will be the correlation between the variables. Variables “tlength” and “weight” showed a high correlation number (=0.89) (Figure I 8), also confirmed before by the output in the Figure I 7.

It was removed the covariate variable one by one (weight and length) to see which one gave a VIF <3, which resulted in both of them. However, the variable length was chosen instead of the weight, because it made more sense to study the relation between the size of the porpoises and the number of whiting eaten by them. Even, the “weight” variable was the one with more NA data, too. So, the variable “weight” was selected as NO, at the Brodgar data viewer in “Variables and Transformations”.



```
Correlations of the variables

           whit_abun      year  season    ICES      sex tlength
whit_abun     1.0000 -0.10670 -0.0704 -0.0149  0.05614 -0.0515
year         -0.1067  1.00000 -0.2368  0.2652  0.00775 -0.0150
season       -0.0704 -0.23684  1.0000 -0.1496  0.06099  0.1227
ICES         -0.0149  0.26521 -0.1496  1.0000 -0.05582  0.0549
sex           0.0561  0.00775  0.0610 -0.0558  1.00000 -0.1559
tlength      -0.0515 -0.01498  0.1227  0.0549 -0.15594  1.0000


Variance inflation factors

          GVIF
whit_abun 1.03
year      1.14
season    1.10
ICES      1.09
sex       1.04
tlength   1.05
```

Figure I 9- Output and pairplot for correlation.
Output without the variable “weight” and the correspondent Pairplot.

In the end, the variable “tlength” presented a GVIF=1.05 and a non-high correlation with the other variables (Figure I 9).

At this point, it was resulted as an explanatory variable the number of whiting, and as response variables the year (1992-2014), season (1,2,3,4), ICES (1,2,3), sex (1,2,3) and porpoise’s length (cm). The next step, it was to go to UNIVARIATE section and to create the models (GAMs).

As demonstrated by histograms and Q-Q plots (Figure I 4, Figure I 5) it was a Poisson distribution. It was selected a log link function, as a specify smoothers “year” and “tlength”, as nominal variables “sex”, “season” and “ICES”, and as response variable “whit_abun”, and the model was run. Several graphics resulted as Figure I 10 shows.

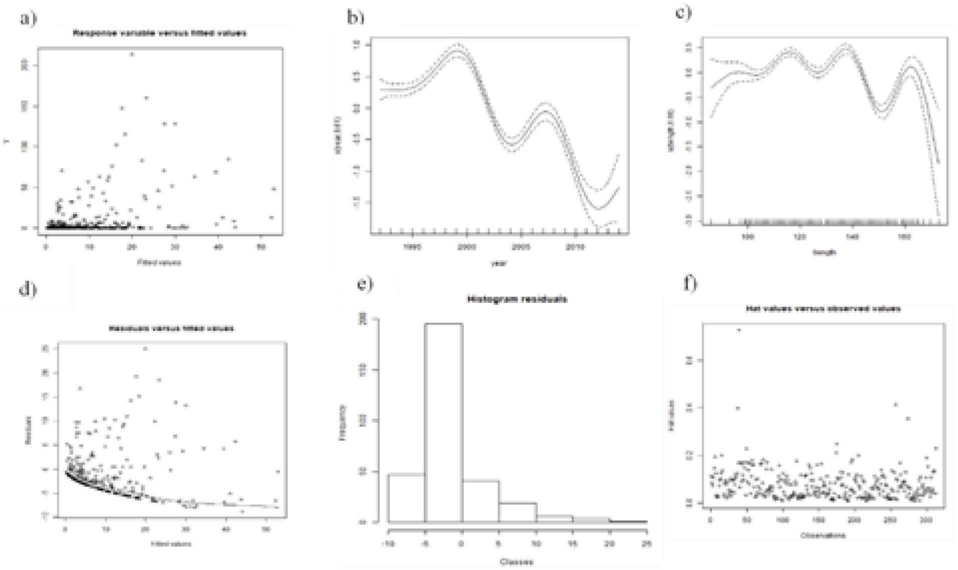
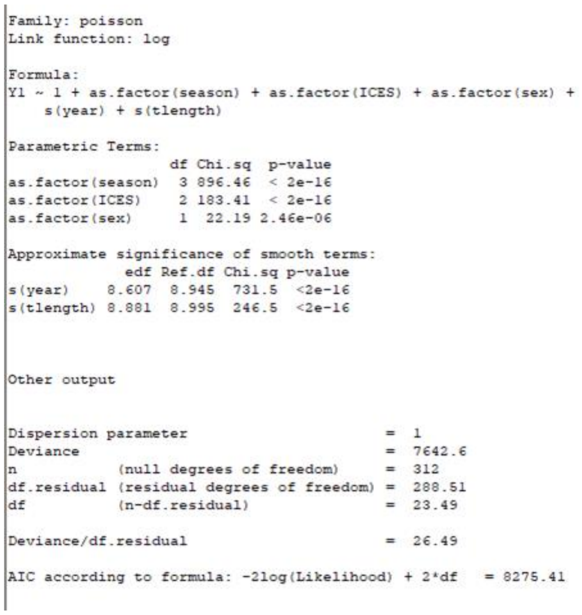


Figure I 10- Brodgar graphics for a Poisson model.
Some of the graphics resultant after plotted a GAM with a Poisson distribution with a log link, for the number of whiting found in harbour porpoise’s stomachs.

From the Figure I 10, the b) and c) suggested a possible fluctuation of the number of whiting with the years (which seemed biological acceptable) and with porpoise’s length, in d) with the residual versus fitted values the interpretation wasn’t clear, from e) the histogram showed a Poisson distribution very skewed at the right side, and in f) it seemed to exist influencers but no (high values <0.70).

As complement for the analysis the numerical output demonstrated a deviance/df.residual equals to 26.49, as shown in the next Figure I 11.



```
Family: poisson
Link function: log

Formula:
Y1 ~ 1 + as.factor(season) + as.factor(ICES) + as.factor(sex) +
    s(year) + s(tlength)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(season)  3 896.46  < 2e-16
as.factor(ICES)    2 183.41  < 2e-16
as.factor(sex)     1  22.19 2.46e-06

Approximate significance of smooth terms:
            edf Ref.df Chi.sq p-value
s(year)    8.607  8.945  731.5  <2e-16
s(tlength) 8.881  8.995  246.5  <2e-16



Other output


Dispersion parameter                        =  1
Deviance                                    =  7642.6
n           (null degrees of freedom)       =  312
df.residual (residual degrees of freedom) =  288.51
df          (n-df.residual)                 =  23.49

Deviance/df.residual                        =  26.49

AIC according to formula: -2log(Likelihood) + 2*df   = 8275.41
```

Figure I 11- Numerical output for data analysis.
Numerical output for the Poisson model with a log link, for the number of whiting n the stomachs.

With a deviance/df. residual higher than 1 and a very right skewed distribution, the next step it was to try a Negative Binomial model.

A Negative Binomial with a log link function, with a Theta between 1 to 10, with the exactly same explanatory and response variables set for the Poisson model, it was plotted and run. However, Brodgar did not let to go forward with this model. So, it was changed the Theta value for 1, and it worked.

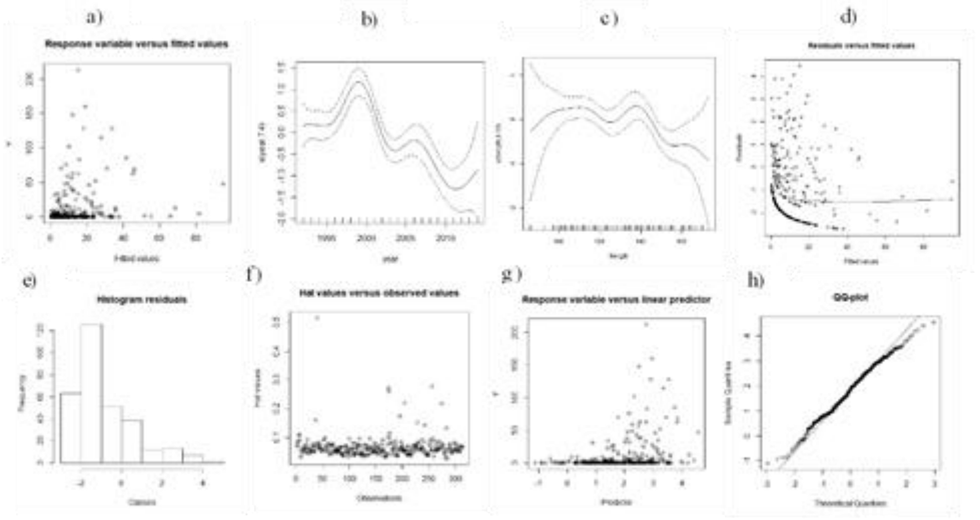


Figure I 12- Brodgar graphics for a Negative Binomial model.

Some of the graphics plotted for a Negative Binomial model with a log link and Theta=1, for the number of whiting found in the stomachs of harbour porpoises.

From the Figure I 12, the graph b) suggested a possible fluctuation with the number of whiting eaten along the years, which seemed biological acceptable. The graph c) showed that the number of whiting eaten by porpoises went up and down based on their length, so it was applied to the smoother "tlength" a change to its base dimension (k), from default to the value of 4. The result it will be showed further on. In d) with the residual versus fitted values the interpretation wasn't clear, but it was possible to see again the presence of outliers, which were considered from the beginning. Histogram e) showed a distribution slightly less right skewed compared with the histogram of the Poisson model (Figure I 10e)). In graph f) it seemed to exist influencers but they were not real because the values stayed near 0.5, and h) demonstrated a non-Normal distribution.

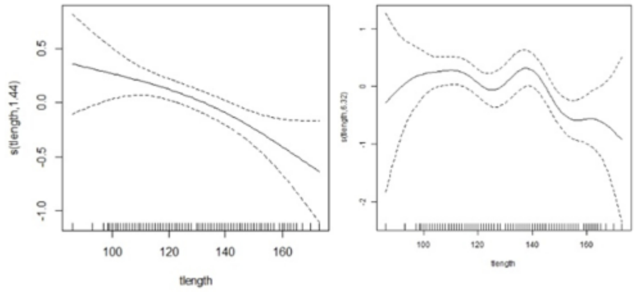
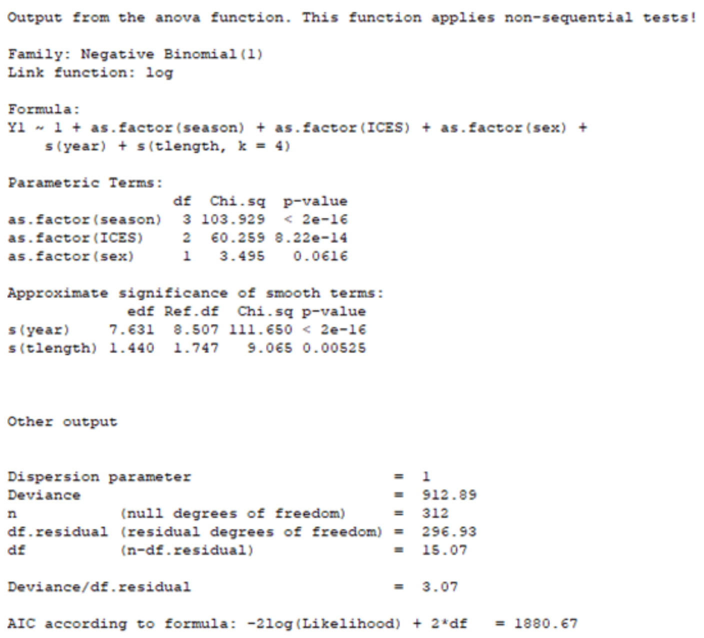


Figure I 13- Smoother graphics for a GAM.
Comparison of "tlength" smoothers: left - "tlength" with k=4, right –"tlength" with k=default.

Figure I 13 shows the transformation made to the smoother "tlength" referred before. Now the GAM suggested a possible trend, not so clear before, between the number of whiting eaten and the porpoise length (Figure I 13- left).

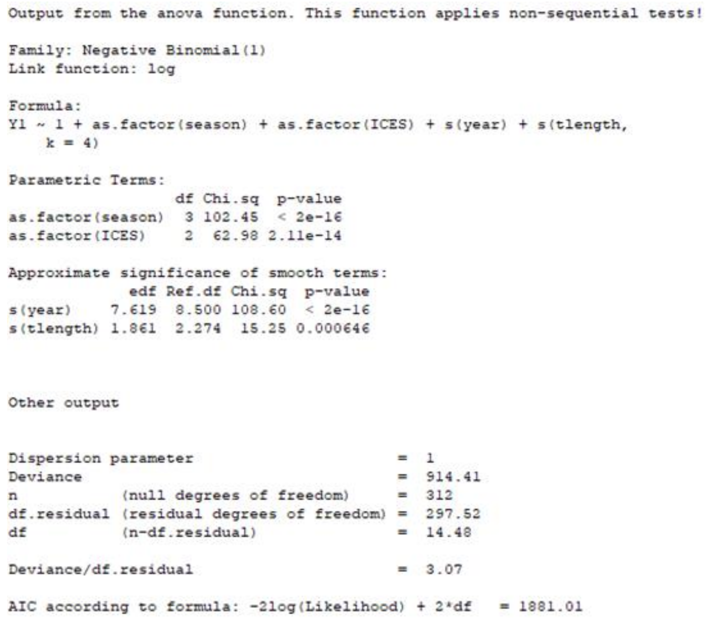
After, it was plotted the numerical output which the ANOVA demonstrated a deviance/df.residual equals to 3.07, and an AIC=1880.67, as shown in the next Figure I 14.



```
Output from the anova function. This function applies non-sequential tests!

Family: Negative Binomial(1)
Link function: log

Formula:
Y1 ~ 1 + as.factor(season) + as.factor(ICES) + as.factor(sex) +
    s(year) + s(tlength, k = 4)

Parametric Terms:
                  df  Chi.sq  p-value
as.factor(season)  3 103.929  < 2e-16
as.factor(ICES)    2  60.259 8.22e-14
as.factor(sex)     1   3.495   0.0616

Approximate significance of smooth terms:
             edf Ref.df  Chi.sq p-value
s(year)    7.631  8.507 111.650 < 2e-16
s(tlength) 1.440  1.747   9.065 0.00525


Other output


Dispersion parameter                          =  1
Deviance                                      =  912.89
n           (null degrees of freedom)        =  312
df.residual (residual degrees of freedom) =  296.93
df          (n-df.residual)                  =  15.07

Deviance/df.residual                          =  3.07

AIC according to formula: -2log(Likelihood) + 2*df   = 1880.67
```

Figure I 14- Numerical output of a Negative Binomial model, 1st model.
Numerical output for the Negative Binomial model with a log link and a Theta=1, with the smoother variable “tlength” with a k=4.

A backward selection in which the variable with the highest p-value was sequentially dropped from the model, was the next step. So, as the output plotted, the least significant variable effect was “sex” with the highest p-value=0.0616, and so a different model was tested without this variable.



```
Output from the anova function. This function applies non-sequential tests!

Family: Negative Binomial(1)
Link function: log

Formula:
Y1 ~ 1 + as.factor(season) + as.factor(ICES) + s(year) + s(tlength,
    k = 4)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(season)  3 102.45  < 2e-16
as.factor(ICES)    2  62.98 2.11e-14

Approximate significance of smooth terms:
             edf Ref.df Chi.sq  p-value
s(year)    7.619  8.500 108.60  < 2e-16
s(tlength) 1.861  2.274  15.25 0.000646


Other output


Dispersion parameter                          =  1
Deviance                                      =  914.41
n           (null degrees of freedom)        =  312
df.residual (residual degrees of freedom) =  297.52
df          (n-df.residual)                  =  14.48

Deviance/df.residual                          =  3.07

AIC according to formula: -2log(Likelihood) + 2*df   = 1881.01
```

Figure I 15- Numerical output of a Negative Binomial model, 2nd model.
Numerical output for the Negative Binomial model with a log link and a Theta=1, with the smoother variable “tlength” with a k=4, and without the variable “sex”.

ANOVA showed for the new model, the deviance/df.residual stayed the same (=3.07) and with a slightly higher AIC=1881.01. All the variables had low p-values (<0.05) and so they were variables with significant effect. Most significant effects were season and year, followed by ICES area and length of the porpoise.

At this point, the models were compared based on their AIC value and deviance/df.residual. This last model only had a slightly higher (<0.5) AIC, but among them it was the simplest one. Therefore, the Negative Binomial model with a log link function and a Theta of 1, with the formula: whit_abun~1+as.factor(season)+as.factor(ICES)+s(year)+s(length, k=4), it seemed the best fitted model.

NOTE: The Negative Binomial model done until now it was done based on a Theta=1, but the real Theta value could be different. Brodgar cannot performed the next steps (e.g. set a theta value), so the statistical analysis was taken in R Studio.

- R Studio

Following the previous work, a Negative Binomial model was tested in R Studio programme.

Before to test any model, it was run in R the followed commands.

Different libraries need to be run at the beginning: **library(nlme), library(mgcv), library(lattice), library (graphics), library (AER).**

Then it was set the directory where the results were saved: **setwd("C:/Users/Ana/Dropbox/R/whitabund")**, followed by the importation of the data from a file saved as a text (MS-DOS): **modeldatafile="whitabundance.txt"**. The data was then read from the file: **defradata<-read.table(file=modeldatafile, fill= TRUE, header=TRUE, dec=".")**.

Before started the analysis, it was explored if the imported data was correct. The text file used here was created especially just for R (details were explained in Chapter III: Methodology). It was used the commands: **view(defradata)**, **names(defradata)**,

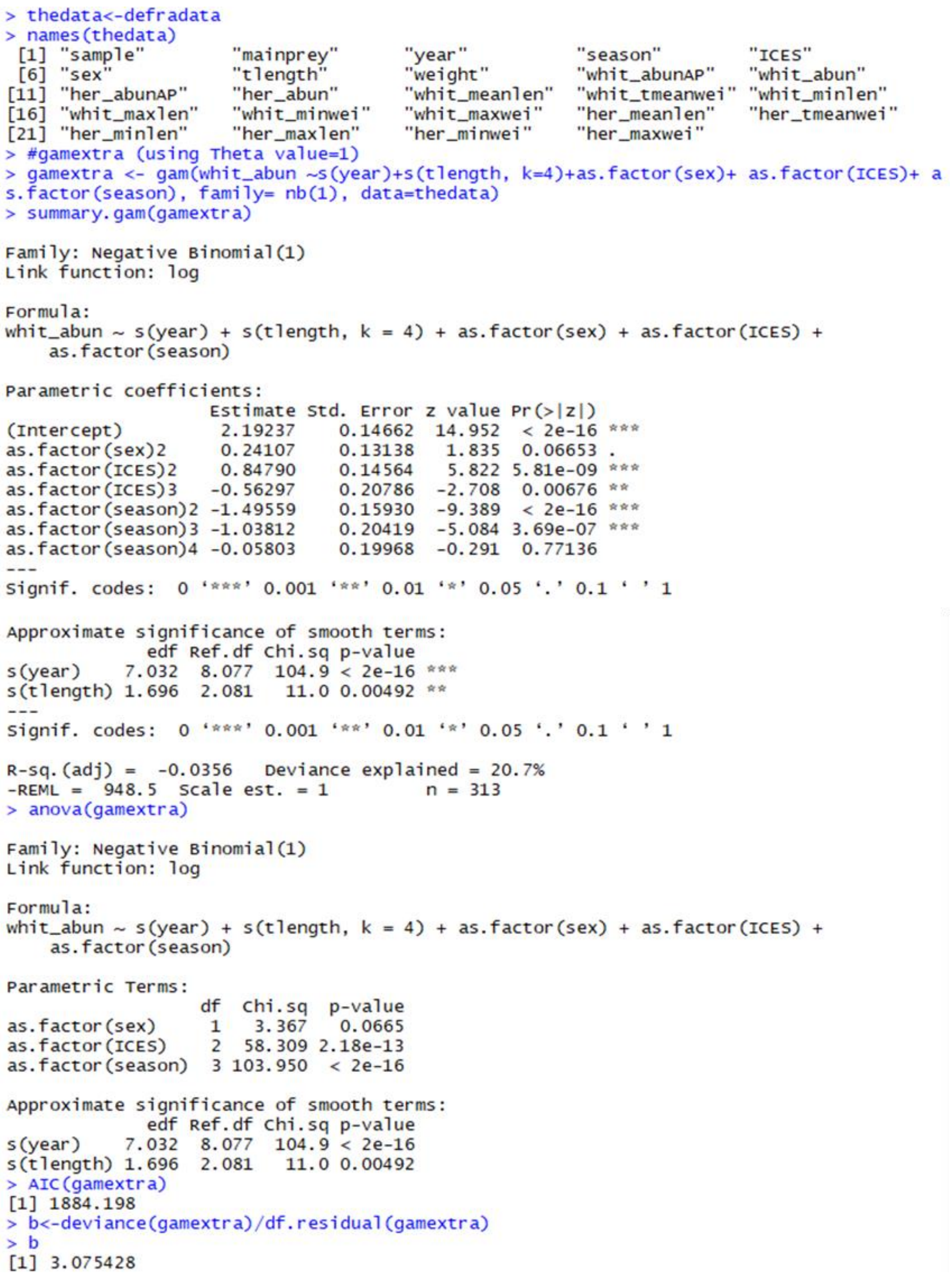
hist(defradata$ICES), hist(defradata$year), hist(defradata$whit_abun), hist(defradata$sex), hist(defradata$season), hist(defradata$tlength), summary (defradata$tlength), summary (defradata$whit_meanlen), summary(defradata$season), summary(defradata$year), summary(defradata$whit_abun). It was detected years misplaced from their column, which had been rectified in the text file.

It was, even, verified how many harbour porpoises did and did not have whiting on their stomachs, resulting from a total data with 314 animals, 160 without and 154 that had eaten whiting. The commands used were: defra2<-subset(defradata,whit_abun==0), defra2$"whit_abun", and defra3$"whit_abun, defra3.

The command **thedata<-defradata** was the last one, and marks the last step used before plot the formula of any model. Until here, all the previous **bold** commands were used to plot the next described models.

Now, to test a Negative Binomial model was done the followed steps.

Firstly, it was tested if the results from R Studio and Brodgar were the same, for a Negative Binomial model plotted with a Theta=1. The first commands used was names(thedata), which helped to write the right headers for the model commands: gam1 <-gam(whit_abun~s(year)+s(tlength)+as.factor(sex)+as.factor(ICES)+ as.factor(season), family= nb(1), data=thedata), summary.gam(gam1). ANOVA function and the AIC value were plotted: anova(gam1), AIC(gam1).

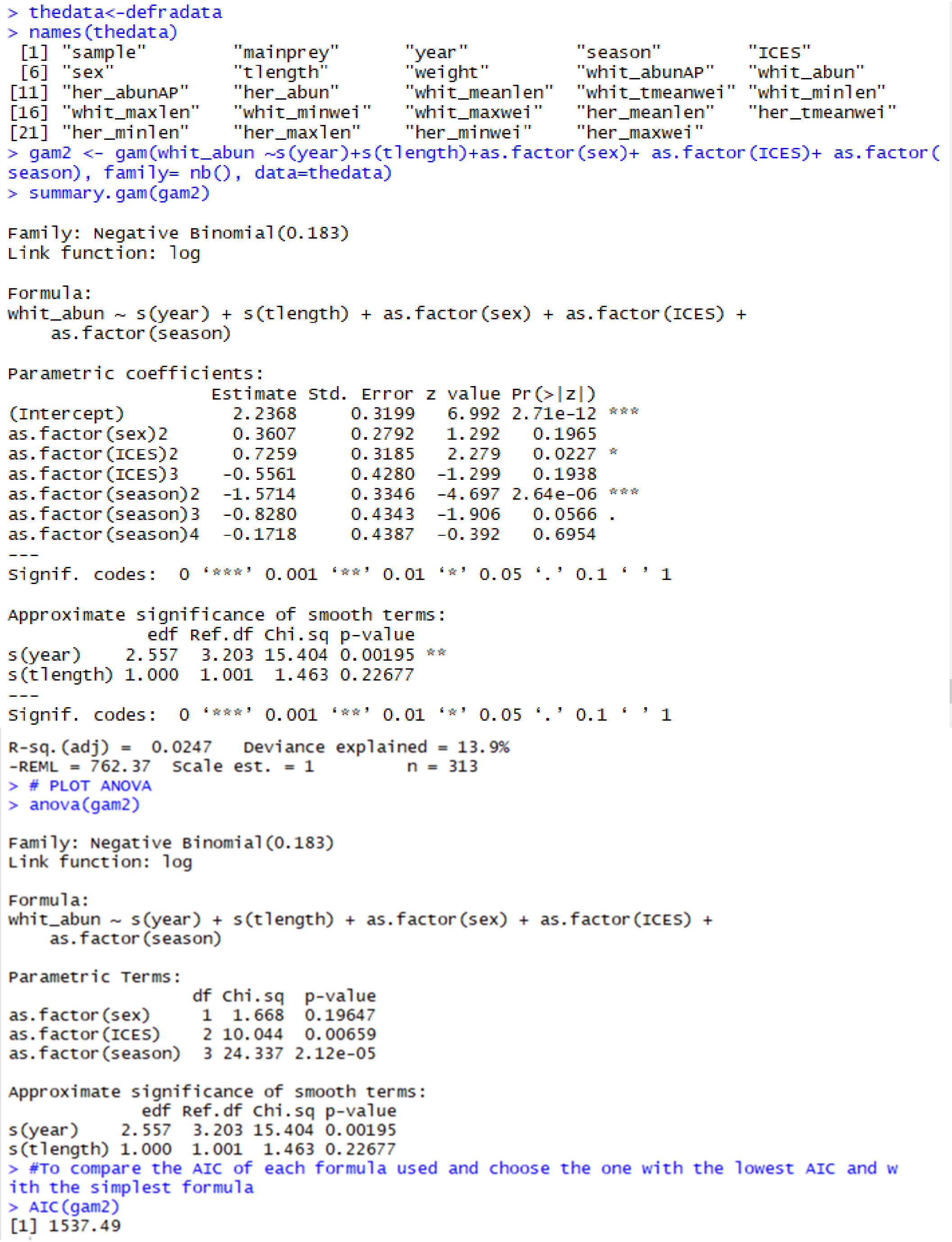


```
> thedata<-defradata
> names(thedata)
 [1] "sample"        "mainprey"      "year"          "season"        "ICES"
 [6] "sex"           "tlength"       "weight"        "whit_abunAP"   "whit_abun"
[11] "her_abunAP"    "her_abun"      "whit_meanlen"  "whit_tmeanwei" "whit_minlen"
[16] "whit_maxlen"   "whit_minwei"   "whit_maxwei"   "her_meanlen"   "her_tmeanwei"
[21] "her_minlen"    "her_maxlen"    "her_minwei"    "her_maxwei"
> #gamextra (using Theta value=1)
> gamextra <- gam(whit_abun ~s(year)+s(tlength, k=4)+as.factor(sex)+ as.factor(ICES)+ a
s.factor(season), family= nb(1), data=thedata)
> summary.gam(gamextra)

Family: Negative Binomial(1)
Link function: log

Formula:
whit_abun ~ s(year) + s(tlength, k = 4) + as.factor(sex) + as.factor(ICES) +
    as.factor(season)

Parametric coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)         2.19237    0.14662  14.952  < 2e-16 ***
as.factor(sex)2     0.24107    0.13138   1.835  0.06653 .
as.factor(ICES)2    0.84790    0.14564   5.822 5.81e-09 ***
as.factor(ICES)3   -0.56297    0.20786  -2.708  0.00676 **
as.factor(season)2 -1.49559    0.15930  -9.389  < 2e-16 ***
as.factor(season)3 -1.03812    0.20419  -5.084 3.69e-07 ***
as.factor(season)4 -0.05803    0.19968  -0.291  0.77136
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value
s(year)    7.032  8.077  104.9 < 2e-16 ***
s(tlength) 1.696  2.081   11.0 0.00492 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  -0.0356   Deviance explained = 20.7%
-REML =  948.5  Scale est. = 1         n = 313
> anova(gamextra)

Family: Negative Binomial(1)
Link function: log

Formula:
whit_abun ~ s(year) + s(tlength, k = 4) + as.factor(sex) + as.factor(ICES) +
    as.factor(season)

Parametric Terms:
                  df  Chi.sq  p-value
as.factor(sex)     1   3.367   0.0665
as.factor(ICES)    2  58.309 2.18e-13
as.factor(season)  3 103.950  < 2e-16

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value
s(year)    7.032  8.077  104.9 < 2e-16
s(tlength) 1.696  2.081   11.0 0.00492
> AIC(gamextra)
[1] 1884.198
> b<-deviance(gamextra)/df.residual(gamextra)
> b
[1] 3.075428
```

Figure I 16- R console output of a Negative Binomial model, for whiting data.
Output of a Negative Binomial model, with Theta=1, and “tlength” with k=4, for the number of whiting.

The R output (Figure I 16) and the Brodgar (Figure I 14) showed similarities in their results, AIC’s close to each other (AIC(R)=1884.2, AIC(Brd)=1880.7), the deviance/df.residual had the same value (3.07) and even for both models the lowest statistically significant variable effect was sex.

However, the objective was to run a Negative Binomial model and to determine the real Theta value. All the above **bold commands** were previously run, then it was set the command with the specific formula: gam2<-gam(whit_abun~s(year)+s(tlength)+as.factor(sex)+as.factor(ICES)+as.factor(season), family= nb(), data=thedata), summary.gam(gam2). ANOVA function and the AIC value were plotted as: anova(gam2), AIC(gam2).

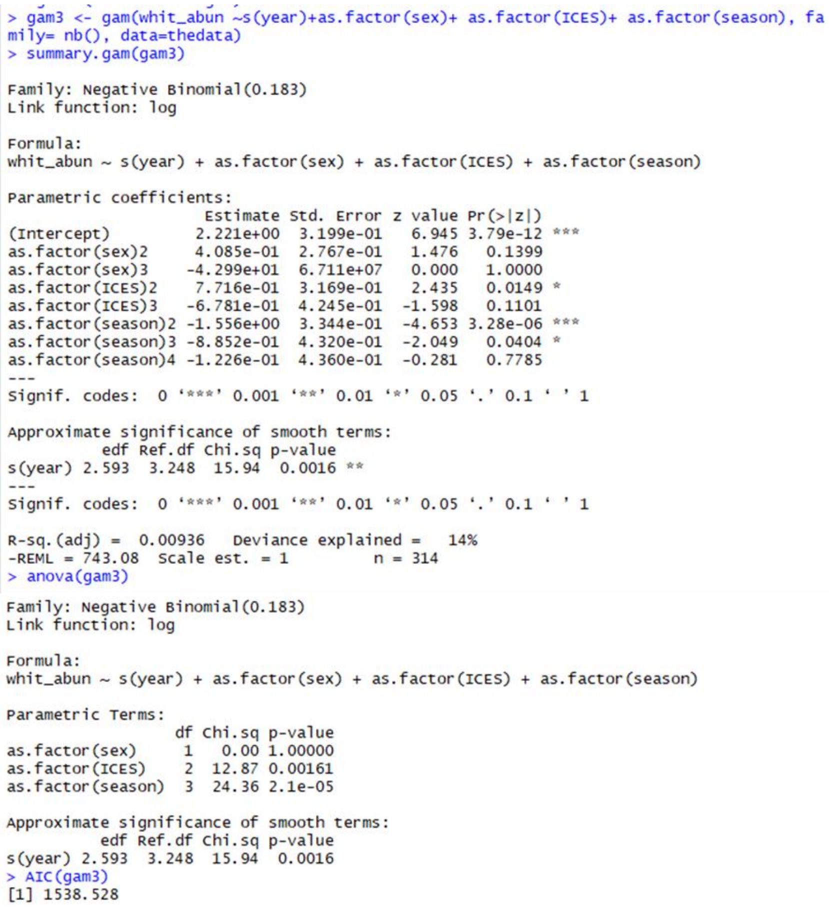


```
> thedata<-defradata
> names(thedata)
 [1] "sample"        "mainprey"      "year"          "season"        "ICES"
 [6] "sex"           "tlength"       "weight"        "whit_abunAP"   "whit_abun"
[11] "her_abunAP"    "her_abun"      "whit_meanlen"  "whit_tmeanwei" "whit_minlen"
[16] "whit_maxlen"   "whit_minwei"   "whit_maxwei"   "her_meanlen"   "her_tmeanwei"
[21] "her_minlen"    "her_maxlen"    "her_minwei"    "her_maxwei"
> gam2 <- gam(whit_abun ~s(year)+s(tlength)+as.factor(sex)+ as.factor(ICES)+ as.factor(
season), family= nb(), data=thedata)
> summary.gam(gam2)

Family: Negative Binomial(0.183) 
Link function: log 

Formula:
whit_abun ~ s(year) + s(tlength) + as.factor(sex) + as.factor(ICES) + 
    as.factor(season)

Parametric coefficients:
                   Estimate Std. Error z value Pr(>|z|)    
(Intercept)          2.2368     0.3199   6.992 2.71e-12 ***
as.factor(sex)2      0.3607     0.2792   1.292   0.1965    
as.factor(ICES)2     0.7259     0.3185   2.279   0.0227 *  
as.factor(ICES)3    -0.5561     0.4280  -1.299   0.1938    
as.factor(season)2  -1.5714     0.3346  -4.697 2.64e-06 ***
as.factor(season)3  -0.8280     0.4343  -1.906   0.0566 .  
as.factor(season)4  -0.1718     0.4387  -0.392   0.6954    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value   
s(year)    2.557  3.203 15.404 0.00195 **
s(tlength) 1.000  1.001  1.463 0.22677   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.0247   Deviance explained = 13.9%
-REML = 762.37  Scale est. = 1         n = 313
> # PLOT ANOVA
> anova(gam2)

Family: Negative Binomial(0.183) 
Link function: log 

Formula:
whit_abun ~ s(year) + s(tlength) + as.factor(sex) + as.factor(ICES) + 
    as.factor(season)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(sex)     1  1.668  0.19647
as.factor(ICES)    2 10.044  0.00659
as.factor(season)  3 24.337 2.12e-05

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value
s(year)    2.557  3.203 15.404 0.00195
s(tlength) 1.000  1.001  1.463 0.22677
> #To compare the AIC of each formula used and choose the one with the lowest AIC and w
ith the simplest formula
> AIC(gam2)
[1] 1537.49
```

Figure I 17- R console output of a Negative Binomial model, 1st model.
Output of a Negative Binomial model, with Theta=0.183, for the number of whiting.

The Theta value resultant was equal to 0.183, and the AIC was 1537.49. A backward selection in which the variable with the highest p-value was sequentially dropped from the model, resulted in the removing of length of the porpoise (p-value=0.22677) and so a new model was test without this variable.

A new command, without length, was run: gam3<-gam(whit_abun~s(year)+as.factor(sex)+as.factor(ICES)+as.factor(season), family= nb(), data=thedata), summary.gam(gam2). ANOVA function and the AIC value were plotted: anova(gam3), AIC(gam3).

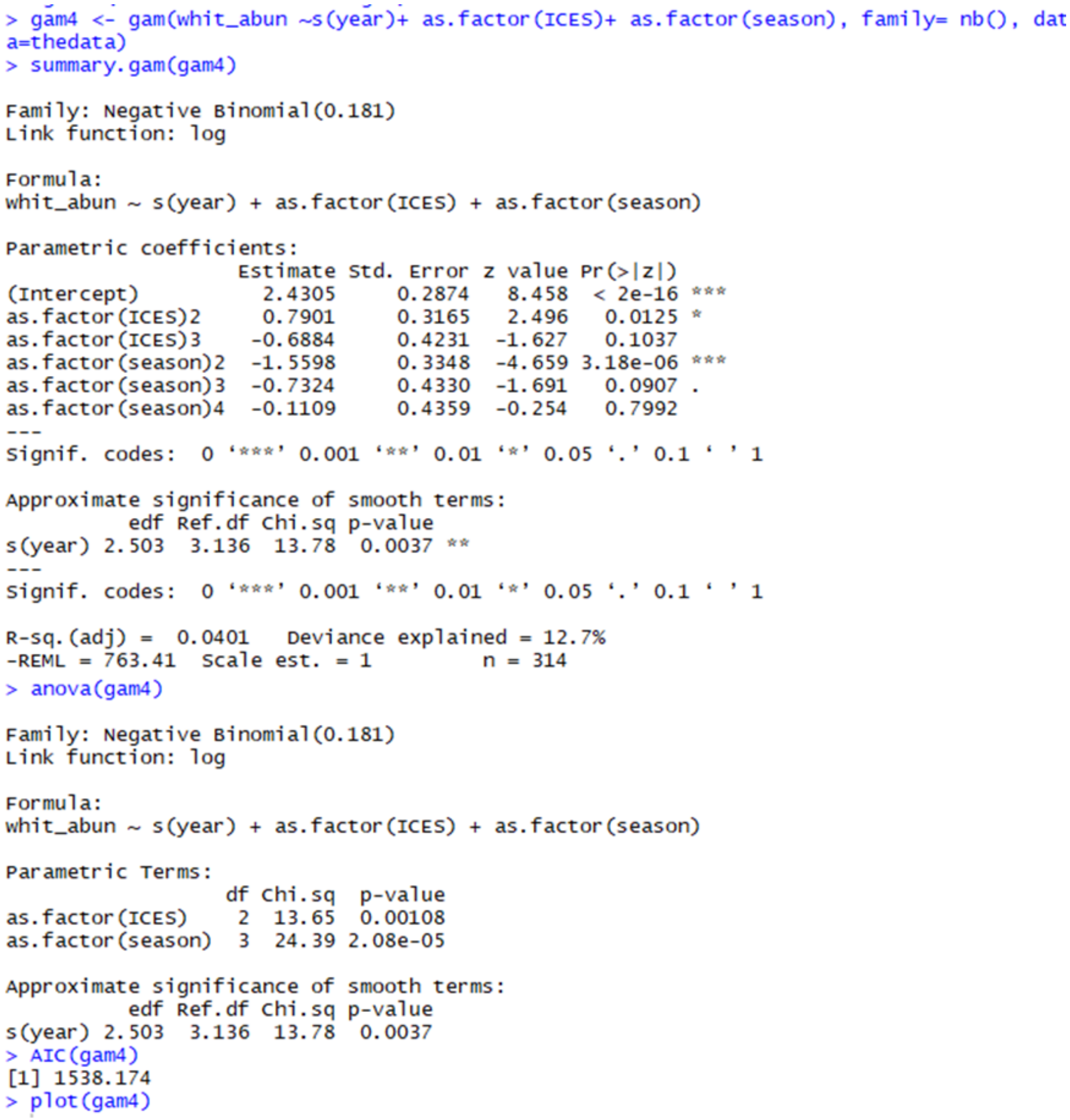


```
> gam3 <- gam(whit_abun ~s(year)+as.factor(sex)+ as.factor(ICES)+ as.factor(season), fa
mily= nb(), data=thedata)
> summary.gam(gam3)

Family: Negative Binomial(0.183)
Link function: log

Formula:
whit_abun ~ s(year) + as.factor(sex) + as.factor(ICES) + as.factor(season)

Parametric coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)         2.221e+00  3.199e-01   6.945 3.79e-12 ***
as.factor(sex)2     4.085e-01  2.767e-01   1.476   0.1399
as.factor(sex)3    -4.299e+01  6.711e+07   0.000   1.0000
as.factor(ICES)2    7.716e-01  3.169e-01   2.435   0.0149 *
as.factor(ICES)3   -6.781e-01  4.245e-01  -1.598   0.1101
as.factor(season)2 -1.556e+00  3.344e-01  -4.653 3.28e-06 ***
as.factor(season)3 -8.852e-01  4.320e-01  -2.049   0.0404 *
as.factor(season)4 -1.226e-01  4.360e-01  -0.281   0.7785
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 2.593  3.248  15.94  0.0016 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.00936   Deviance explained =   14%
-REML = 743.08  Scale est. = 1         n = 314
> anova(gam3)

Family: Negative Binomial(0.183)
Link function: log

Formula:
whit_abun ~ s(year) + as.factor(sex) + as.factor(ICES) + as.factor(season)

Parametric Terms:
                  df Chi.sq p-value
as.factor(sex)     1   0.00 1.00000
as.factor(ICES)    2  12.87 0.00161
as.factor(season)  3  24.36 2.1e-05

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 2.593  3.248  15.94  0.0016
> AIC(gam3)
[1] 1538.528
```

Figure I 18- R console output of a Negative Binomial model, 2nd model.
Output of a Negative Binomial model, with Theta=0.183, without variable length.

For this 2nd formula inserted, the Theta value resultant it did not change. AIC was slightly higher (=1538.528). Sex was the variable with the least significant effect with a p-value=1, and so it was removed to test a 3rd model.

The 3rd model command, without length and sex, was: gam4<-gam(whit_abun~s(year)+as.factor(ICES)+as.factor(season), family= nb(), data=thedata), summary.gam(gam4). Again ANOVA function and the AIC value for the 3rd model were plotted: anova(gam4), AIC(gam4).

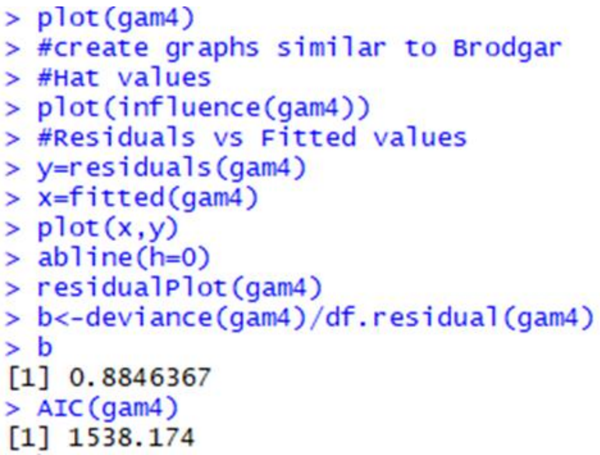


```
> gam4 <- gam(whit_abun ~s(year)+ as.factor(ICES)+ as.factor(season), family= nb(), dat
a=thedata)
> summary.gam(gam4)

Family: Negative Binomial(0.181)
Link function: log

Formula:
whit_abun ~ s(year) + as.factor(ICES) + as.factor(season)

Parametric coefficients:
                   Estimate Std. Error z value Pr(>|z|)    
(Intercept)          2.4305     0.2874   8.458  < 2e-16 ***
as.factor(ICES)2     0.7901     0.3165   2.496   0.0125 *  
as.factor(ICES)3    -0.6884     0.4231  -1.627   0.1037    
as.factor(season)2  -1.5598     0.3348  -4.659 3.18e-06 ***
as.factor(season)3  -0.7324     0.4330  -1.691   0.0907 .  
as.factor(season)4  -0.1109     0.4359  -0.254   0.7992    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value   
s(year) 2.503  3.136  13.78  0.0037 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.0401   Deviance explained = 12.7%
-REML = 763.41  Scale est. = 1         n = 314
> anova(gam4)

Family: Negative Binomial(0.181)
Link function: log

Formula:
whit_abun ~ s(year) + as.factor(ICES) + as.factor(season)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(ICES)    2  13.65  0.00108
as.factor(season)  3  24.39 2.08e-05

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 2.503  3.136  13.78  0.0037
> AIC(gam4)
[1] 1538.174
> plot(gam4)
```

Figure I 19- R console output for a Negative Binomial model, 3rd model.
Output of a Negative Binomial model, with Theta=0.181, without variable length and sex.

For the 3rd model, Theta value resultant it was 0.181. AIC was slightly smaller (=1538.174). ICES area, season and year had significant effect, as Figure I 19 showed (season>ICES area>year).

After compared all the AIC values from the 3 models and choosing the simplest one, the best Negative Binomial that fitted whiting data was the last one. For a complete analysis, it was plotted different types of graphics in R.



```
> plot(gam4)
> #create graphs similar to Brodgar
> #Hat values
> plot(influence(gam4))
> #Residuals vs Fitted values
> y=residuals(gam4)
> x=fitted(gam4)
> plot(x,y)
> abline(h=0)
> residualPlot(gam4)
> b<-deviance(gam4)/df.residual(gam4)
> b
[1] 0.8846367
> AIC(gam4)
[1] 1538.174
```

Figure I 20- R commands used to create graphics.
Commands used to plot some graphics for the best fitted Binomial model, with Theta=0.181, for the whiting abundance data.

Example of graphics that were plotted with the previous R commands, Figure I 21.

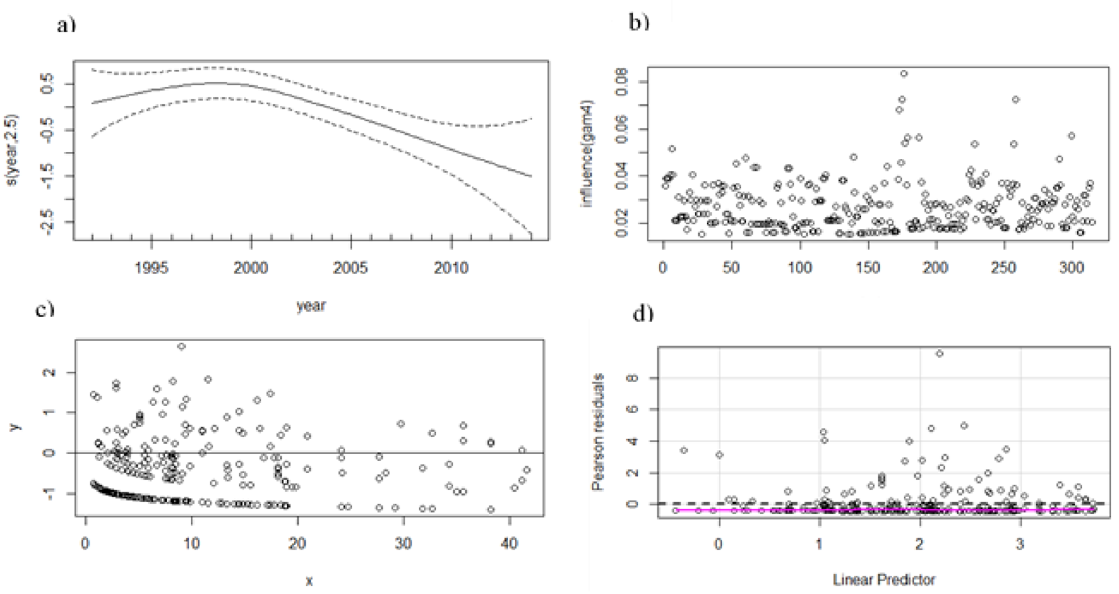


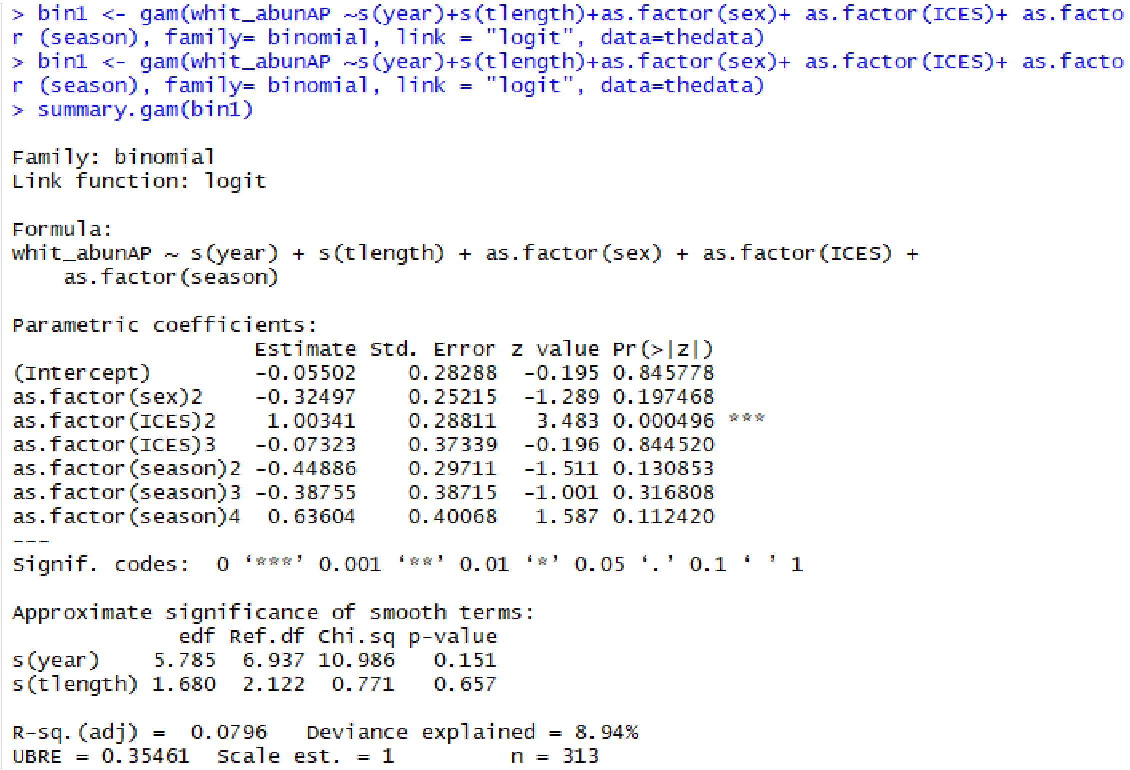
Figure I 21- Plotted graphics with R commands
a) Pattern between whiting abundance along the years. b) Hat values. c) Residual vs fitted values. d) Pearson predictor vs linear.

NOTE: Comparing the two Negative Binomial models, one which the Theta value (=1) that was stated by the programme and the other which the real Theta value (=0.181) was calculated by the programme, it was possible to check their different outcomes.

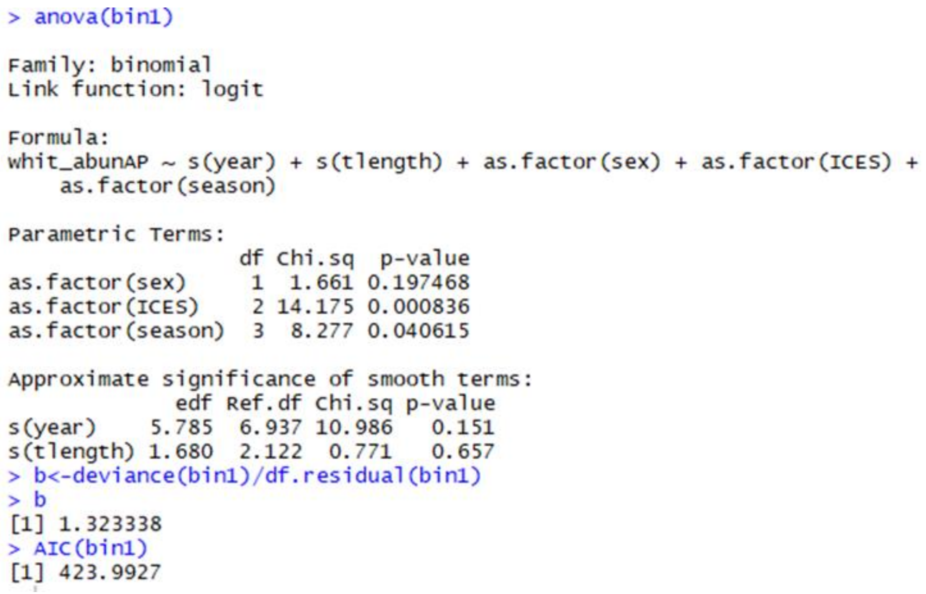
Until here, a Poisson and a Negative Binomial model were tested, but due to a high number of 0 whiting found in the stomachs, a Binomial model was investigated, too.

To test the Binomial model, in R Studio, it was required a transformation of the original file. The number of whiting eaten equal to 0 stayed zeros, and the number of whiting eaten higher that 0 were changed to 1's. The new file was imported to R Studio as "BNabundanceAP.txt". Then all the above **bold commands** used for the Negative Binomial were run, with the difference on the file's name written in the command: modeldatafile=" BNabundanceAP.txt ".

The exploration phase had been already done for the Negative Binomial model in Brodgar. The next step was to run the full model formula: bin1<-gam(whit_abunAP~s(year)+s(length)+as.factor (sex)+as.factor(ICES)+as.factor(season), family= binomial, link="logit", data=thedata). A summary of the data, summary.gam(bin1), the ANOVA function, anova(bin1), the AIC value, AIC(bin1), and the deviance/df.residual, deviance (bin1)/df.residual (bin1), were plotted, too.



```
> bin1 <- gam(whit_abunAP ~s(year)+s(tlength)+as.factor(sex)+ as.factor(ICES)+ as.facto
r (season), family= binomial, link = "logit", data=thedata)
> bin1 <- gam(whit_abunAP ~s(year)+s(tlength)+as.factor(sex)+ as.factor(ICES)+ as.facto
r (season), family= binomial, link = "logit", data=thedata)
> summary.gam(bin1)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + s(tlength) + as.factor(sex) + as.factor(ICES) +
    as.factor(season)

Parametric coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)        -0.05502    0.28288  -0.195 0.845778
as.factor(sex)2    -0.32497    0.25215  -1.289 0.197468
as.factor(ICES)2    1.00341    0.28811   3.483 0.000496 ***
as.factor(ICES)3   -0.07323    0.37339  -0.196 0.844520
as.factor(season)2 -0.44886    0.29711  -1.511 0.130853
as.factor(season)3 -0.38755    0.38715  -1.001 0.316808
as.factor(season)4  0.63604    0.40068   1.587 0.112420
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value
s(year)    5.785  6.937 10.986   0.151
s(tlength) 1.680  2.122  0.771   0.657

R-sq.(adj) =  0.0796   Deviance explained = 8.94%
UBRE = 0.35461  Scale est. = 1         n = 313
```

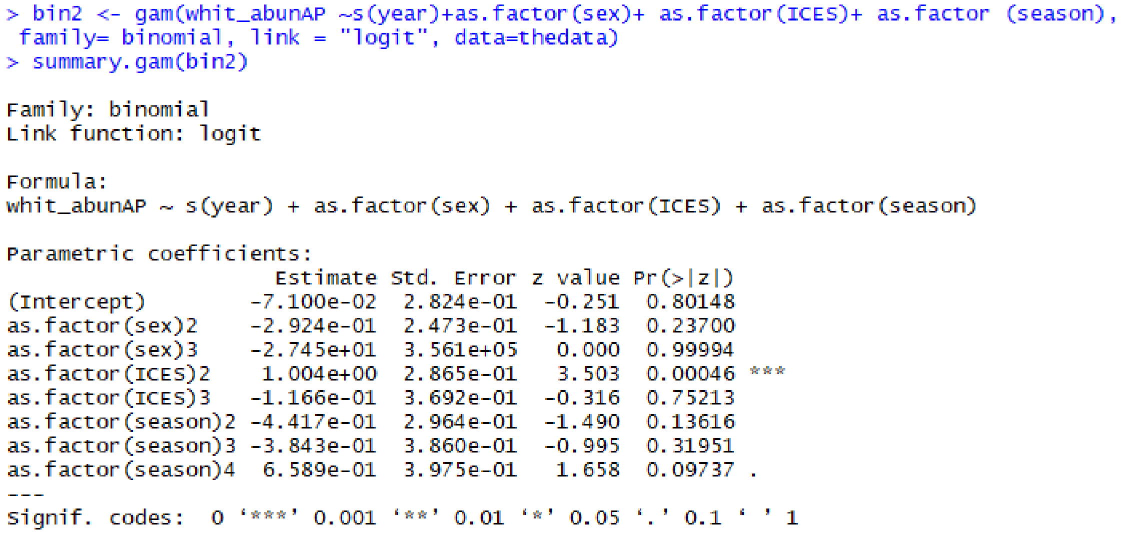


```
> anova(bin1)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + s(tlength) + as.factor(sex) + as.factor(ICES) +
    as.factor(season)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(sex)     1  1.661 0.197468
as.factor(ICES)    2 14.175 0.000836
as.factor(season)  3  8.277 0.040615

Approximate significance of smooth terms:
             edf Ref.df Chi.sq p-value
s(year)    5.785  6.937 10.986   0.151
s(tlength) 1.680  2.122  0.771   0.657
> b<-deviance(bin1)/df.residual(bin1)
> b
[1] 1.323338
> AIC(bin1)
[1] 423.9927
```

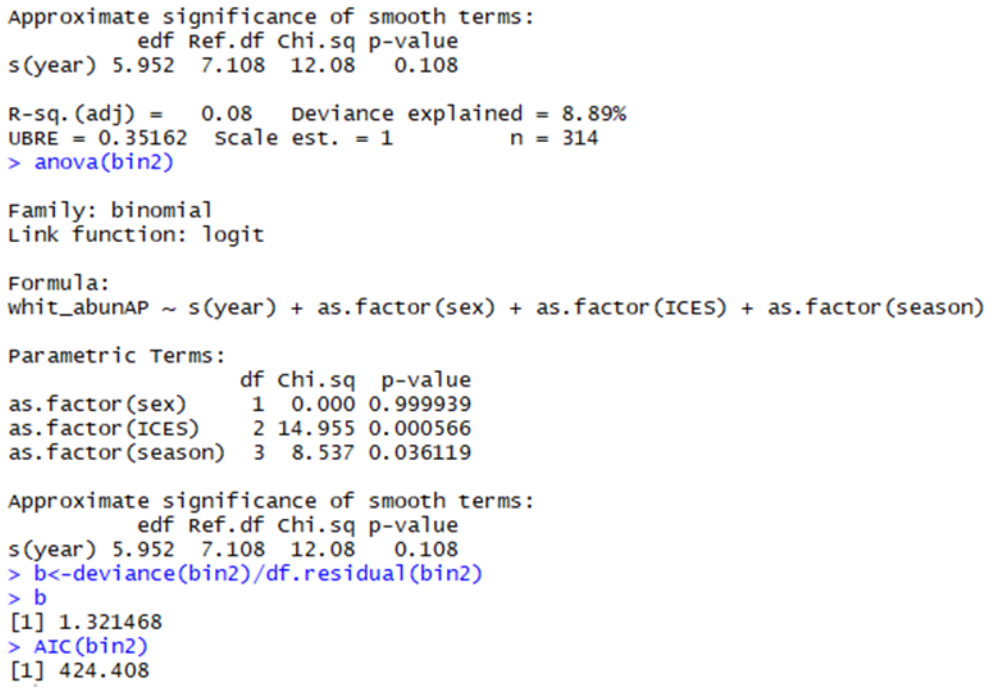
Figure I 22- R console output of a Binomial model, for whiting data.
Output of the Binomial model (bin1), for the number of whiting eaten by porpoises.

It resulted in an AIC= 423.99, and with porpoise length as the least statistically significant effect (p-value=0.657).

Next full model command plotted was without length: bin2<-gam(whit_abunAP~s(year)+as.factor (sex)+as.factor(ICES)+as.factor(season), family= binomial, link=”logit”, data=thedata). The model summary, the ANOVA function, AIC and the deviance/df. residual resulted in the next outcomes (Figure I 23).



```
> bin2 <- gam(whit_abunAP ~s(year)+as.factor(sex)+ as.factor(ICES)+ as.factor (season),
 family= binomial, link = "logit", data=thedata)
> summary.gam(bin2)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + as.factor(sex) + as.factor(ICES) + as.factor(season)

Parametric coefficients:
                    Estimate Std. Error z value Pr(>|z|)    
(Intercept)        -7.100e-02  2.824e-01  -0.251  0.80148    
as.factor(sex)2    -2.924e-01  2.473e-01  -1.183  0.23700    
as.factor(sex)3    -2.745e+01  3.561e+05   0.000  0.99994    
as.factor(ICES)2    1.004e+00  2.865e-01   3.503  0.00046 ***
as.factor(ICES)3   -1.166e-01  3.692e-01  -0.316  0.75213    
as.factor(season)2 -4.417e-01  2.964e-01  -1.490  0.13616    
as.factor(season)3 -3.843e-01  3.860e-01  -0.995  0.31951    
as.factor(season)4  6.589e-01  3.975e-01   1.658  0.09737 .  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

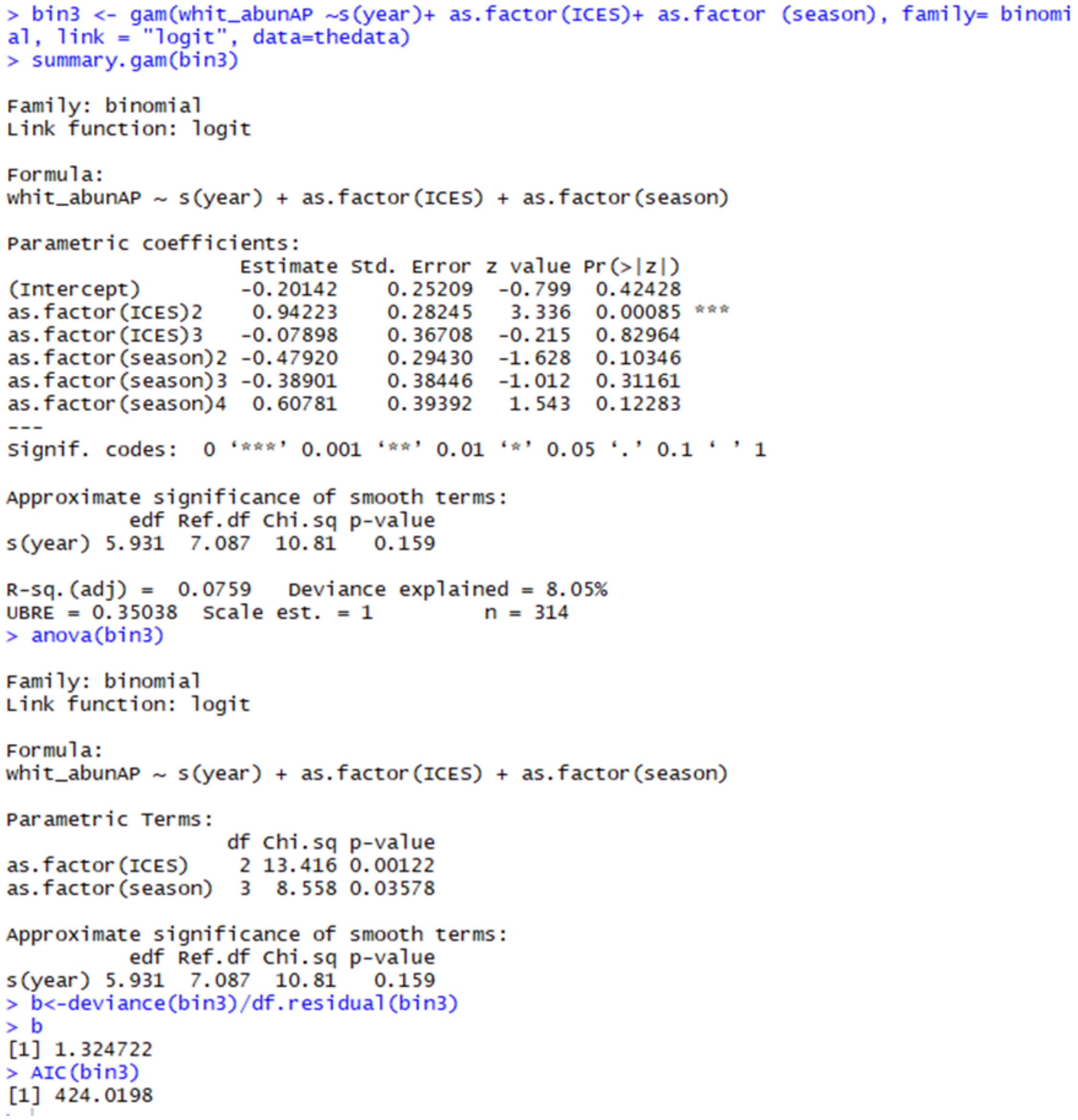


```
Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 5.952  7.108  12.08   0.108

R-sq.(adj) =   0.08   Deviance explained = 8.89%
UBRE = 0.35162  Scale est. = 1         n = 314
> anova(bin2)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + as.factor(sex) + as.factor(ICES) + as.factor(season)

Parametric Terms:
                  df Chi.sq  p-value
as.factor(sex)     1  0.000 0.999939
as.factor(ICES)    2 14.955 0.000566
as.factor(season)  3  8.537 0.036119

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 5.952  7.108  12.08   0.108
> b<-deviance(bin2)/df.residual(bin2)
> b
[1] 1.321468
> AIC(bin2)
[1] 424.408
```

Figure I 23- R console output of a Binomial model, 1st model.
Output for the Binomial model (bin2), without variable length

It resulted in an AIC= 424.40, and with porpoise sex as the least statistically significant effect (p-value=0.999). Again, a backward selection in which the variable with the highest p-value will be dropped from the model, resulted in the removing of sex variable.

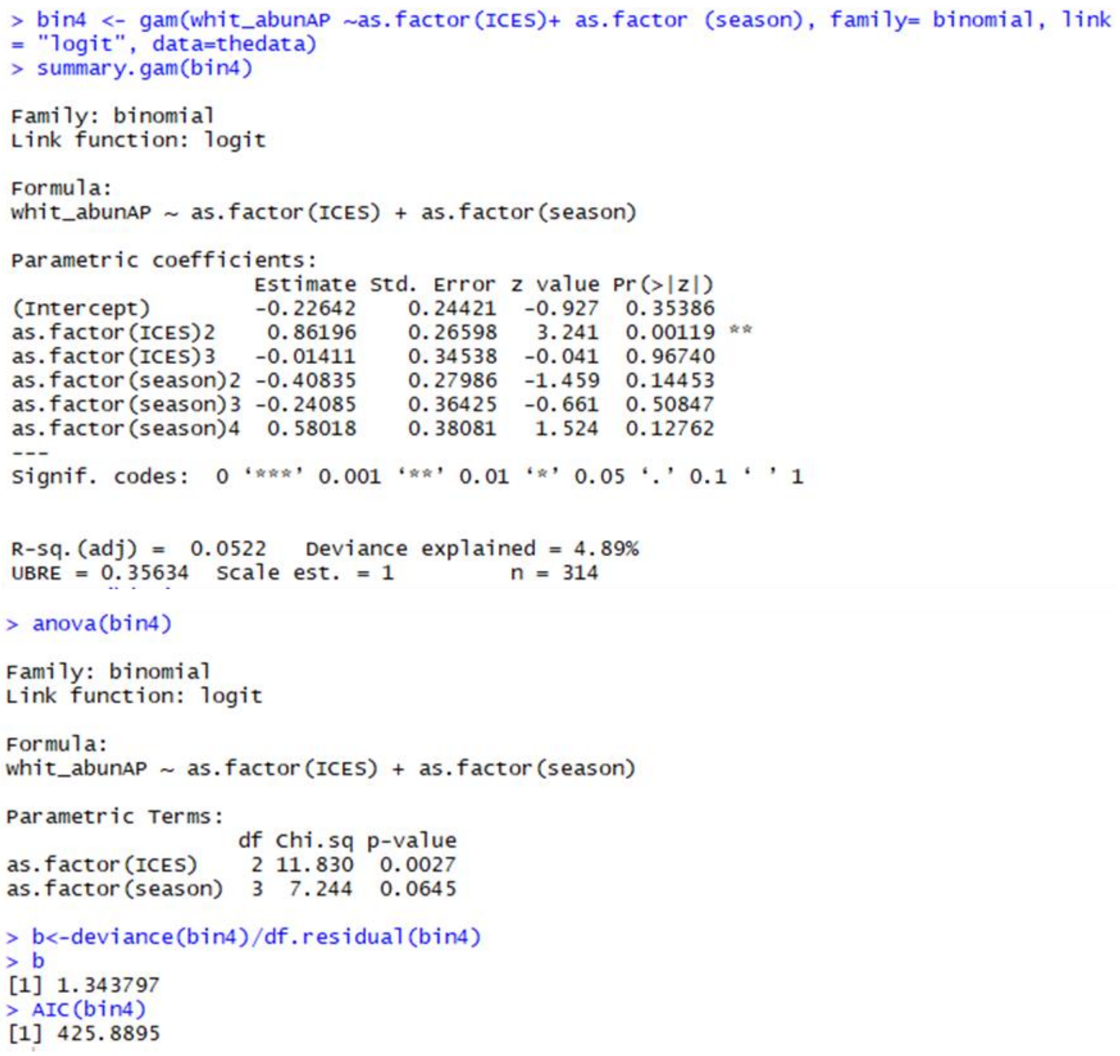
The 3rd full model command plotted was without length and sex: bin3<-gam(whit_abunAP~s(year)+as.factor(ICES)+as.factor(season), family= binomial, link=”logit”, data=thedata). The model summary, the ANOVA function, AIC and the deviance/df. residual resulted in the next outcomes (Figure I 24).



```
> bin3 <- gam(whit_abunAP ~s(year)+ as.factor(ICES)+ as.factor (season), family= binomi
al, link = "logit", data=thedata)
> summary.gam(bin3)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + as.factor(ICES) + as.factor(season)

Parametric coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)          -0.20142    0.25209  -0.799  0.42428
as.factor(ICES)2      0.94223    0.28245   3.336  0.00085 ***
as.factor(ICES)3     -0.07898    0.36708  -0.215  0.82964
as.factor(season)2 -0.47920    0.29430  -1.628  0.10346
as.factor(season)3 -0.38901    0.38446  -1.012  0.31161
as.factor(season)4  0.60781    0.39392   1.543  0.12283
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 5.931  7.087  10.81   0.159

R-sq.(adj) =  0.0759   Deviance explained = 8.05%
UBRE = 0.35038  Scale est. = 1         n = 314
> anova(bin3)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ s(year) + as.factor(ICES) + as.factor(season)

Parametric Terms:
                  df Chi.sq p-value
as.factor(ICES)    2 13.416 0.00122
as.factor(season)  3  8.558 0.03578

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(year) 5.931  7.087  10.81   0.159
> b<-deviance(bin3)/df.residual(bin3)
> b
[1] 1.324722
> AIC(bin3)
[1] 424.0198
```

Figure I 24- R console output of the Binomial model, 2nd model.
Output of the Binomial model (bin3), without variable length and sex.

It resulted in an AIC= 424.019, and with year as the least statistically significant effect (p-value=0.159). Again, to test other model, year variable was removed.



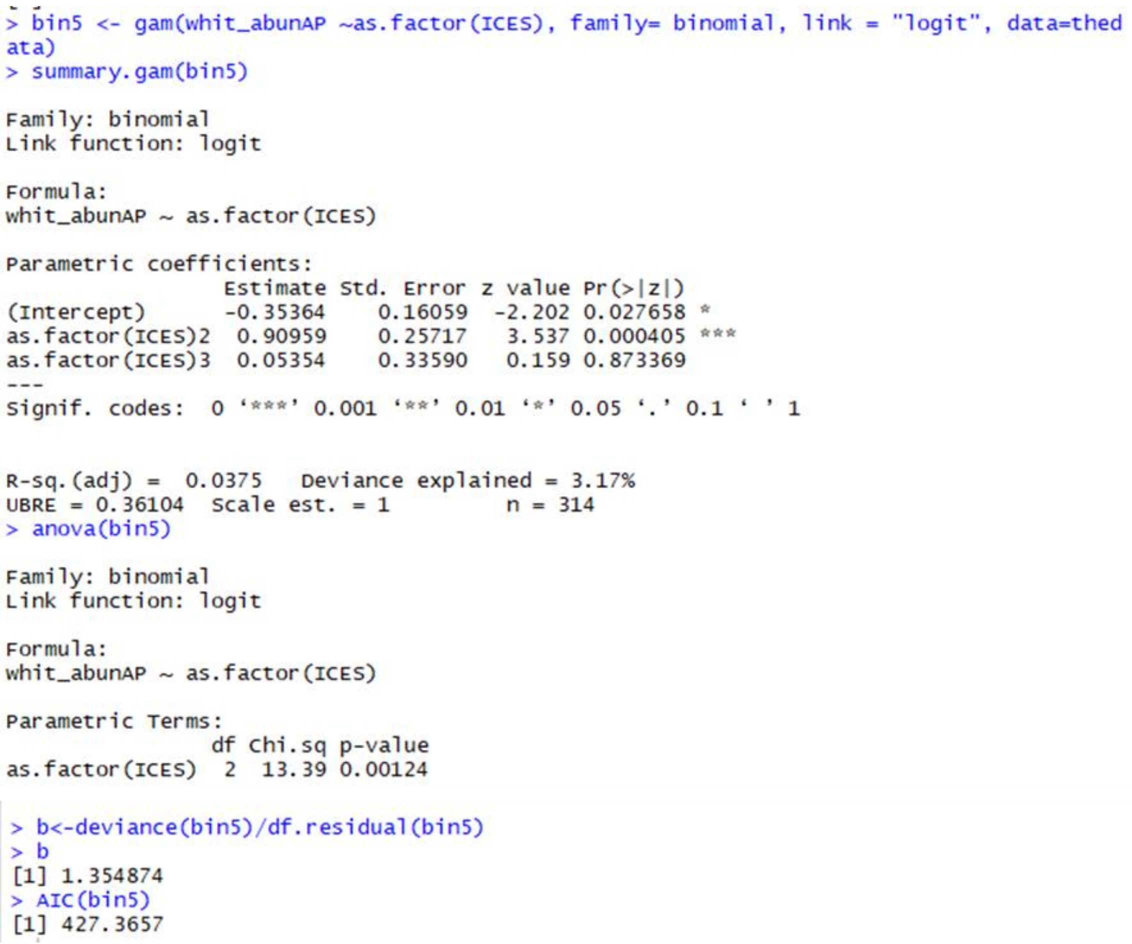
```
> bin4 <- gam(whit_abunAP ~as.factor(ICES)+ as.factor (season), family= binomial, link
= "logit", data=thedata)
> summary.gam(bin4)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ as.factor(ICES) + as.factor(season)

Parametric coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)        -0.22642    0.24421  -0.927  0.35386
as.factor(ICES)2    0.86196    0.26598   3.241  0.00119 **
as.factor(ICES)3   -0.01411    0.34538  -0.041  0.96740
as.factor(season)2 -0.40835    0.27986  -1.459  0.14453
as.factor(season)3 -0.24085    0.36425  -0.661  0.50847
as.factor(season)4  0.58018    0.38081   1.524  0.12762
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


R-sq.(adj) =  0.0522   Deviance explained = 4.89%
UBRE = 0.35634  Scale est. = 1         n = 314

> anova(bin4)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ as.factor(ICES) + as.factor(season)

Parametric Terms:
                  df Chi.sq p-value
as.factor(ICES)    2 11.830  0.0027
as.factor(season)  3  7.244  0.0645

> b<-deviance(bin4)/df.residual(bin4)
> b
[1] 1.343797
> AIC(bin4)
[1] 425.8895
```

Figure I 25- R console output of the Binomial model, 3rd model.
Output for the Binomial model (bin4), without variable length, sex and year.

The followed full model, without length, sex and year, had: bin4<-gam(whit_abunAP~as.factor(ICES)+as.factor(season), family= binomial, link=”logit”, data=thedata). The model summary, the ANOVA function, AIC and the deviance/df. residual were plotted, too (Figure I 25).

As the 4rd model tested, Figure I 25 shows for the model an AIC=425.88, with ICES area as the only statistically significant effect (p-value=0.0027).

The last model, without length, sex, year and season, had: bin5<-gam(whit_abunAP~as.factor(ICES), family= binomial, link=”logit”, data=thedata). Also, the model summary, the ANOVA function, AIC and the deviance/df. residual were plotted (Figure I 26).



```
> bin5 <- gam(whit_abunAP ~as.factor(ICES), family= binomial, link = "logit", data=thed
ata)
> summary.gam(bin5)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ as.factor(ICES)

Parametric coefficients:
                 Estimate Std. Error z value Pr(>|z|)
(Intercept)      -0.35364    0.16059  -2.202 0.027658 *
as.factor(ICES)2  0.90959    0.25717   3.537 0.000405 ***
as.factor(ICES)3  0.05354    0.33590   0.159 0.873369
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


R-sq.(adj) =  0.0375   Deviance explained = 3.17%
UBRE = 0.36104  Scale est. = 1         n = 314
> anova(bin5)

Family: binomial
Link function: logit

Formula:
whit_abunAP ~ as.factor(ICES)

Parametric Terms:
                df Chi.sq p-value
as.factor(ICES)  2  13.39 0.00124

> b<-deviance(bin5)/df.residual(bin5)
> b
[1] 1.354874
> AIC(bin5)
[1] 427.3657
```

Figure I 26- R console output of a Binomial model, 4th model.
Output for the Binomial model (bin5), without variable length, sex, year and season.

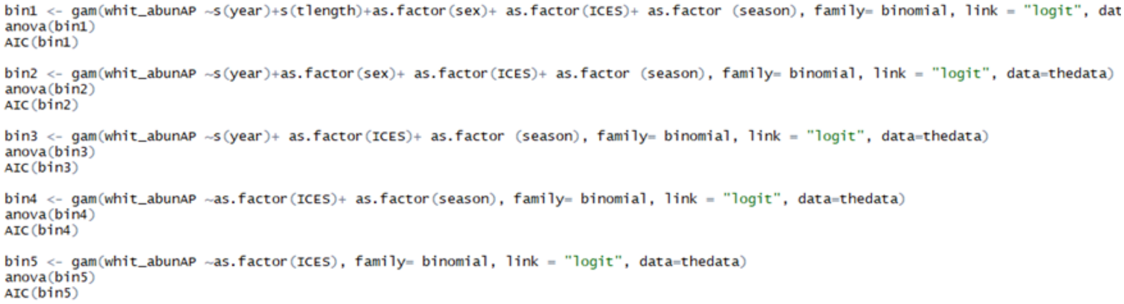
As the last model tested, Figure I 26 shows for the model an AIC=427.36, again with ICES area as the only variable with statistically significant effect (p-value=0.00124).

NOTE: Binomial model it was done in R Studio programme and in Brodgar software, and both had given the same results.

After compare all the 5 Binomial models and to try to choose the simplest one, the AIC values were similar, and to complicate, the simplest model had the highest AIC value. Therefore, a comparing ANOVA table between all the 5 models was the next step.

To compare all the models, R only accepted data with the same "size". A new adjustment was done: whitingbinom<-defradata, whitingbinom, whitingbinomless1row <- subset(whitingbinom, sex!=3), thedata<-whitingbinomless1row.

One by one the models were run with the next commands, Figure I 27.

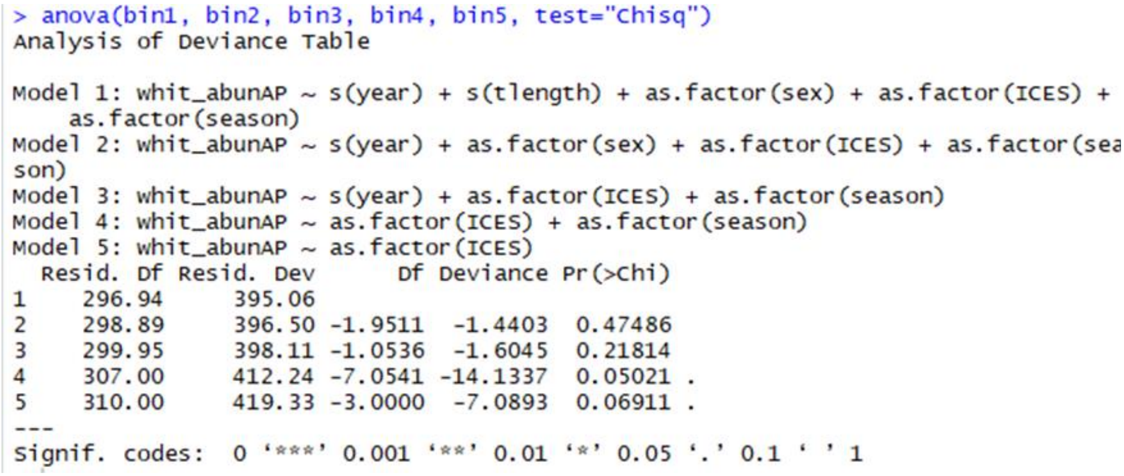


```
bin1 <- gam(whit_abunAP ~s(year)+s(tlength)+as.factor(sex)+ as.factor(ICES)+ as.factor (season), family= binomial, link = "logit", dat
anova(bin1)
AIC(bin1)

bin2 <- gam(whit_abunAP ~s(year)+as.factor(sex)+ as.factor(ICES)+ as.factor (season), family= binomial, link = "logit", data=thedata)
anova(bin2)
AIC(bin2)

bin3 <- gam(whit_abunAP ~s(year)+ as.factor(ICES)+ as.factor (season), family= binomial, link = "logit", data=thedata)
anova(bin3)
AIC(bin3)

bin4 <- gam(whit_abunAP ~as.factor(ICES)+ as.factor(season), family= binomial, link = "logit", data=thedata)
anova(bin4)
AIC(bin4)

bin5 <- gam(whit_abunAP ~as.factor(ICES), family= binomial, link = "logit", data=thedata)
anova(bin5)
AIC(bin5)
```

Figure I 27- R commands of the final models.
Binomial models which will be after compared.

A new series of AIC values resulted: 423.99 (bin1), 422.40 (bin2), 421.90 (bin3), 424.24 (bin4), 425.33 (bin5).

A Chi-squared test was performed between the models: anova(bin1, bin2, test="Chisq"), anova(bin2, bin3, test="Chisq"), anova(bin3, bin4, test="Chisq"), anova(bin4, bin5, test="Chisq") or anova(bin1, bin2, bin3, bin4, bin5, test="Chisq").



```
> anova(bin1, bin2, bin3, bin4, bin5, test="Chisq")
Analysis of Deviance Table

Model 1: whit_abunAP ~ s(year) + s(tlength) + as.factor(sex) + as.factor(ICES) +
    as.factor(season)
Model 2: whit_abunAP ~ s(year) + as.factor(sex) + as.factor(ICES) + as.factor(sea
son)
Model 3: whit_abunAP ~ s(year) + as.factor(ICES) + as.factor(season)
Model 4: whit_abunAP ~ as.factor(ICES) + as.factor(season)
Model 5: whit_abunAP ~ as.factor(ICES)
  Resid. Df Resid. Dev      Df Deviance Pr(>Chi)
1    296.94     395.06
2    298.89     396.50 -1.9511  -1.4403  0.47486
3    299.95     398.11 -1.0536  -1.6045  0.21814
4    307.00     412.24 -7.0541 -14.1337  0.05021 .
5    310.00     419.33 -3.0000  -7.0893  0.06911 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure I 28- R console output of the Chi-squared test.
Analysis of deviance table with a Chi-squared test.

At the end the chosen best fitted model was bin4, with an AIC=424.2414, a deviance explained=4.98% (n=313), with the remaining variables ICES (p-value=0.0025) and season (p-value=0.0761). From bin4 some graphics can be plotted with the same commands as the ones used before for the Negative Binomial model.