On the Ecology of Rorqual Whales (Balaenopteridae) in Irish Waters using Intrinsic Markers

PhD Thesis

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BSc. (Hons) Zoology

Submitted for fulfillment of Doctorate of Philosophy

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Submitted to the Higher Education and Training Awards Council

October 2012

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Abstract:

Despite repeated calls for an ecosystem-based approach to the management of marine resources, little information exists on the ecosystem role and requirements of top predators such as baleen whales in Irish waters. Considering that they are long-lived and highly migratory, baleen whales such as rorquals (Family Balaenopteridae) are potentially useful bio-monitors of anthropogenic environmental change. However, their use as environmental indicators is precluded by an insufficient understanding of their basic ecology. Following centuries of whaling and other emerging threats to their survival including pollution, bycatch and ship-strikes, the conservation status of most rorqual species is difficult to assess due to a shortfall in our knowledge. This thesis aims to facilitate more informed management decision-making and the implementation of more effective conservation measures by bridging key gaps in our knowledge. Pertinent questions on feeding and migration ecology are addressed using intrinsic markers, *i.e.* chemicals whose stable properties ensure they move through biotic systems and are detectable in tissues. Intrinsic markers include naturally occurring (*e.g.* stable isotopes, DNA, trace elements) and anthropogenic compounds (*e.g* persistent organic pollutants or POPs). In Chapter 1, current knowledge on the ecology of blue (*Balaenoptera musculus*), fin (*B. physalus*), humpback (*Megaptera novaengliae*) and minke whales (*B. acutorostrata*) in Irish waters is reviewed. In

chapter 2, stable isotope analysis of baleen from these four species is used to approximate the ecological niche and thus to examine resource partitioning. Chapter 3 is a methodological precursor to Chapter 4 where stable isotope Bayesian mixing models are used to investigate the preferred diet of fin and humpback whales in the Celtic Sea. In Chapter 3, unpredictable effects of pre-analytical lipid-extraction of skin and blubber biopsies were found for both $\delta^{13}C$ and $\delta^{15}N$ values. Chapter 5 is a methodological chapter, concluding that the amount of lipid remaining in blubber biopsies is not representative of blubber *in situ.* This finding was relevant to Chapter 6 which used POP concentrations to investigate elements of population structure of humpback whales in the North Atlantic. Chapter 7 discusses how the key findings such as the diet preferences of Celtic Sea fin whales (Euphausiids such as *Meganyctiphanes norvegica* and *Nyctiphanes couchii,* and year-0 sprat (*Sprattus sprattus*) and herring (*Clupea harengus*)) and humpback whales (chiefly sprat and herring) may be used to facilitate an informed ecosystem-based approach to fisheries management.

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____, Candidate

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Date: 12-10-2012

Acknowledgements

To Simon Berrow, Brendan McHugh, Ian O'Connor and David McGrath, thanks for having the faith in me to carry out this thoroughly rewarding work. Without your faith in my ability, this thesis would not have been achievable. To Brendan in particular, your encouragement and guidance was beyond the call of duty, for which I am very thankful. I am very grateful for the opportunity to pursue what truly has been my passion since I was a young child. Thanks to my parents Liz and Richie, brothers Cian and John and my sister Amy who nurtured this passion – they are responsible for my first ever encounter with a whale – a fin whale that stranded at Ballyheigue, Co. Kerry in 1994 (IRBp94001, Table 1, Chapter 2!). The floods of curious questions that entered my mind that day, as I rested my two hands on such a giant animal that once roamed the cold rough seas, continue to challenge me. Thanks to my examiners, Florence Caurant and Deirdre Brophy, for their constructive suggestions which helped to improve the final draft.

Thanks to my partner Vivi, who had to put up with frustration, distant haziness and constant change of plans throughout the toil. She continues to be an endless source of encouragement and support, and I'm so glad we got to share such memorable days together in the field. Thanks to Jim Wilson, from whom I contracted a highly infectious love of wildlife and passion for conserving what ought to be enjoyed by generations to come. To his son Peter, equally afflicted by this condition, I am also very grateful – he taught me never to underestimate the inspirational power of conversation over a few pints, whether on Cape Clear or in the Donk' at home in Cobh.

I am indebted to Clive Trueman and Chris Harrod for helping me to take a look from another angle and for helpful discussions about stable isotopes in ecology. To those friendly faces in the Marine Institute, especially Brian O'Boyle, Poppy and Philip – I am grateful for your patience and unending help in the chemistry lab. Thanks to Joanne O'Brien who helped me see the wood from the trees, and for always being available for a chat any hour of the day. Joanne and Simon have

taught me to always to raise the bar higher and jump at every opportunity. To Per Palsbøll, Martine Bérubé and everyone at the conservation genetics labs in Stockholm and Groningen, I am very grateful for the opportunity to work with such great scientists. Obrigado to the Cape Verde humpback research community, namely Pedro Lopez Suárez, Fred Wenzel and Beatrice Jann for welcoming a stranger with open arms, into what is normally a 'competitive field'. It is so refreshing to find like-minded people whose mutual passion for understanding and conserving whales is not blemished by ego or competition. For the encouragement and samples I received from UK-based cetacean community (Rob Deaville, Nick Davison, Collin MacLeod, James Barnett and Paul Jepson), many thanks. Thanks to Richard Sabin for one of the most memorable days of my life, not because I travelled the London Underground with a box of blubber samples at rush hour, but because I was privileged enough to drill baleen samples for a whole day inside the London Natural History Museum cetacean collection in Wimbledon.

To Pádraig Whooley, Nick Massett, Andrew Malcolm, Mick O'Connell, Paddy Roche, Deirdre Slevin and all those involved in the IWDG sightings and strandings schemes – I am indebted to you for being so generous with your time. Thanks to Ronan Cosgrove, Pádraig Whooley and John Coveney for permission to use their photographs. To Commodore Mark Mellett, the Commanding Officers and crews of L.É. Orla and the R.V. Celtic Explorer, to Darren Craig, Colin Barnes, Mick Sheeran, Martin Colfer, Alessandro Pierini and Fien deRaedemaeker: thanks for your skilled navigation and for keeping a cool head during those close approaches to the whales! To my drinking and surfing buddies in Galway (Alessandro Pierini, Bernie O'Neill, Clementine Harma, Fien deRaedemaeker, Paula Haynes, Stephen Comerford, Weibke Schmidt and Marieke Volkenandt)– your companionship through tough times was a saving grace ... the shneaky pints and mid-week surf sessions kept despair at bay!

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Chapter 1.
General Introduction

"Few things provoke like the presence of wild animals. They pull at us like tidal currents with questions of volition, of ethical involvement, of ancestry"

Barry Lopez, "Arctic Dreams" (1986)

1.1. Ecology of Rorquals

1.1.1 Systematics

There are 11 extant species within four families of the Suborder Mysticeti (Flower 1864), or baleen whales. Mysticete is derived from the Greek for moustache which refers to the frilled keratinous baleen plates that form a filtration system, a character trait that distinguishes them from the other major cetacean clade; the Suborder Odontoceti (toothed whales). This study focuses on four of the most abundant baleen whale species in Irish waters which belong to the largest family, Balaenopteridae (Gray 1864). These include the humpback whale *Megaptera novaengliae* (Borowski 1781), monotypic in the subfamily *Megapterinae* (Gray 1864), and the minke *Balaenoptera acutorostrata* (Lacépède 1804), fin *B. physalus* (Linnaeus 1758) and blue *B. musculus* (L. 1758) whales, of the subfamily Balaenopterinae (Brandt 1872). The vernacular name for this family is rorqual which derives from the Norwegian *røyrkval* meaning 'furrowed whale' referring to the diagnostic expandable throat pleats of the buccal cavity. Molecular phylogenetic analysis has revealed that rorqual species radiated rapidly from a common ancestor between 23 and 10 MYBP (Nikaido *et al.* 2006, Jackson *et al.* 2009).

1.1.2 Life History, Reproduction and Migration

Reproductive success is best explained within the theoretical framework of life history theory (Stearns 1976). Life history theory maintains that evolutionary fitness is dictated by lifetime reproductive success which itself is determined largely by reproductive strategy. The life history of rorquals is characterised by obligate migration to unproductive tropical waters coupled with protracted periods of fasting (Mackintosh 1965). The selective advantages conferred by these strategies remain unresolved and one of the most enigmatic problems in evolutionary biology (Corkeron & Connor 1999). It has been proposed that large body size and highly-derived blubber for energy stores comprise exaptations for the evolution of polygyny

in marine mammals (Bartholomew 1970). Notwithstanding the evolutionary explanation, the life cycle of rorquals revolves around seasonal migration (Lockyer 1984).

While the social structures of most rorquals remain poorly understood, humpback whales are the exception. Owing to their abundance close to shore, high site fidelity and propensity for fluking (lifting the tail fluke above the water's surface thus enabling researchers to identify individuals by photo identification of unique ventral fluke pigmentation), humpback whales are the most accessible species for longitudinal research on life history traits (Clapham 1996). Humpback whales exhibit a 'floating lek' mating system, where both males and females are promiscuous and where reproductive success of individual males is not skewed by competition for mates (Clapham & Palsbøll 1997, Cerchio *et al.* 2005). The mating systems of other rorquals are poorly known but are likely to involve sperm competition and possible individual guarding of females (Brownell Jr & Ralls 1986, Boness *et al.* 2002). Rorquals give birth to a single offspring. Reproductive output in rorquals is limited by deferred sexual maturity (> 5 yr), protracted parental care and extraordinarily long gestation and inter-birth-interval (12 months and 2—3 yr respectively) (Lockyer 1984, Aguilar & Lockyer 1987).

The migrations carried out by some rorquals are the longest of any mammal (Rasmussen *et al.* 2007). While this allows researchers to gain unique insights into the evolutionary ecological basis for migration, it also presents formidable logistical challenges for researchers and management alike. The reasons for migration have been the subject of much debate (Corkeron & Connor 1999, Clapham 2001). Brodie (1975) hypothesised that baleen whales counter thermoregulatory challenges in order to optimise their energy budgets (and those of their neonates) by migrating to warm waters to give birth, when productivity in high latitudes is lowest, *i.e.* during winter. Observations of several rorqual species at high latitudes during winter however, challenges this theory (Jonsgård 1966, Clark & Charif 1998, Whooley *et al.* 2011). The paradox that bowhead whales (*Balaena mysticetus*) do not migrate, but give birth in polar waters, was accounted for by the observation that rorquals being negatively buoyant must constantly maintain forward motion (hence increasing

energy expenditure), whereas bowhead whales do not. Evans (1987) proposed that migration is a behavioural vestige or tradition carried over from a period when high productivity occurred at lower latitudes, but has since shifted pole-ward due to changes in ocean circulation coinciding with the closure of the Tethys Sea *circa* 20 MYBP. However this hypothesis is strongly contested by some (C. Hazevoet 2012, pers. comm.).

Recent evidence supports the hypothesis that some species, *e.g.* fin whales, may track productive zones as they progress with the seasons, thus potentially enabling them to feed continually (Visser *et al.* 2011). This would permit concomitant calving and foraging at lower latitudes, and may account for the apparent lack of discrete calving grounds for blue, fin and minke whales (unlike humpback whales) considering that the optimal foraging zone may vary annually. The decreased threat of predation chiefly by killer whales was suggested to account for migratory habits in baleen whales (Corkeron & Connor 1999). However, the greater threat of attacks by killer whales during migration, rather than on primary feeding grounds, lends little merit to this explanation (Clapham 2001). The most plausible hypothesis however relates to the reduced energy expenditure in calves, which are more thermally conductive than adults given their higher body surface area to volume ratio (Norris 1967). If born in cold waters, an energy deficit would be conferred to thermal stasis rather than growth requiring longer time for calves to attain maturity. This is supported in terms of reproductive success and from studies on terrestrial mammals, whereby offspring born at lower latitudes have a greater chance of surviving to recruitment than those born at higher latitudes (Clapham 2001). Therefore optimisation for rapid growth in calves most likely accounts for migration in rorquals (Norris 1967).

1.1.3 Feeding

Key to reproductive success and hence fitness is the ability to acquire nutrients for survival. Rorquals are capital breeders and must therefore store energy (in the form of blubber lipid) at a rate that far exceeds energy expenditure. Feeding under optimal foraging conditions over prolonged periods in an environment where food distribution is patchy and ephemeral is challenging. It is unknown how mysticetes locate their prey at both large and small spatial scales. While they lack the ability of fine-scale echolocation exhibited by odontocetes, mysticetes have two nares, exhibit olfaction and may therefore locate prey using directional sense of smell (Thewissen *et al.* 2011). For example, euphausiids emit dimethylsulfide that birds such as petrels (Procelariiformes) use to locate prey (Nevitt 1999, Thewissen *et al.* 2011). For those species that predate on herring, it is possible that sounds produced by bubble release from herring swim-bladders (as they ascend the water column) may be used to locate schools of fish (Wahlberg & Westerberg 2003). Exemplary of our poor understanding of the feeding ecology of rorquals was the very recent discovery of a sensory organ that is critical to the coordinating the biomechanics of lunge-feeding (Pyenson *et al.* 2012).

Undoubtedly the most distinctive attribute of rorquals is their large body size, which in blue whales is unprecedented in the history of life on earth. The ability to exploit the greater biomass afforded by lower trophic levels, came as a result of feeding adaptations such as baleen (Werth 2000, Deméré *et al.* 2008). Baleen is a highly derived keratinous tissue that functions as a filter which permits batch-feeding. All rorquals feed by lunge-feeding: 'the greatest biomechanical action in the animal kingdom' (Brodie 1993). Prey-laden water is engulfed by distension of the buccal cavity (to as much as 100 percent of the body volume), with extension of the fronto-mandibular stay in order to allow dislocation and rotation of the mandibles to accommodate the load (Brodie 1993, Goldbogen *et al.* 2006) (Figure 1). Once closed, the mouth is sealed by re-articulation of the suborbital and the mandibles and the buccal cavity is drained through the baleen. Distention of the buccal cavity has been shown to be controlled by elastic energy stored in the ventral groove blubber and

fronto-mandibular stay, coupled with the pressure of oncoming flow of water and a rebounding wave within the mouth (Lambertsen *et al.* 1995, Potvin *et al.* 2009). The lunge feeding process however is not completely passive nor compliant, rather an energetically costly process requiring coordinated muscular activity with increased fluke strokes (Goldbogen *et al.* 2008, 2011, Potvin *et al.* 2009). Engulfing large volumes of cold water presents obvious thermoregulatory challenges, which may increase the energetic cost of lunge-feeding. The tongue however is adapted as a highly thermoregulatory organ, with a counter-current heat exchange afforded by vascular bundles called *lingual rete* (Heyning & Mead 1997, Reidenberg 2007, Werth 2007).

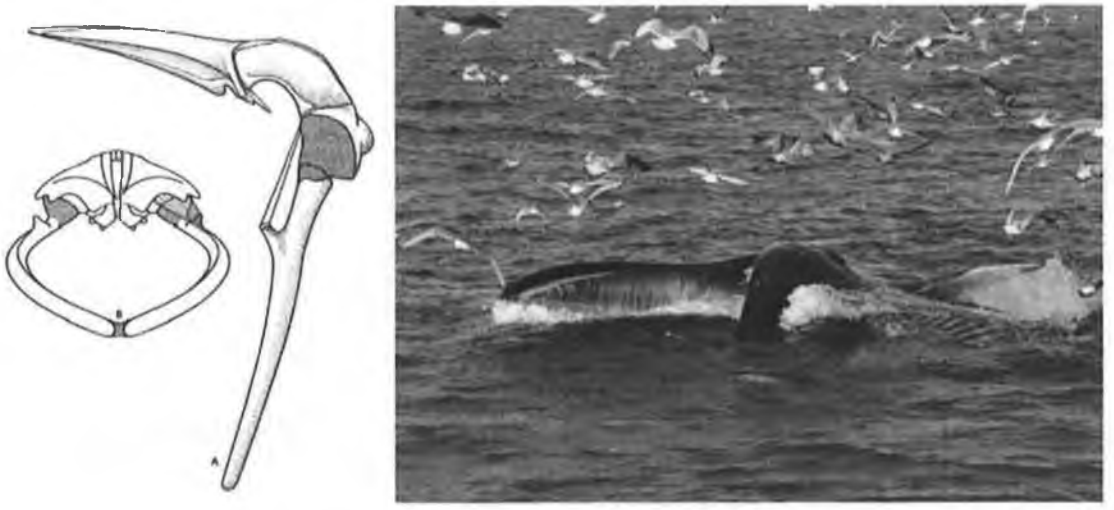

Figure 1. Left: the cranium and mandibles of a rorqual showing the fronto-mandibular stay (parallel lines), and disarticulations of the mandibles at the suborbital articulation and the mandibular symphysis (shaded) (Lambertsen *et al.* 1995). Right: fin whale lunge-feeding in West Cork, Ireland, showing distension of buccal cavity, ventral grooves, baleen, and asymmetric white pigment on the right jaw (submerged). Photograph by Conor Ryan.

Obligate batch filter-feeding and large body sizes necessitate the acquisition of large quantities of food in order to make foraging profitable in terms of energy expenditure (Goldbogen *et al.* 2008). Prey fields of suitable magnitude and density are critical for optimal foraging leading to threshold foraging behaviour (Piatt & Methven 1992,

Goldbogen *et al.* 2011). The patchiness of prey and suitable foraging conditions in the marine environment has given rise to sympatric positive density-dependent foraging by fin and humpback whales (Piatt & Methven 1992, Skern-Mauritzen *et al.* 2011). While difficult to test, it has been suggested that facilitation may occur between species *e.g.* where feeding whales benefit from prey contained by diving seabirds and vice versa. Given that rorquals have evolved to be obligate batch-feeders, facilitation among these species, as well as seabirds and other marine predators, may be necessary to both locate and contain prey (Rudd *et al.* 2011). In light of this, the trade-off between positive density-dependent foraging and resource partitioning likely culminates in complex community structure among rorquals. Although constrained to filter-feeding by their shared filter-feeding adaptation, there is a diversity of different feeding techniques employed among fin, humpback, minke and blue whales. Humpback whales exhibit the highest diversity of feeding techniques among rorquals, curtailing their prey using bubble-net curtains (exhaled air used to create a barrier for prey), lob-tailing (stunning prey using the force of the tail fluke) and barriers such as the benthos or cliff faces (Hays *et al.* 1985, Weinrich *et al.* 1992, Hain *et al.* 1995). The marked asymmetry in fin whale pigmentation may be due to chirality ('handedness') in the direction of roll when lunge feeding, where one side of the head maintains counter-shading while lunge-feeding (Figure 1). In minke whales, individual specialization of foraging strategy has been shown to vary with individuals showing favoured lunge-feeding techniques that persist over several years (Hoelzel *et al.* 1989). Although sub-surface feeding strategies are difficult to observe, tags which contain multi-axis accelerometers are advancing our knowledge on optimal foraging in baleen whales (Goldbogen *et al.* 2011).

Rorquals consume a variety of prey across several trophic levels, although some species are more stenotypic than others, *e.g.* blue whales are exclusively planktivorous while minke whales are piscivorous. Prey preference and relative trophic level among species may differ between regions. For example, fin whales in the Gulf of St. Lawrence are thought to be piscivorous, whereas those of the Bay of Biscay and Mediterranean Sea feed almost exclusively on euphausiids (mostly *Meganyctiphanes norvegica*) (Relini *et al.* 1992, Borobia *et al.* 1995, Borrell *et al.* 2012). The fishes consumed by fin and humpback whales in the North Atlantic are

chiefly capelin (*Maillots villotus*), sand eel (*Ammodytes* spp.), herring (*Clupea harengus*), polar cod (*Boregadus saida*) (Piatt *et al.* 1989, Skern-Mauritzen *et al.* 2011). Minke whales primarily feed on capelin, sand eel, herring, sprat (*Sprattus sprattus*), cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melangrammus aeglefinus*) in the North Atlantic (Haug *et al.* 1995, Lindstrøm *et al.* 2002, Macleod *et al.* 2004, Pierce *et al.* 2004).

1.1.4 Distribution & Population Structure

Fin, humpback, minke and blue whales have a cosmopolitan distribution although the fin whale is the only species found in the Mediterranean Sea. The seasonally dictated migratory nature of rorquals has given rise to population structuring in the absence of physical barriers. This has come about due to seasonally opposed distribution given that northern and southern populations will be six months out of phase (Mackintosh 1965). Non-migratory populations have become reproductively isolated, for example humpback and blue whales in the Indian Ocean (Mikhalev 1997, Branch *et al.* 2007). Population structuring in humpback whales has been intensively studied (Smith *et al.* 1999, Palsboll *et al.* 2001, Stevick *et al.* 2003), revealing maternally directed philopatry to breeding and feeding grounds (Palumbi & Baker 1994). In the North Atlantic, humpback whales breed at two breeding grounds – The West Indies and Cape Verde (Smith *et al.* 1999). Whales from both populations converge on shared feeding grounds – Iceland and the Barents Sea (Figure 2). Whales breeding in Cape Verde have not been recorded in the western North Atlantic (Smith *et al.* 1999, Jann *et al.* 2003, Wenzel *et al.* 2009). The extent to which genetic exchange occurs between the two breeding grounds remains unknown, and the small population size of the Cape Verdean breeding population is a conservation concern (Punt *et al.* 2007, Wenzel *et al.* 2009).

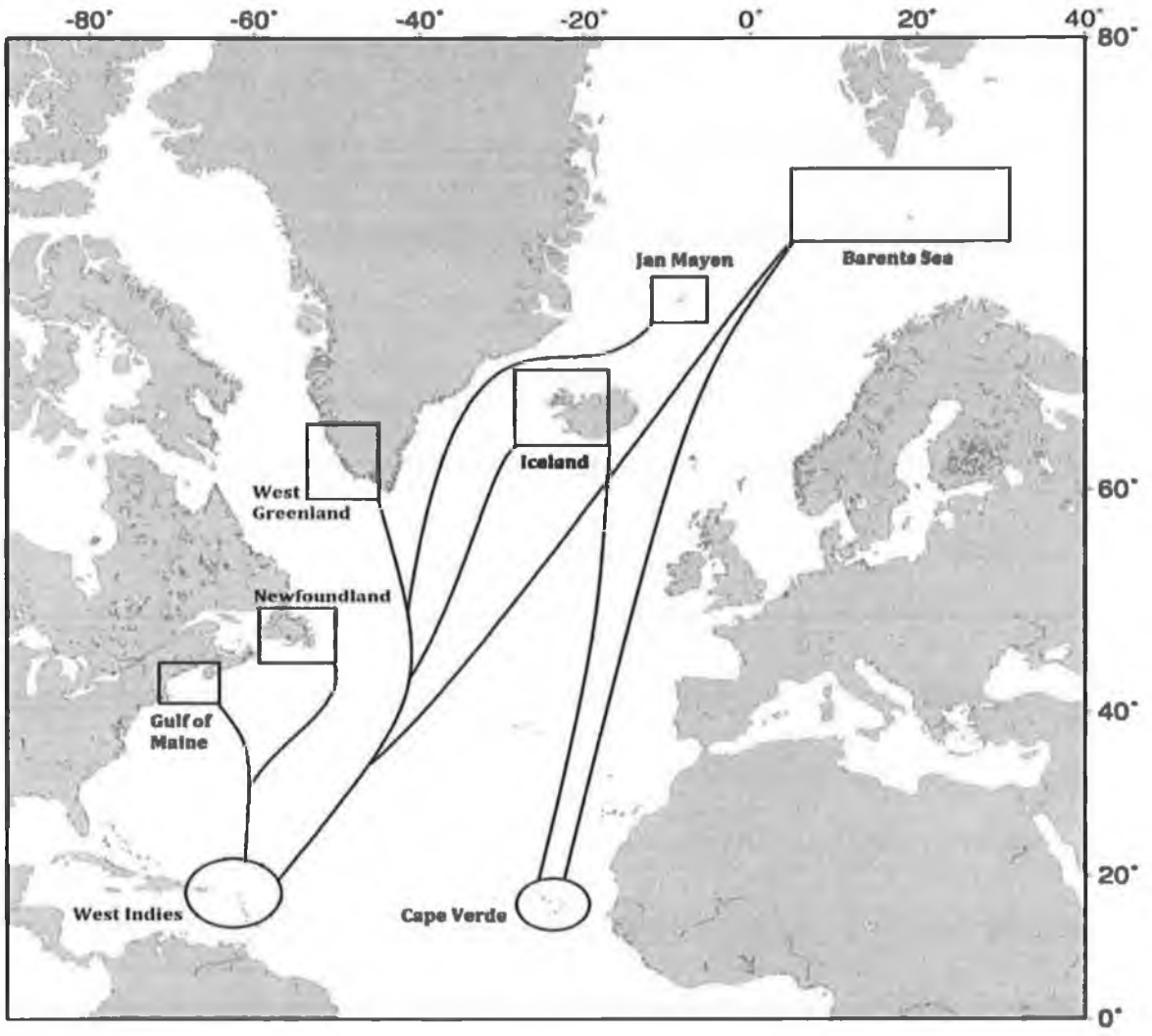

Figure 2. Map showing migration between major feeding grounds (squares) and known breeding grounds (circles) of humpback whales in the North Atlantic. The tracks are approximations as the true migration routes are not known.

The population structures of minke, fin and blue whales in the North Atlantic are poorly resolved owing to unidentified (or perhaps spatially non-explicit) breeding grounds, confounded by mixing of several populations on feeding grounds where sampling is primarily carried out (Donovan 1991, Reeves *et al.* 2004, International Whaling Commission 2007, Anderwald *et al.* 2011). Molecular genetic analysis of minke whales in the North Atlantic suggests a cryptic stock structure with possibly two breeding grounds (Anderwald *et al.* 2011). The location of neither is known however. Conflicting findings from genetic studies based on allozyme, mitochondrial

and nuclear markers has resulted in confusion over the degree of population structuring in North Atlantic fin whales (Danielsdottir *et al.* 1992, Bérubé *et al.* 1998, Palsbøll *et al.* 2004). A model of recurrent gene flow confounded by regional over-exploitation best explains the current poorly defined population within the North Atlantic (Palsbøll *et al.* 2004) It was thought that blue whales in the North Atlantic may constitute several discrete feeding populations, however an ocean-basin scale acoustic study did not support this view (Sigurjónsson & Gunnlaugsson 1988, Clark 1994). In light of continued and past exploitation of rorquals in the North Atlantic, and in spite of an international moratorium on whaling, knowledge on ecologically relevant population structuring is required if conservation goals are to be identified and realised.

1.2. Ecological Tools and Sampling

1.2.1 Stable Isotope Analysis

There are few tools available to ecologists that yield quantitative information on movements, diet and community structure. Stable isotope analysis exploits the fact that light elements (C,N,O,H) are abundant in animal tissues where the ratio of light to heavy isotopes of these elements reflect the environment from which they are derived (subject to predictable metabolic processes). As such, stable isotopes are intrinsic markers that track energy and nutrients through complex food-webs to provide information on the provenance of food and on diet generally (Peterson & Fry 1987). Biological processes manipulate isotopic compositions *e.g.* the relative abundance of heavy (^{13}C) to light (^{12}C) carbon in the tissue of a plant is dependent on the carbon fixation pathways used by that plant (CAM, C_3 or C_4 metabolism) (Smith & Epstein 1971). In the marine environment, the baseline carbon isotopic composition of food-webs is dictated principally by the concentration of molecular carbon dioxide [$CO_2(aq)$] of the seawater which varies spatially given that it is ultimately a function of sea temperature (Rau *et al.* 1992, Goericke & Fry 1994). Baseline nitrogen isotopes reflect the availability and types of nutrients that supply energy to a food-web, *i.e.*

ammonium, nitrate or N_2. As with carbon, the nitrogen isotopic composition in the marine environment is controlled by dynamic physical and chemical processes such as sea temperature and vertical mixing or upwelling. The spatially predictable forces that are responsible for ultimate baseline isotopic compositions give rise to large scale patterns including enrichment of ^{15}N towards the shore and depletion of ^{13}C with latitude and distance from shore. By assaying tissues of predators, broad-scale mapping of animal movements through an isotopic landscape ('isoscape') can be carried out provided that baseline variability (both spatial and temporal) is known (West *et al.* 2010, MacKenzie *et al.* 2011). However, some stable isotopes are subject to fractionation due to metabolic partitioning in the tissues of consumers. This is best exemplified in nitrogen isotopes of animal tissues, whereby successive enrichment of *circa* 3.4 ‰ per trophic level occurs, where ^{15}N is enriched relative to ^{14}N, thus allowing for trophic and hence ecological community studies (DeNiro & Epstein 1981, Layman *et al.* 2007).

Stable isotope compositions of samples are measured by mass spectrometry, an analytical technique where the mass-to-charge ratio (specific to each isotope) is measured in ionized compounds of the sample. In stable isotope analysis, standard delta notation has been adopted in order to standardise values; to render them directly comparable between studies. This is achieved by measuring the isotopic abundances of samples and known international standard references simultaneously. Thus the stable isotope value used is a ratio of the heavy to light isotope divided by that of a standard. The standards used are certified by the International Atomic Energy Association and include Pee Dee Belemnite (originally calcite from a fossil *Belemnitella americana* found in the 'Pee Dee Formation') for $\delta^{13}C$ and atmospheric N_2 (air) for $\delta^{15}N$. Given the high accuracy and precision of the technique, coupled with the small variation in isotope concentrations that are of interest, the ratio is presented as parts per thousand as opposed to per cent:

$$\delta^{Y}X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R denotes the heavier to lighter isotope ratio and Y is the atomic mass of the stable isotope X ($\delta^{13}C$ or $\delta^{15}N$)

1.2.2 Persistent Organic Pollutants

Persistent organic pollutants (POPs) are synthesised chemicals that are highly resistant to natural degradation. It is this property that made them fit for purpose for a wide range of applications including insecticides (Chlordanes (CHs), Dichlorodiphenyltrichloroethanes (DDTs), Hexachlorocyclohexanes (HCHs)) lubricants and insulators (Polychlorinated biphenyls (PCBs)). It was discovered that these compounds comprise xenobiotics: posing significant health and environmental threats. The risks to biota include endocrine-disruption, cancer and immunotoxicity, leading to bans on their usage in many nations. However, their use in developing nations continues, particularly in insect-borne disease control such as malaria (Roberts *et al.* 1997). Despite restrictions on their usage, POPs are ubiquitous in the environment due largely to atmospheric transport and bioaccumulation which results from their high lippophilicity. Known as the 'global distillation effect', the liquid vapour pressure (*i.e.* boiling point pressure) which is dependent on temperature, is such that at cool temperatures, most POPs are readily distilled from the atmosphere (Simonich & Hites 1995). Environmental POP concentrations in colder regions such as the Arctic Basin can therefore be elevated despite the fact that they are far from the pollution source, namely the large industrialised cities of mid-latitudes (Muir *et al.* 1988). Thus long-lived, high trophic level predators with a lipid-rich prey sourced in high latitudes, such as marine mammals, will be especially at risk from detrimental concentrations of POPs (Tanabe *et al.* 1988, 1994, Tanabe 2002). POP burdens are offloaded to offspring by placental transfer and *postpartum* in the lipid-rich milk (Aguilar & Borrell 1994). As such, sex is a crucial co-factor for consideration when interpreting POP concentration data. Given that patterns of POP contaminants in the environment are spatially explicit over large geographical scales, they can be used as a tracer in highly migratory animals to assist in resolving population structure where multiple feeding populations mix on breeding grounds. Although an unfortunate and destructive by-product of the industrialised human enterprise, POPs are fortuitous

intrinsic markers that can shed light on the ecology of poorly studied migratory populations such as baleen whales (Elfes *et al.* 2010).

1.2.3 Biopsy Sampling

The intrinsic markers harboured in tissues may be used to measure ecological parameters, to track migrations and to understand population connectivity, particularly in highly migratory species such as baleen whales. Considering the threatened status of many cetacean species, minimally invasive sampling techniques such as biopsy sampling have been to developed to allow scientific research without the need for lethal sampling (Lambertsen 1987, Barrett-Lennard *et al.* 1996). Projectiles fired using a crossbow or modified rifle can collect both skin and blubber samples for use in *inter alia* stable isotope, POP, molecular genetic, trace element and immunohistochemical analyses. The bolt (biopsy dart) comprises a long shaft with flights, a compressed foam stop-collar and a removable sampling tip with internal barbs and a sharpened cutting edge. Samples are taken from an area about the dorsal fin as this is presented clear of the water during surfacing and the blubber there is both thick and biochemically representative of the entire blubber envelope (Aguilar & Borrell 1990, 1991). The effects of this sampling technique on whale behaviour are not severe, usually momentary and only detectable for half of the successful hits (Weinrich *et al.* 1992, Clapham & Mattila 1993). Furthermore, biopsy wound healing occurs quickly which reduces the risk of infection posed by piercing the integument (Krtüzen *et al.* 2002).

1.3. Conservation Status

1.3.1 History of Whaling in Irish Waters

The first attempt at commercial whaling in Irish waters was carried out in Donegal Bay in the middle of the eighteenth century (Went 1968). Too few whales were caught to make the venture commercially viable, however the invention of the

explosive swivel harpoon gun by Thomas Nesbitt subsequently paved the way for efficient large-scale whaling (McGonigle 2008). After a long hiatus in whaling during the nineteenth century, plans for the establishment of Norwegian whaling operations in northwest Ireland were vehemently opposed by locals. This protest was grounded in conservation concerns for the local herring fishery, chiefly the effects of effluent and the use of explosives (Fairley 1981). However in 1908 commercial whaling recommenced at the Blacksod Whaling Company in Co. Mayo and by 1910 a second station was operational nearby on the Iniskea Islands, collectively operating six whaling vessels (Fairley 1981). Between 1908 and 1920 an estimated 525 fin whales, 87 blue whales, 77 sei whales (*B. borealis*), 18 right whales (*Eubalaena glacialis*) and just 5 humpback whales were landed (Went 1968). The small numbers of humpback whales landed probably reflects the widespread over-exploitation elsewhere: on the West Indies and Cape Verde breeding grounds by Yankee whalers to the extent that voyages there were no longer profitable by the late 1880s (Smith & Reeves 2003). Shore-based whaling in Irish waters ceased in 1922, however a fleet of 10 Norwegian registered vessels continued to catch whales in Irish waters until 1976 when the Wildlife Act imposed a ban on the killing of cetaceans in Irish waters (Fairley 1981).

Figure 3. A whaling ship attempts to catch a fin or blue whale in 1911off the coast of Co. Mayo (Visible in the background). Picture courtesy of Leslie Hamilton Wilson.

1.3.2 Legislation

Cetaceans in Irish waters are protected by several national and international legislative instruments. The first appearance of legislation for the protection of baleen whales came in the first decade of the twentieth century when bye-laws were implemented to manage the whale fisheries of the northwest of Ireland (Fairley 1981). This was followed by the Whale Fisheries Act of 1908 which regulated the industry and allowed for public consultation in the issuing of licences (Fairley 1981). Following summonsing of the International Whaling Convention by the League of Nations, Ireland enacted the Whale Fisheries Act of 1937 which afforded more stringent protection including an outright ban on whaling within the Irish economic exclusion zone (EEZ). This act was reinforced by the Wildlife Act of 1976 many years later, galvanising political will for the conservation of cetaceans in Irish waters by prohibiting hunting, injury, destruction of breeding places and willful interference. The establishment of a whale and dolphin sanctuary in Ireland, the first of its kind in Europe, was not supported by any additional legislative instruments. As such the effectiveness of this political gesture in conserving cetaceans is questionable (Rogan & Berrow 1995).

Ireland is signatory to several relevant international conventions including the Bonn Convention (Conservation of Migratory Species of Wild Animals), the OSPAR Convention (The Convention for the Protection of the Marine Environment of the Northeast Atlantic) and CITES (The Convention on International Trade in Endangered Species). Ireland is not party to the ASCOBANS (Agreement on the Conservation of Small Cetaceans in the Baltic Sea and North Sea) despite Irish waters being included in its remit. Ireland joined the International Whaling Commission in 1985 and in 1997 the Irish delegation announced 'The Irish Proposal'. This was a radical proposal which aimed to address the ongoing stalemate in the IWC as to whether limited whaling should be agreed upon considering the ubiquitous exploitation of loop-holes (*e.g.* scientific whaling) by some nations.

Perhaps the most ambitious legislation to date however has been the EU Habitats Directive (1992) which legally enforces Ireland to establish a network of Special Areas of Conservation for harbour porpoises (*Phocoena phocoena*) and bottlenose dolphins (*Tursiops truncatus*). It provides protection for all cetaceans including baleen whales (listed in Annex IV) for the extent of the EEZ and requires Ireland to achieve and maintain a favourable conservation status for these species, enforcing financial penalties for non-compliance. However, the means by which favourable conservation status can be defined and monitored remains a challenge, particularly considering the scale of the Irish EEZ which occupies an area eight times that of the landmass (Figure 5). In 2004, the EU Council agreed that EU member states must also monitor fisheries bycatch of cetaceans, take mitigation measures and report on these measures annually (Council Regulation 812/2004).

1.3.3 Threats

The most significant threats faced by humpback and minke whales in Irish waters are believed to be fisheries bycatch (Anonymous 2009). This is presumably due to their near-shore distributions where there is a greater chance for interacting with intensive fishing activity, and would therefore also apply to fin whales that forage close to shore. Both minke and humpback whales have fallen victim to entanglement in fishing gear, particularly lobster creels (IWDG, unpublished data). Indeed, entanglement in creel buoy-lines and ground-lines is a major source of mortality for humpback whales throughout the North Atlantic (Johnson *et al.* 2005). Sound pollution and ship strikes have been identified as potential threats to fin and blue whales, but not humpback whales (Smiddy 1990, Anonymous 2009). Given their similar ecologies, it is likely that these threats equally apply to humpback whales in Irish waters, but perhaps a lack of data precludes assessment of major threats to survival here. The effects of anthropogenic ecosystem alterations such as trophic cascades due to over-exploitation on rorquals are axiomatic yet they have not been

listed as threats to cetaceans on the conservation plan for cetaceans in Irish waters (Pauly *et al.* 1998, Anonymous 2009).

1.4. Background and Rationale of the Study

On an ocean-basin scale, the movements, population structure and feeding ecologies of most rorqual species are poorly understood. This has hampered efforts to effectively manage them as a resource that is highly mobile across international boundaries (Donovan 1991, International Whaling Commission 2007). This issue is complicated further by culturally entrenched differences on how whales ought to be managed – whether conserved for their bequest value, or utilised for human consumption (Clapham *et al.* 2007). Irrespective of perceived cultural differences, it is incumbent on management bodies to implement effective measures to prevent further loss of species which remain threatened due to harvesting or indirect anthropogenic effects including pollution, bycatch, climatic change and trophic cascades. Information on the movements, population discrimination and preferred prey of rorquals throughout most of their range is currently lacking, but is required to make informed decisions to this end.

From a national economic perspective, the resource requirements, preferred prey and spatiotemporal occurrence of top predators such as rorquals will be of key concern to industries such as ecotourism and fisheries. Critical to achieving sustainable exploitation of marine biological resources is an understanding of trophic interactions and hence the potential interactions between industries such as fisheries and ecotourism. Only then can mutually effective management procedures be implemented (Duffy 2003, Hoyt 2005). In order to place into context the relevance and necessity of this research project, the following paragraphs summarise the paucity of available information on rorquals in Irish waters,.

1.4.1 Current Knowledge on Rorquals in Irish Waters

A review of recent research on cetaceans in Irish waters was carried out by O'Brien *et al.* (2009) which identified key gaps in knowledge that need to be addressed in order to form policies that maintain conservation objectives. Despite a lack of the most basic information on their ecology in Irish waters (including stock affinity, location of breeding grounds, preferred diet and abundance) both fin and minke whales have been assigned a 'good' conservation status, while humpback and blue whales are categorized as 'unknown' (Anonymous 2009). The Irish Whale and Dolphin Group (IWDG) records strandings and sightings in Irish waters from which a recent review was compiled (Berrow *et al.* 2010). Both stranding and sighting records suggest that minke whales are the most abundant rorqual species, followed by fin and humpback whales, while blue whale records are exceptionally rare (Wall *et al.* 2009, Berrow *et al.* 2010). The peak in sightings reported to the IWDG occurs in August for minke whales, September to November for humpback whales, and November for fin whales (Berrow *et al.* 2010).

In abyssal waters to the west of Ireland, a static hydrophone array known as the Sound Surveillance System (SOSUS) was used to monitor rorquals during the late 1990s (Clark & Charif 1998). Detections of fin whales occurred in every month of the year and detections (relative abundance) peaked in September but reached a minimum during May to July (Clark & Charif 1998). Similarly in the Celtic Sea, sighting data in coastal waters reveal a peak in November and a minimum in April (Whooley *et al.* 2011). It is interesting to note that an increase in fin whale acoustic activity during March in the Mid-Atlantic Ridge (MAR) corresponds with a decrease in Irish waters (Nieukirk *et al.* 2004). Although it is not possible to determine whether these trends are related, it is consistent with a southwesterly movement of fin whales from Ireland towards the MAR in spring. The suggestion that fin whales exhibit a seasonal southerly movement into the Bay of Biscay is currently unsubstantiated by any evidence (Anonymous 2009). A single fin whale tagged in the Faro Islands was tracked for 116 days moving south towards Madeira/Azores before spending two months in Irish waters (Mikkelsen *et al.* 2007), covering average of 82 km per day

near the continental shelf edge (Figure 4). Although based on a single sample, this shows that Ireland may constitute an important feeding ground, rather than just a migratory corridor for fin whales. Using photo identification, a high fidelity to the coastal waters of the Celtic Sea was found for fin whales, with 18% of individually recognisable whales recorded inter-annually (Whooley *et al.* 2011).

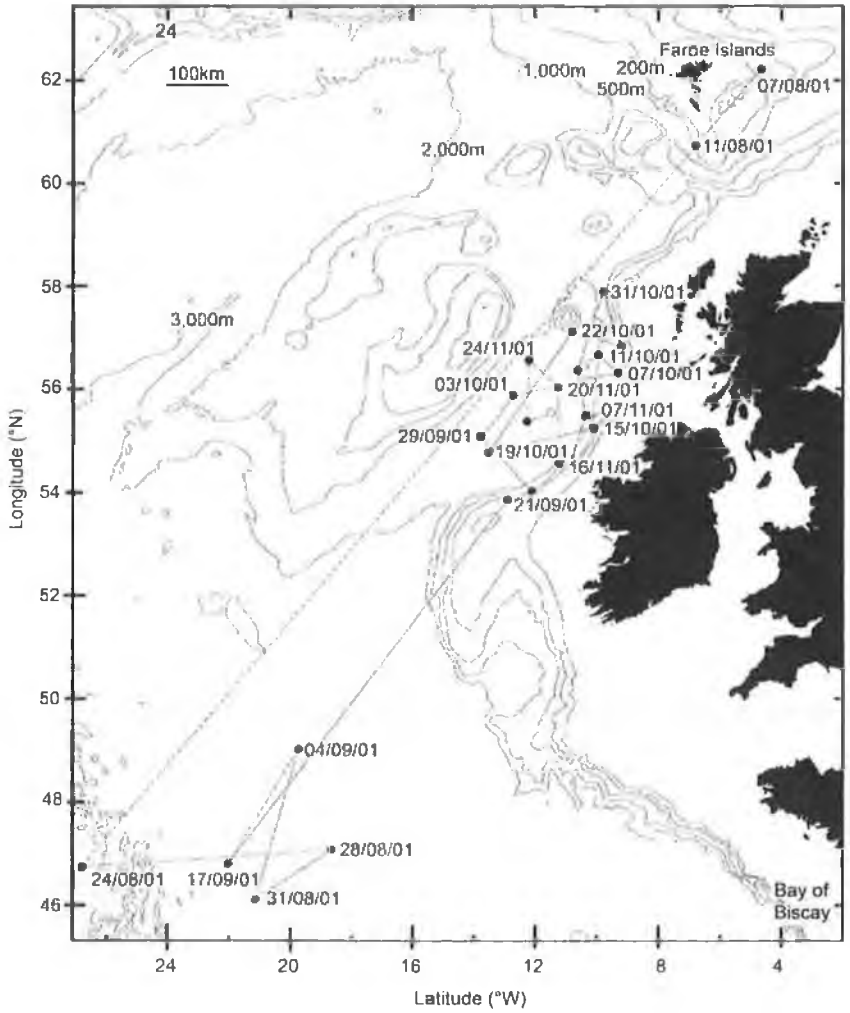

Figure 4. The track of a fin whale tagged in Faro Islands on 7 August 2001 for positions received every fourth day (solid line) or less frequently than every fourth day (dashed line). From Mikkelsen *et al.* (2007).

Seasonal patterns in recordings of humpback whales made by SOSUS off the west coast of Ireland are consistent with a southerly migration from Arctic waters (with no corresponding northerly migration), primarily towards the West Indies breeding grounds (Charif *et al.* 2001). The peak number of whales detected by hydrophone

arrays closes to Ireland occurred in March (Charif *et al.* 2001). A longitudinal photo-identification study of humpback whales has been carried out by the Irish Whale and Dolphin Group since 2001. Of the 20 individually identified whales, 45% have been recorded in multiple years, mostly in the Celtic Sea, though this is likely to reflect the high research effort there (Berrow *et al.* 2012). Photo-identification images are lodged with the North Atlantic Humpback Whale Catalogue curated by Allied Whale, College of the Atlantic, however no matches have been made to date (Berrow *et al.* 2012). A single juvenile whale recorded in the Celtic Sea had been recorded previously and subsequently in The Netherlands (Berrow *et al.* 2012). Blue whales were recorded acoustically during all months of the year to the west of Ireland, with a peak in abundance between September and December (Clark & Charif 1998).

There have been no studies on the diet of baleen whales in Irish waters in recent times (O'Brien *et al.* 2009). Many of those that strand undergo rapid autolysation thus obtaining useful stomach a sample is logistically challenging. The only available information on the diet of baleen whales from Irish waters comes from observations made during 1911 of stomach contents of whales (mostly fin whales) landed at the Blacksod and Inishkea whaling stations in Co. Mayo, northwest Ireland (Fairley 1981). Fin whales were found to be feeding almost exclusively on euphausiids ('krill'), although the species was not determined (Burfield 1913). The author, a visiting biologist, noted that the crew of the whaling ships recognised two 'forms' of fin whales: one with an apparently specialised diet of herring, short in length, dark in colour and more difficult to catch. Another 'form' that was lighter in colour, larger, more easily approached and fed mainly on euphausiids (Burfield 1913). Blue, humpback and sei whales landed at the whaling stations were also feeding primarily on euphausiids (Burfield 1913, Fairley 1981). Based on observations of foraging activity, it has been suggested that fin and blue whales were feeding on northern krill (*Meganyctiphanes norvegica*) over the continental shelf edge to the west of Ireland, while the seasonal distribution of fin whales in coastal waters appears to be linked with the locations of spawning herring (Wall *et al.* 2009, Whooley *et al.* 2011).

1.4.2 Celtic Sea Ecosystem and Fisheries

The Celtic Sea is a shallow temperate sea that is highly productive and is therefore subject to intense fisheries for benthic and pelagic fishes (Figure 5). Discarding of non-target catch is a serious concern: for example almost 63% of the 186 million fish caught in the Celtic Sea and adjacent waters between 2002 and 2005 were discarded (Enever *et al.* 2007). Marine mammal bycatch is also a management concern with common dolphin (*Delphinus delphis*) and harbour porpoise (*Phocoena phocoena*) particularly at risk (Tregenza *et al.* 1997a, b, Berrow *et al.* 1998). Evidence of widespread alterations in the ecological community structure of the Celtic Sea has been found (Pinnegar *et al.* 2002). In 2003 a marine protected area called the Biologically Sensitive Area (BSA) was established in order to protect biological sensitivity and the commercial importance of this region. The BSA follows the 200m contour from Slyne Head to Waterford Harbour and encompasses about 40% of the Celtic Sea (Figure 5). One of the most abundant species of zooplankton (up to 35% of zooplankton biomass) is *Nyctiphanes couchii* (Williams & Fragopoulu 1985). At the continental shelf edge, however *M. norvegica* are more abundant and both species confer significant biomass to subsequent trophic levels in the CS (Lindley 1982).

From a global fisheries management perspective, the Celtic Sea herring fishery is perhaps an unusual case in that a carefully considered monitoring scheme was already in place before industrial scale exploitation of fish stocks commenced. Although carefully monitored, some fisheries do not recognise stock substructure. In the case of Celtic Sea herring a single fishery targets two stocks that are subject to different trends in recruitment (Harma *et al.* 2012). Although they are believed to be a key species in the Celtic Sea ecosystem (Chivers *et al.* 2012), sprat is subject to a fishery with an open quota. This is in spite of the fact that their life history, stock structure and spawning grounds are poorly known.

The indirect effects of fisheries on top predators may be significant, for example, discard practices may provision scavenging seabird species such as gulls, thus augmenting population sizes (Furness 2003, Votier *et al.* 2004). The number of

trophic levels in the Celtic Sea ecosystem is declining due to the systematic removal of predatory fish (Pinnegar *et al.* 2002, 2003). As in the North Sea, this may have a competitive release effect on lower trophic species such as sandeels (*Ammodytes* spp.) or clupeids that may benefit certain predators that specialise on these species (Furness 2003). While a more simplified trophic system may benefit some specific fisheries and predators, it may also be less resilient to perturbations arising from climate change and pollution (Folke *et al.* 2004).

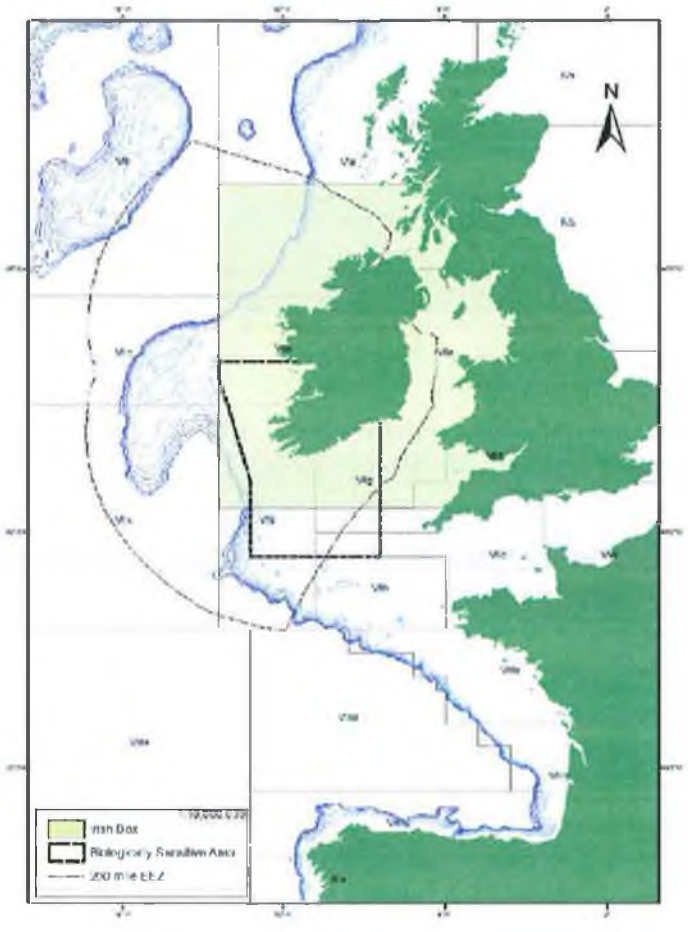

Figure 5. Map of the Irish Economic Exclusion Zone (EEZ), showing the Biologically Sensitive Area (BSA), Celtic Sea and continental shelf edge. Courtesy of the Beaufort Project, Queens University Belfast.

1.5. Objectives and Outline of the Study

1.5.1 Primary Aims

The overall aim of this thesis is to investigate the feeding ecology and migration of baleen whales that occur in Irish waters, and chiefly in the Celtic Sea. Despite a persistent call for an ecosystem approach to resource management of marine resources, little information exists on the ecosystem role and requirements of baleen whales in Irish waters. This thesis aims to bridge gaps in knowledge on this important and charismatic taxon, with its history marred by over-exploitation, in order to facilitate more informed management decisions and the implementation of more effective conservation measures.

The thesis comprises seven chapters including a general introduction and conclusion. The main body of the thesis consists of five peer-review articles which form chapters. Although each paper can be considered an autonomous study, they are organised in line with a logical progression to form a cohesive thesis. Each of these papers has already been, or will be, subjected to peer-review with a view to publishing in order to disseminate the findings. Below is a brief overview of each chapter outlining the primary aims and the citation of each publication.

1.5.2 Summary of Chapters

Chapter 2: Stable isotope analysis of baleen reveals resource partitioning among four sympatric species of rorqual and population structure in fin whales in the Northeast Atlantic

In order to investigate resource partitioning among blue (*Balaenoptera musculus*), fin (*B. physalus*), humpback (*Megaptera novaengliae*) and minke whales (*B. acutorostrata*), the stable isotopic composition of carbon and nitrogen in keratin was

measured along the growth axis of baleen plates. Samples were sourced from collections and strandings between 1892 and 2010 in order to delineate the isotopic niche of each species as a proxy for resource use to investigate resource partitioning. This was carried out using Bayesian inference through SIBER (Stable Isotope Bayesian Ellipses in R). Primary aims:

- To investigate whether rorquals which occur sympatrically in Irish waters occupy discrete isotopic niches that are persistent over time.
- To infer resource partitioning and degree of diet specialisation among the four species.
- To identify evidence for population structure that may exist between fin whales in the Northeast Atlantic (Ireland & UK) and the Mediterranean.

To be published in Marine Ecology Progress Series (subject to re-review).

Chapter 3: Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of skin and blubber of balaenopterid whales

Stable isotope analysis has promising potential and many applications in ecology. However many fundamental assumptions of the technique are transferred to unrelated taxa or left untested. Stable carbon isotopes are subject to bias as they are depleted in ^{13}C and are subject to complex metabolic pathways. To account for this, two approaches are used: mathematical correction ('normalization') and lipid-extraction. Both are considered equally valid, however the extraction process may alter the $\delta^{15}N$ values which are usually measured simultaneously to reduce the cost and amount of tissue required. As biopsy samples are small and difficult to acquire, single analysis with *a posteriori* normalization is preferable, however the accuracy of normalization models and effects of lipid extraction on sample integrity remain poorly known. The primary aims of this study are:

- To test the accuracy and precision of six normalization models to correct $\delta^{13}C$ values for lipid content in skin and blubber from fin, humpback and minke whales

- To identify suitable normalization models to correct $\delta^{13}C$ values for use in ecological applications such as diet solution mixing models.
- To measure the effects and predictability of lipid-extraction on the $\delta^{15}N$ value

Ryan, C., McHugh, B., Trueman, C.N., Harrod, C., Berrow, S.D. and O'Connor, I. (2012) Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of skin and blubber of balaenopterid whales. Rapid Communications in Mass Spectrometry 26:2745-2754.

Chapter 4: Prey Preferences of Sympatric Fin (*Balaenoptera physalus*) and Humpback (*Megaptera novaengliae*) Whales Revealed by Stable Isotope Mixing Models.

The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) composition of skin biopsies and tissues from euphausiids (*Meganyctiphanes norvegica* and *Nyctiphanes couchii*) and clupeids (*Clupea harengus* and *Sprattus sprattus*) collected in the Celtic Sea were measured. The proportionate contribution of putative prey (sources) in feasible diet solutions of fin and humpback whales (mixture) is analysed using stable isotope mixing models in Bayesian framework known as SIAR (Stable Isotope Analysis in R). The primary aims are to:

- Identify most likely prey sources of fin and humpback whales occurring in the Celtic Sea.
- Identify age-class prey choice by fin and humpback whales
- Shed light on the ecosystem role of baleen whales in light of managed (herring) and unmanaged (sprat) fisheries which exploit fish stocks that are strongly influenced by complex environmental factors (*e.g.* climate change).

Ryan, C., Berrow, S.D., McHugh, B., O'Donnell, C., Trueman, C.N. and O'Connor, I. (In Review) Prey preferences of sympatric fin (*Balaenoptera physalus*) and humpback (*Megaptera novaengliae*) whales revealed by stable isotope mixing models. Marine Mammal Science.

Chapter 5: Methodological Study: Sampling Effect of the Biopsy Technique Leading to Significant Lipid Loss

Biopsy sampling has revolutionised our understanding of cetaceans, by facilitating studies on pollutants, stable isotopes and molecular genetics. However some of the underlying assumptions of the technique remain untested. Here, lipid content of the blubber portion of biopsy samples are considered given their importance in estimating nutritive condition (*i.e.* fattening) and tissue concentrations of lipophilic pollutants. The main aims are to:

- Test if lipid loss occurs in blubber due to biopsy sampling.
- Test if lipid loss occurs as a function of 'soak time' in sea water prior to sample retrieval.

Ryan, C., McHugh, B., O'Connor, I., Berrow, S. (2012) Lipid content in blubber biopsies is not representative of blubber in situ for fin whales (*Balaenoptera physalus*). Marine Mammal Science, 28: In Press.

Chapter 6: Levels of Persistent Organic Pollutants in Northeast Atlantic Humpback Whales

Cape Verde Islands constitutes the second known breeding ground for humpback whales in the North Atlantic. The putative Northeast Atlantic (NEA) population was re-discovered in the mid 1990s and is estimated to number just 200 individuals. Persistent organic pollutants (POPs) pose a potential threat to reproductive capacity and hence the maintenance of threatened populations. Patterns of POP burdens may also be used as a fortuitous tracer, given that whales integrate profiles which reflect those of the environment which are geographically determinate due to differential inputs and latitudinal gradients. The primary aims are:

- To estimate baseline levels of PCBs, DDTs, HCHs and chlordanes in the poorly studied NEA humpback whales

- To investigate linkages between Irish and Cape Verdean humpback whales, POPs are used as a tracer to infer geographically explicit groups, using western Atlantic (Gulf of Maine) samples as an out-group.
- To infer possible feeding ground fidelity based on qualitative and quantitative patterns of POP burdens in Cape Verdean humpback whales.

Proposed Journal: Endangered Species Research

Chapter 7: Conclusion

In the concluding chapter, overarching themes including foraging ecology and migrations, primarily of fin and humpback whales (but including minke and blue whales) are drawn upon from the five studies to discuss the relevance of the main findings for conservation and management of whales in Irish waters.

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Chapter 2.

Stable isotope analysis of baleen reveals resource partitioning among four sympatric species of rorqual and population structure in fin whales in the Northeast Atlantic

"Nature holds the key to our aesthetic, intellectual, cognitive and even spiritual satisfaction"

Edward Osborne Wilson

This chapter is reproduced from the following paper which has been reviewed, and may be accepted subject to changes:

Ryan C, McHugh B, Trueman, CN, Sabin, R, Deaville, R, Harrod, C, Berrow SD and O'Connor I (In Review) Stable isotope analysis of baleen reveals resource partitioning among four sympatric species of rorqual and population structure in fin whales in the Northeast Atlantic. Marine Ecology Progress Series.

2.1 Abstract

Stable isotope analysis is a useful tool for investigating diet, migrations and niche in ecological communities by tracing energy through food-webs. In this study, the stable isotopic composition of carbon and nitrogen in keratin was measured at growth increments of baleen plates from four sympatric species of rorquals (*Balaenoptera acutrostrata, B. musculus, B. physalus* and *Megaptera noveaengliae*) which died between 1892 and 2010 in Irish and contiguous waters. Bivariate ellipses were used to plot isotopic niches and standard ellipse area parameters were estimated *via* Bayesian inference using the SIBER routine in the SIAR package in R. Evidence of resource partitioning was thus found among fin, humpback and minke whales. Significantly larger niche area and higher overall $\delta^{15}N$ and $\delta^{13}C$ values found in fin whales from Irish/UK waters compared to those sampled in adjacent regions (Bay of Biscay and Mediterranean) suggest inshore foraging that may be unique to fin whales in Ireland and the UK. Highest $\delta^{15}N$ values were found in minke whales followed by humpback, fin and blue whales. Baleen from calves was enriched in ^{15}N which was attributed to suckling. Comparison between Irish/UK, Biscayan and Mediterranean

fin whales support the current IWC stock assessment of an isolated Mediterranean population. Isotopic profiles support spatial overlap but different foraging strategies between fin whales sampled in Ireland/UK and the Bay of Biscay. Stable isotope analysis of baleen could provide an additional means for identifying ecological units, thus supporting more effective management for the conservation of baleen whales.

2.2 Introduction

Following two centuries of commercial whaling, the current ecological assemblage and population sizes of rorquals (Family Balaenopteridae) are believed to be very different to those of pre-whaling times (Roman & Palumbi 2003). While we know little of their ecosystem role, the near extirpation of some species of baleen whales has been associated with subsequent trophic cascades (Springer *et al.* 2003, Ballance *et al.* 2006). Poorly managed fisheries may exert a top-down control on marine food webs, assuming the functional role of apex predators such as cetaceans and hence replacing them (Trites *et al.* 2006). Policies for sustainable management of marine resources are attempting to move from single species based approaches to include ecosystem-scale effects, but designing effective ecosystem-based management requires knowledge of species interactions within a spatio-temporal context (Pauly *et al.* 2002, Gerber *et al.* 2009). These data are often missing, particularly for highly migratory, high trophic level predators. Basic biological information on foraging strategies, relative trophic positions and resource partitioning is also needed for effective species-based management and maintenance of cetacean populations. In this study, the foraging ecology of four sympatric species of rorqual: blue whales

(*Balaenoptera musculus*), fin whales (*B. physalus*), humpback whales (*Megaptera noveangliae*) and minke whales (*B. acutrostratus*) is investigated over a century using stable isotope analysis of baleen plates.

In the North Atlantic, fin, minke and humpback whales are considered to be generalist predators, consuming pelagic schooling fishes, primarily capelin (*Mallotus villosus*) and herring (*Clupea harengus*), and zooplankton such as krill (Euphausiacea) and amphipods (Piatt & Methven 1992, Lindstrøm *et al.* 2002, Laidre *et al.* 2010, Skern-Mauritzen *et al.* 2011). Fin whales in the North-east Atlantic (NEA) and Mediterranean Sea have been shown to feed chiefly on northern krill (*Meganyctiphanes norvegica*) and other zooplankton (Relini *et al.* 1992, Aguilar 2008, Skern-Mauritzen *et al.* 2011, Visser *et al.* 2011). However in Ireland, where they occur in coastal waters for up to 10 months of the year, they have been colloquially referred to as 'herring hogs': reflecting the fact that they associate strongly with an inshore movement of spawning herring (Fairley 1981, Whooley *et al.* 2011). Gut content-analysis of fin whales landed in 1912 at a whaling station in Ireland found that fin whales were feeding on both clupeids ('herrings') and northern krill, while blue whales fed exclusively on the latter (Burfield 1913, Fairley 1981). Two studies at separate sites in Scotland yielded consistent findings that minke whales there had a piscivorous diet (Macleod *et al.* 2004, Pierce *et al.* 2004). Blue whale sightings in the NEA are exceptionally rare, however they have been observed in association with fin whales feeding in pelagic waters on northern krill (Pike *et al.* 2009, Wall *et al.* 2009).

Rorquals are difficult to study as they can be elusive and often occur offshore, ranging over large geographical areas. Rapid onset of autolysis in stranded specimens due to

their large body sizes usually renders stomach content analysis impractical. A moratorium on whaling, along with conservation and ethical issues preclude lethal sampling for research. Considering these challenges, stable isotope analysis is a tool that is being used with increasing frequency in marine mammal research to address gaps in our knowledge (Newsome *et al.* 2010, Ramos & González-Solís 2012). It presents an alternative to more conventional approaches to studying diet with the benefit of providing proxy data on habitat use and movements (Lee *et al.* 2005, Newsome *et al.* 2010, Ramos & González-Solís 2012). While conventional diet analyses such as stomach content analysis offers high taxonomic resolution, it provides information over a short timescale and is subject to several sources of uncertainty and bias (Layman *et al.* 2007, Pierce *et al.* 2007).

Stable isotope ratios in tissues reflect those of the environment and prey from which the tissues were synthesised (DeNiro & Epstein 1978). Both provenance of feeding and trophic status can be inferred using $\delta^{13}C$ and $\delta^{15}N$ values respectively on the basis that isotopic values are transferred from prey to consumers in a predictable manner (DeNiro & Epstein 1978, McConnaughey & McRoy 1979). Stable carbon and nitrogen isotope values of tissues are thus intrinsic markers which trace nutrients and energy through food webs. Although less spatially and temporally explicit than extrinsic markers (such as satellite or radio tags), stable isotope analysis can provide information on migratory movements and foraging habits of animals provided that they feed along an isotopic gradient where isotopic baseline is known (Harrod *et al.* 2005, MacKenzie *et al.* 2011, Trueman *et al.* 2012a). The technique can provide quantitative data to investigate *inter alia* resource partitioning and other complex processes that govern structure in ecological communities (Jackson *et al.* 2012).

However metabolic (*e.g.* fractionation) and baseline sources of isotopic variation are non-trivial, necessitating careful consideration when interpreting stable isotope values in an ecological context (*e.g.* Graham *et al.* 2010, Trueman *et al.* 2012).

Marked fractionation of nitrogen isotopes occurs in at each trophic level in animal tissues whereby ^{15}N is progressively concentrated relative to ^{14}N given that the latter is preferentially excreted in nitrogenous waste (DeNiro & Epstein 1981). The magnitude of trophic enrichment for a given tissue, termed the tissue-diet discrimination factor, is both species and tissue-specific, *e.g.* mean (SD) $\delta^{15}N$ = 2.7 (± 0.2) ‰ in fin whale baleen (Borrell *et al.* 2012). Trophic enrichment does not occur to the same degree in carbon isotopes where values more closely reflect those of primary production (DeNiro & Epstein 1978), hence its preferred use for inferring provenance of feeding. During biosynthesis of lipids, isotopically heavier ^{13}C is discriminated-against by up to 8 ‰ by comparison with other tissue components (DeNiro & Epstein 1978, Peterson & Fry 1987), similarly, biomineral carbonates are relatively enriched in ^{13}C compared to organic tissues. Consequently, where tissues contain mixtures of proteins, lipids and/or mineral carbonates, $\delta^{13}C$ values will vary with the relative contributions of differing tissue types. Chemical extraction of non-proteinaceous tissue components from ecological samples may introduce ecologically significant error depending on the technique employed (Kiljunen *et al.* 2006, Post *et al.* 2007, Søreide *et al.* 2007). A protein-rich tissue such as baleen, composed almost exclusively of keratin, is therefore preferable in stable isotope analysis when estimating sources of assimilated nutrients from the diet.

In the marine environment $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter (POM) vary with both latitude and proximity to shore. These broad geographic patterns are driven by variation in the degree of isotopic fractionation during photosynthesis, and the isotopic composition of the inorganic carbon utilised by phytoplankton (Goericke & Fry 1994, Hofmann *et al.* 2000, Fry 2006, Tagliabue & Bopp 2008, Graham *et al.* 2010). The concentration and isotopic composition of dissolved molecular carbon dioxide [CO_2(aq)] is a complex function of sea surface temperature (SST) (Rau *et al.* 1992, Goericke & Fry 1994). The degree of biological fractionation of carbon isotopes during phytoplankton photosynthesis varies according to the geometry and growth rate of phytoplankton which are also influenced by SST and day length (Popp *et al.* 1998). Consequently, differential biological fractionation of carbon isotopes in primary production broadly co-varies with SST, giving rise to latitudinal gradients in $\delta^{13}C$ POM values (Hofmann *et al.* 2000). Because of its sensitivity to plankton growth rates and seawater carbonate chemistry, the isotopic composition of POM is subject to dynamic climatic and oceanographic forcings at all spatio temporal scales (Newsome, Etnier, *et al.* 2007, MacKenzie *et al.* 2011, Trueman *et al.* 2012a), and despite predictable gradients in stable carbon isotope ratios at an ocean-basin scale, their usage in assigning geographic location to marine animals is confounded by additional temporal and small-scale spatial variations (due to, *e.g.* upwelling) which may be difficult to predict (Rau *et al.* 1992, Hofmann *et al.* 2000, MacKenzie *et al.* 2011).

Spatial heterogeneity is also found in the nitrogen stable isotope compositions of marine primary producers due to differential fractionation (Montoya 2007) and differences in the isotopic composition of nitrogen assimilated during call growth. One study in the coastal seas around the UK showed that 77% of variability in $\delta^{15}N$ values of whiting (*Merlangius merlangus*) was attributed to differences at the base of the food chain (up to 2 ‰ per 100 km) rather than trophic level (Jennings & Warr 2003). Baseline phytoplankton $\delta^{15}N$ values are dictated by the availability and types of nutrients (ammonium, nitrate or N_2) from which they are sourced. Broad latitudinal regions are characterised by specific mechanisms of nitrogen fixation, for example N_2-fixation in sub-tropical gyres and nitrates in vertically-mixed mid to high latitude regions. Considering that nitrogen availability to phytoplankton and its isotopic composition dictates baseline $\delta^{15}N$ values, inputs of terrestrial nitrogen can manifest as a gradient of isotopic enrichment towards the shore in marine food webs (Jennings & Warr 2003). Furthermore, due to progressive enrichment in ^{15}N with trophic level, tissue $\delta^{15}N$ values may be enriched in animals that are suckling or fasting (Hobson & Schell 1998, Knoff *et al.* 2008). It has been demonstrated that in fasting birds, catabolism of muscle leads to successive loss of ^{14}N which elevates whole-body $\delta^{15}N$ values (Hobson *et al.* 1993). During prolonged fasting associated with migration, ^{15}N enrichment may similarly be reflected in baleen resulting in an apparently elevated trophic position (Hobson *et al.* 2004).

Stable isotope analysis may provide taxonomically coarse trophic information over broad timescales. Incremental sampling of accretionary tissues for example can provide a longitudinal record of isotopic proxies for both diet and habitat use (Mendes *et al.* 2007, Newsome *et al.* 2009). From an evolutionary viewpoint, baleen is

a highly-derived tissue and one of the most significant adaptations among cetaceans as it permitted batch-feeding in mysticetes enabling them to exploit large quantities of prey and in doing so attain unprecedented body sizes (Deméré *et al.* 2008). Synthesized from metabolites of the bloodstream and composed almost exclusively of keratin, baleen is an accretionary tissue suitable for time-integrated stable isotope analysis (Schell *et al.* 1989a). In one study where minke whale baleen was sampled along the growth axis, isotopic values from the most recent 10 mm segment of baleen were found to reach equilibrium with those of muscle (Hobson *et al.* 2004). Thus baleen isotope values exhibit similar carbon and nitrogen assimilation rates to muscle (several months), although this is not established experimentally) representing metabolic pathways of proteins rather than mobilized lipid stores (Hobson *et al.* 2004).

The ecological niche concept formally defined by Hutchinson (1957), has become fundamental in community ecology theory. Defined as an "*n*-dimensional hypervolume", a niche is a space that encloses *n* limiting dimensions of the environment in which an organism can occur (Hutchinson 1957). It was recently proposed that the range of stable isotope compositions measured in individuals could be used to quantify trophic niche dimensions, contributing to a resurgence of interest in the niche concept and giving rise to the 'isotopic niche' or 'δ-space' (Newsome, Martinez del Rio, *et al.* 2007). Theoretically, the isotopic niche approximates the trophic niche (Bearhop *et al.* 2004, Layman *et al.* 2007). However, this is unlikely where either spatial or temporal baseline variation is not accounted-for. The use of Euclidean methods, such as convex hulls to define the isotopic niche space of a species in a community (Layman *et al.* 2007) is subject to sampling biases, and is

sensitive to sample size (Jackson *et al.* 2011). Standard area ellipses estimated by Bayesian inference can incorporate uncertainties such as sampling biases and small sample sizes into niche metrics (Jackson *et al.* 2011). In this study, photographic evidence of apparently shared prey resources prompted an investigation into resource partitioning using the SIBER (Stable Isotope Bayesian Ellipses in R) routine in SIAR; a package in the R programming environment, to examine variation in $\delta^{13}C$ and $\delta^{15}N$ values and estimate isotopic niche space (Jackson *et al.* 2011, R Development Core Team 2011). This approach is similar to bootstrapping in that it iteratively assigns measures of uncertainty, in this case based on Monte Carlo Markov-Chain simulation to construct parameters of ellipses. By applying this technique to specimens from museum collections and recently stranded individuals we examine resource partitioning among four species of rorqual which are sympatric in the NEA.

The International Whaling Commission aims to manage baleen whale populations based on identification of geographically explicit and biologically relevant units (Donovan 1991). However management units are difficult to define. In the management of fin whales in particular, 'stock' discrimination remains poorly resolved in the North Atlantic(International Whaling Commission 2007). We employ isotopic niches estimated *via* a Bayesian inference framework to infer population connectivity in fin whales sampled in three adjacent geographic locations: Ireland/UK, Bay of Biscay and The Mediterranean Sea.

2.3 Methods and Materials

Photographs of surface-active feeding rorquals were collected opportunistically from both research and fishing vessels in both inshore and offshore waters of the study area from 2008 to 2011. Visible prey were categorised into coarse taxonomic groups to provide some baseline evidence on the diet of fin, humpback and minke whales. Baleen plates from Fin (n=13), Minke (n=8) and Humpback (n=4) whales found stranded around Ireland and the United Kingdom (UK) were sampled from collections or taken directly from carcases in the case of recent samples. A plate from single blue whale (*B. musculus*) stranded in Ireland in 1891 was also included. Samples were also taken from three fin whales landed at Caneliñas whaling station in Northern Spain before the international moratorium on whaling. These fin whales were killed in the Bay of Biscay near Cape Finisterre at unknown dates between July and August of 1985. While length of these animals was unknown, they were assumed to be >17m in accordance with catch regulations at that time.

All baleen plates were air-dried and stored at room temperature, scoured using steel wool to remove extraneous material and cleaned with 1:1 methanol-chloroform solution to remove lipids. Incremental sampling was carried out longitudinally (*i.e.* along the growth axis) on each plate at locations measured using demarcated adhesive tape (due to curvature of some plates when dried), from the gingival end to the distal tip. Along the transverse axis, tissue synthesis is uniform (Schell *et al.* 1989b). Samples were taken within 0 to 20 mm of the non-frilled (outer) edge, where the growth axis is longest (Bentaleb *et al.* 2011). The following increments were used: fin and blue whale, 50 mm; humpback, 20 mm and minke whale, 10 mm, although

two fin whale specimens (SW.1914.10 and SW1924.12) were sampled at 10 mm increments. Samples were simultaneously removed and homogenised using a rotary engraving tool with a stainless steel tip (1 mm diameter) which was cleaned between samples using deionised water. *Circa* 1.5 (± 0.5) mg of tissue were weighed into tin cups and analysed for carbon and nitrogen isotope ratios using a continuous flow isotope ratio mass spectrometer. Routine analysis of in-house laboratory standards was used to estimate instrument accuracy and precision. Carbon and nitrogen isotope ratios were determined against a reference standard, glutamic acid, which itself was referenced with IAEA Vienna Pee Dee Belemnite and atmospheric air.

The elemental carbon to nitrogen ratio (C:N) was used as quality assurance to assess stable isotope values before subsequent analyses. Samples with C:N ratios outside of the theoretical value of 3.4 ± 0.5 for pure keratin were omitted from all analyses (O'Connell & Hedges 1999, Bentaleb *et al.* 2011). Calves were defined as < 5.7 m for minke (Stewart & Leatherwood 1985), < 8 m for humpback (Clapham & Mead 1999), and < 11.5 m for fin whale (Gaskin 1976), while the single blue whale sample was from an adult measuring 25 m in length (Scharff 1900). A continuous growth rate of baleen was assumed to be 20 cm yr^{-1} for fin (Bentaleb et al 2010) and 12 cm yr^{-1} for minke (Mitani et al 2006) whales. The growth rate of humpback baleen is not known, however, based on proportionate body size, it is assumed to be intermediate between fin and minke whales and thus 15 cm yr^{-1} was assumed. Grey whales are of similar body length and weight and are known to have a baleen growth rate of between 10 and 20 cm yr^{-1} (Sumich 2001). The growth rate of blue whale baleen is not known but is likely to be similar to that of fin whales given their similarity in dimensions and life-histories. Growth rates were used to confirm that samples comprised at least one

year of growth for each individual sampled. For some plates, the gingival end was missing thereby precluding the construction of time-series from a known point in time.

Bivariate ellipses and convex hulls were used to delineate isotopic niche space ($\delta^{15}N$ - $\delta^{13}C$ 95 % confidence interval ellipses) and the total extent of isotopic niche respectively among species (Jackson *et al.* 2011). Bayesian standard ellipses (SEA_C), corrected for small sample sizes by two standard deviations (*n*-1 for each axis) were then plotted separately for adults and calves using the SIBER routine (Stable Isotope Bayesian Ellipses in R) in the SIAR package in R (version 2.14.1) (Jackson *et al.* 2011, R Development Core Team 2011). Niche area and overlap ($‰^2$) were estimated based on 100,000 posterior draws of the SEA_C parameters which are comparable among and within ecological communities (Jackson *et al.* 2011). By including bivariate isotopic data for individual whales for increments representing > 1 year of baleen growth, isotopic variation in diet, space and time was incorporated into the posterior distribution. Niche space and area were thus comparable among the four species. Similarly, niche space and area were compared between fin whales in order to investigate for population structure between three sampling regions: Ireland/UK, Bay of Biscay (data from present study) and the Mediterranean (Bentaleb *et al.* 2011), where samples were collected and analysed using a consistent sampling protocol.

2.4 Results

Photographic evidence showed that fin, humpback and minke whales fed on forage fishes (most likely *Clupea harengus* or *Sprattus sprattus*) in inshore waters, while fin

and humpback whales fed on zooplankton in coastal and pelagic waters respectively (Figure 6). There was circumstantial evidence of common prey species targeted by fin, humpback and minke whales, strengthened by consistent evidence from the literature (Aguilar 2008, Burfield 1913, Fairley 1981, Whooley *et al.* 2011). In order to investigate resource partitioning among these species, baleen plates were sampled from collections for stable isotope analysis (Figure 7). Only specimens for which data on stranding location, date, species and total body length were included (Table 1). From three separate runs, the minimum instrumental precision (SD) was < 0.1 ‰ for both $\delta^{15}N$ and $\delta^{13}C$ values respectively for the CF-IRMS. A single fin whale observation had a C:N ratio of 4.2 and was therefore outside of the range of theoretical values for pure keratin. This observation was omitted from subsequent analyses. Baleen plates from 13 fin, eight minke, four humpback and one blue whale were sub-sampled. The mean C:N (range) of fin, humpback, minke and blue whale baleen subsamples was 3.3 (3.0—3.7); 3.5 (3.3—3.7); 3.4 (3.1—3.5) and 3.2 (3.2—3.3) respectively. All plates analysed comprised greater than one year of growth according to known or estimated baleen growth rates for the species concerned.

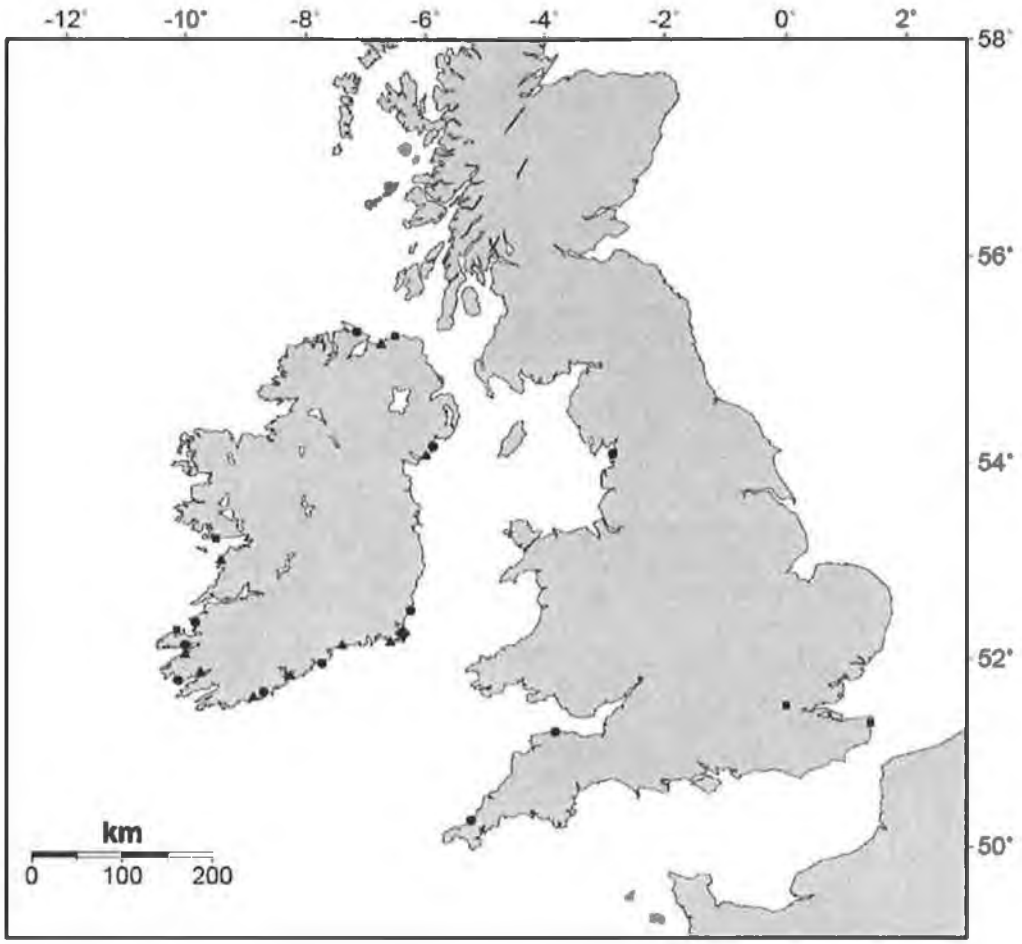


Figure 6. Locations of Blue (diamond), Fin (circle), Humpback (square) and Minke (triangle) strandings listed in Table 1.

Figure 7. Photographic evidence of prey types being consumed by rorquals, taken opportunistically during research cruises in Irish waters. From top left: humpback feeding on Clupeids; humpback feeding on coastal zooplankton (unknown spp.); fin whale feeding in coastal waters on Clupeids; minke feeding in coastal waters on large Clupeids; minke feeding in coastal waters on large Clupeids; orange scats of fin whales in pelagic waters, suggesting a diet of zooplankton (*e.g.* Euphausiidae), 140 km southwest of Ireland 22 August 2008.

Table 1 Dates and locations of strandings with corresponding body lengths and sex. Calves (see methods for definitions) are underlined. Records were sourced from www.iwdg.ie/strandings and the British Natural History Museum database. [a] These genders were determined genetically from skin samples (See (Bérubé & Palsbøll 1996) for methods). [b] Length of this specimen was taken from (Scharff 1900).

Specimen	Date	Species	Location	Length	Sex
ZD.1892.3.1.1	21-Mar-1891	Blue Whale	Rosslare, Co. Wexford, Ireland	25[b]	?
SW.1914.10	28-Feb-1914	Fin Whale	Derrynane, Co. Kerry, Ireland	18.3	?
SW.1924.12	12-Jun-1924	Fin Whale	Ballough, Co. Down, UK	5.2	?
SPBp85001	31-Jul-1985	Fin Whale	*c.*100nm NW Cape Finnistere, Spain	>17	?
SPBp85002	31-Jul-1985	Fin Whale	*c.*100nm NW Cape Finnistere, Spain	>17	?
SPBp85003	31-Jul-1985	Fin Whale	*c.*100nm NW Cape Finnistere, Spain	>17	?
IRBp94001	01-Apr-1994	Fin Whale	Ballyheigue, Co. Kerry, Ireland	24	F
GBBp00001	27-Nov-2000	Fin Whale	Morecambe Bay, Lancashire, UK	11.1	F
IRBp01001	05-Dec-2001	Fin Whale	Whiting Bay, Co. Waterford, Ireland	18.9	M
GBBp04001	01-Feb-2004	Fin Whale	Isle of Coll, Scotland, UK	17.1	F
IRBp08012	11-Oct-2008	Fin Whale	Inch, Co. Kerry, Ireland	18.5	F
IRBp09001	15-Jan-2009	Fin Whale	Courtmacsherry, Co. Cork, Ireland	19.7	F
IRBp09002	01-Feb-2009	Fin Whale	Ballyduboy, Co. Wexford, Ireland	16	M[a]
GBBp10001	23-Feb-2010	Fin Whale	Porthtowan, Cornwall, UK	16.9	F
IRMn96001	25-Aug-1996	Humpback Whale	Brandon Bay, Co. Kerry, Ireland	?	F[a]
IRMn04001	13-Sep-2004	Humpback Whale	Giant's Causeway, Co. Antrim, UK	8	M
IRMn06001	26-Jul-2006	Humpback Whale	Inverin, Co. Galway, Ireland	6	M
GBMn09001	12-Sept-2009	Humpback Whale	QE2 Bridge, London, UK	9.5	M
SW.1924.23	03-Jun-1924	Minke Whale	Kilmore Quay, Co.Wexford, Ireland	7.5	?
SW.1924.17	28-Jul-1924	Minke Whale	Kilkeel, Co. Down, UK	9.1	?
IRBa93001	11-Jul-1993	Minke Whale	Portstewart, Co. Derry, UK	9.4	F
IRBa01001	12-Jul-2001	Minke Whale	Roches Point, Co. Cork, Ireland	4.8	M
IRBa01002	04-Dec-2001	Minke Whale	Ballydowane, Co.Waterford, Ireland	7.84	M
IRBa08001	20-Sep-2008	Minke Whale	Duneen, Co. Cork, Ireland	7.47	M
IRBa10002	28-May-2010	Minke Whale	Doolin, Co. Clare, Ireland	4.43	F

Fluctuations in $\delta^{15}N$ and $\delta^{13}C$ values over time

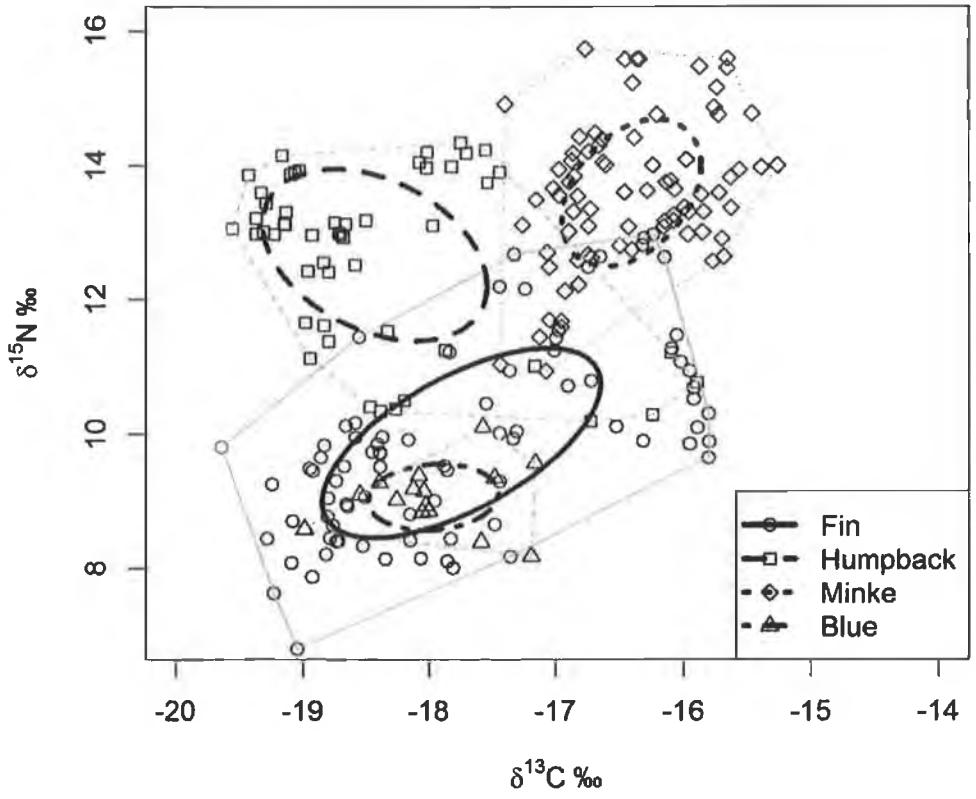
Covariation in $\delta^{15}N$ and $\delta^{13}C$ values occurs markedly in marine ecosystems, possibly due to co-enrichment due to biological fractionation (Kelly 2000). Decoupling of stable carbon and nitrogen values can potentially indicate nutrient assimilation from different food webs (Aita *et al.* 2011). The relationship between $\delta^{13}C$ and $\delta^{15}N$ values for each individual whale was examined using Spearman's rank correlation to test for significance given the non-normal distribution of the response variable ($\delta^{15}N$ values). There were no significant correlations found indicating that decoupling of both isotope values occurred for individual whales.

Isotopic Niche Areas

The probability of differential isotopic niche area for each species (irrespective of niche overlap) was measured by calculating the proportion of ellipse areas of posterior distributions (D) given the model (M), presented in Table 2 and graphically in Figure 8. None of the four species exhibited the same niche area, although the probability that humpback niche area was less than that of fin whales was small ($Pr(M|D) = 0.22$). Fin whales sampled in Ireland/UK exhibited a niche area significantly greater than those of other regions ($Pr(M|D) < 0.001$). The probability that posterior standard ellipse areas of Biscayan fin whales were less than those of Mediterranean fin whales was 0.074. Calves occupied smaller niche areas than adults of the same species (Figure 9).

Table 2. Probability, for the posterior distribution (based on 100,000 draws) of the parameters of model *M* given the prior data *D*, that isotopic niche area is of Species A is less than that of Species B (horizontal), and that the proportion of overlap in niche space for Species A is less than that of Species B (vertical). These probabilities are derived by Bayesian inference, whereby lower Pr values imply lower support of the hypothesis.

	Species A	Pr($M\|D$) area is less than			
		Fin	Humpback	Minke	Blue
Species B Pr($M\|D$) overlap	Fin	.	0.22	0	< 0.001
	Humpback	< 0.001	.	< 0.001	< 0.001
	Minke	< 0.001	0	.	< 0.001
	Blue	0.93	< 0.001	0	.


16
14
12
10
8
δ15N ‰
-20
-19
-18
-17
-16
-15
-14
δ13C ‰
Fin
Humpback
Minke
Blue

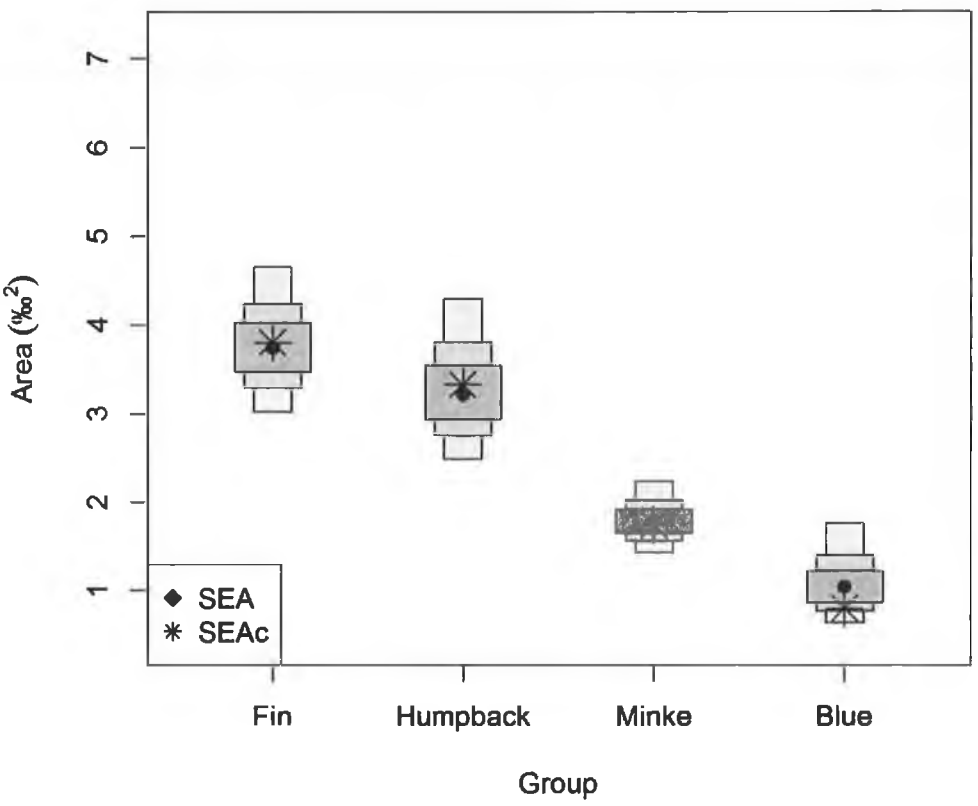

7
6
5
4
3
2
1
Area (‰2)
SEA
SEAc
Fin
Humpback
Minke
Blue
Group

Figure 8 Above: Variation in $\delta^{13}C$ and $\delta^{15}N$ values in the four species. Black and grey lines denote 95% confidence interval bivariate ellipses and convex hulls respectively of $\delta^{13}C$ and $\delta^{15}N$ values plotted according to species, for adults only (see Table 1), demonstrating the significant isotopic niche partitioning among the four rorqual species. Below: Measures of uncertainty and central tendency (black symbols = mode) of Bayesian standard ellipse areas (SEAc, corrected for small samples in a bivariate distribution for 2 degrees of freedom) based on 100,000 posterior draws of parameters showing 95%, 75% and 50% credibility intervals from light to dark grey respectively. See Results section for probabilities of differences in area for species.

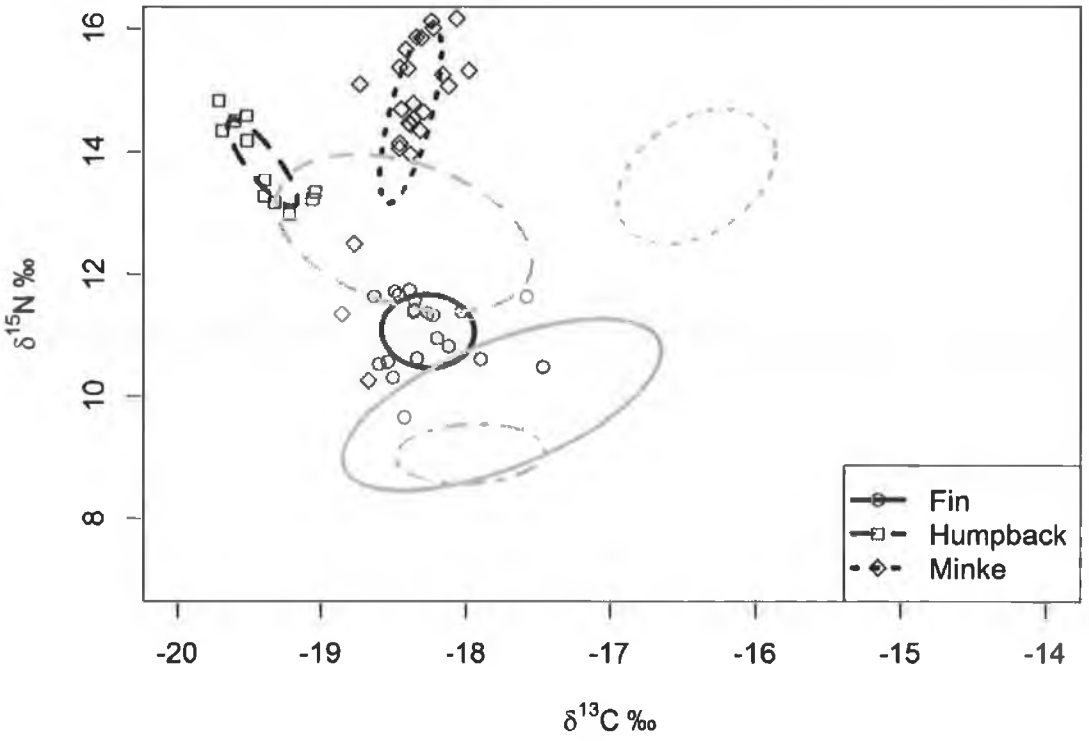
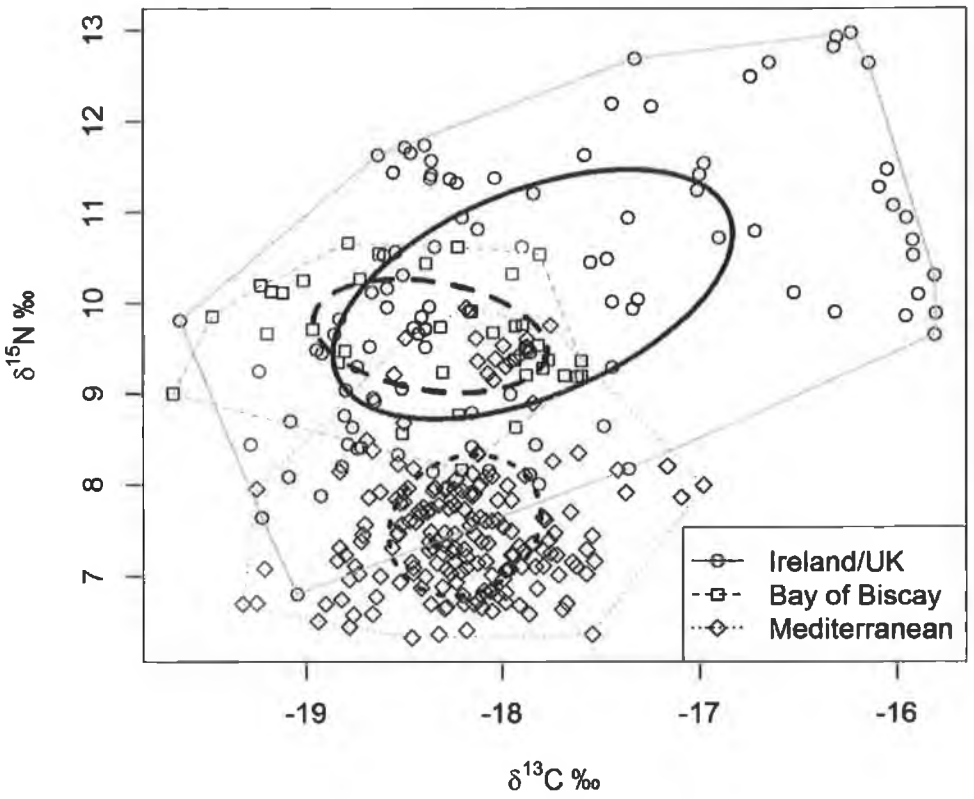


Figure 9. Black lines denote 95% confidence interval bivariate ellipses of $\delta^{13}C$ and $\delta^{15}N$ values plotted according to species, for calves only (see Table 1). Grey lines are plotted for adult whales at the same scale for comparison (see Figure 8). Distinct isotopic niches can be seen between calves and adults for each species. Enriched $\delta^{15}N$ values are attributed to suckling.


δ15N ‰
δ13C ‰
Ireland/UK
Bay of Biscay
Mediterranean


Area (‰2)
SEA
SEAc
Ireland/UK
Bay of Biscay
Mediterranean

Figure 10. Above: Black and grey lines denote 95% confidence interval bivariate ellipses and convex hulls respectively of $\delta^{13}C$ and $\delta^{15}N$ values plotted according to sampling area, for adult fin whales (see Table 1), showing significant isotopic niche partitioning between fin whales from the Mediterranean and other sampling regions. Fin whales from the Bay of Biscay and Ireland/UK occupy common isotopic niche areas. Note, data from a previously published study are included as diamond symbols (Bentaleb *et al.* 2011). Below: Measures of uncertainty and central tendency (black symbols = mode) of the Bayesian standard ellipse areas (SEAc, corrected for small samples in a bivariate distribution for 2 degrees of freedom) based on 100,000 posterior draws of parameters showing 95%, 75% and 50% credibility intervals from light to dark grey respectively. While fin whales from Ireland/UK share the same isotopic niche space as those from Bay of Biscay, the niche area of the former is significantly greater.

2.5 Discussion

Four of the six rorqual species occurring sympatrically in the NEA were considered in this study. Apart from observational evidence, knowledge on the diet of blue, fin and humpback whales from recent times in the NEA is lacking (O'Brien *et al.* 2009, Wall *et al.* 2009, Whooley *et al.* 2011). However fin whales in both the Mediterranean Sea and the Bay of Biscay, and minke whales in Scotland are known to feed preferentially on northern krill and both lesser sandeel (*Ammodytes marinus*) and herring respectively (Relini *et al.* 1992, Pierce *et al.* 2004, Aguilar 2008). A review of the literature coupled with photographic evidence of potential resource overlap between these species in the NEA prompted the use of stable isotope analysis to investigate resource partitioning using isotopic niches. Stable isotope analysis of baleen was used to

elucidate long-term isotopic niche, which it has been argued, is a function of trophic status and habitat use (Layman *et al.* 2007). C:N ratios confirmed that the baleen samples were within the theoretical range for pure keratin 317 out of 318 samples analysed (O'Connell & Hedges 1999). Those C:N ratios presented here are the first in the literature for humpback and blue whale baleen and indicate that a value of 3.4 is conservative across taxa for this tissue. This provides as a useful reference for quality assurance of corresponding $\delta^{15}N$ and $\delta^{13}C$ values for future studies using CF-IRMS.

As capital breeders blue, fin, humpback and minke whales are all believed to undergo prolonged migration for reproduction and show high site fidelity to feeding grounds (Jonsgård 1966, Gill & Fairbainrns 1995, Stevick *et al.* 2006, Whooley *et al.* 2011). However the timing, routes taken and location of breeding areas are not known for these species in this region (Anderwald *et al.* 2011). Decoupling (lack of correlation) of stable carbon and nitrogen isotope values indicates that baleen from each of the four species was synthesised from more than one isotopic source (*i.e.* more than one set of baseline isotope compositions).This could occur due to differential isotopic 'routing' (the differential allocation of dietary isotope components to different consumer tissues) due to feeding in more than one area (*e.g.* during migration) or differential metabolic fractionation of $\delta^{13}C$ and $\delta^{15}N$ such as that associated with catabolism (Schwarcz 1991, Hobson *et al.* 1993, 2004, Gannes *et al.* 1997). While a definitive interpretation of this pattern is not possible with these data alone, compound-specific stable isotope analysis may shed light on the underlying causes.

Isotopic Niche Space

Standard ellipse areas corrected for small sample sizes (SEA_C) delineated three distinct isotopic niches among fin, humpback and minke whales, indicating significant resource partitioning in Ireland/UK where they occur sympatrically. This is surprising considering observational evidence of shared resources (namely clupeids) in coastal waters. While SEA_C showed distinct isotopic niches, overlap between convex hulls did occur at $\delta^{15}N$ values of *circa* 11‰ and at $\delta^{13}C$ of *circa* -17‰ (Figure 8). All else being equal, Irish/UK fin whales would be expected to exhibit more depleted $\delta^{13}C$ values compared to their southern counterparts considering that $\delta^{13}C$ values generally decrease with latitude, particularly at latitudes > 50°N. The opposite was observed, thus latitudinal variations in baseline C isotopes cannot explain the distinctive isotopic composition whales from Ireland/UK whales which is consistent with an inshore component to their diet, a pattern unique to whales from the Ireland/UK sample ($\delta^{13}C$ values are enriched both with latitude and proximity to shore). Fin and humpback whales exhibited the same isotopic niche size which was significantly larger than that of either minke or blue whales. Assuming that both minke and blue whales assimilated nutrients from a baseline subject to similar spatiotemporal variation, these results suggest a more stenophagous diet for both species in the study area. Fin whales exhibited the broadest range of $\delta^{13}C$ values suggestive of feeding over latitudinal gradients (during migration) or in more than one food-web, *i.e.* in both pelagic and coastal waters. The $\delta^{15}N$ values observed for calves were enriched by comparison to adults which is consistent with a diet of maternal milk.

To draw ecological inferences from a comparison of isotopic variability between species or populations, assumptions concerning spatial variability of basal $\delta^{15}N$ and $\delta^{13}C$ values must be made. At present, our understanding of spatial and temporal variability in marine isotopic baselines is incomplete and more baseline isotopic data are required for robust interpretation of either isotope niche dimensions or geographic assignment using an isoscapes (stable isotope maps) approach (West *et al.* 2010, Trueman *et al.* 2012b). Compound-specific stable isotope analysis can be used to decouple the relative contributions of trophic and baseline variation in stable carbon and isotope compositions of proteins (Popp *et al.* 2007). However, currently these techniques are time consuming and costly, prohibiting routine application.

Implications for Conservation and Management

Stable isotopes have been used as a complementary tool towards resolving population structure (Clegg *et al.* 2003, Foote *et al.* 2009, Newsome *et al.* 2009). Our isotopic niche models indicate that fin whales sampled from Ireland/UK and the Bay of Biscay were spatially explicit from those of the Mediterranean Sea. The isotopic niche area however was different between the three groups of fin whales analysed, possibly reflecting either different foraging strategies between fin whales from Ireland/UK and Bay of Biscay or differential variability in isotopic baselines. The International Whaling Commission (IWC) recognises two fin whale stocks ('British Isles – Spain – Portugal' and 'Mediterranean') from the sampling regions included in this study (Donovan 1991). Our results support the 'British Isles–Spain–Portugal' management unit as ecologically relevant given that considerable overlap in isotopic

niche was found in whales from both the Bay of Biscay and Ireland and the UK. Comparing our data with those published by Bentaleb *et al.* (2011) also supports the hypothesis that Mediterranean fin whales constitute a distinct population and should be considered as a separate management unit (Bérubé *et al.* 1998). Enriched $\delta^{13}C$ values > -17‰ observed in samples from Ireland and the UK but not the Mediterranean or the Bay of Biscay is surprising in light of the expected depletion in baseline $\delta^{13}C$ values with latitude. This pattern is therefore consistent with a significant coastal diet component unique to fin whales from Ireland and the UK, where they have been observed feeding on forage fishes close to shore (Whooley *et al.* 2011). While direct evidence from satellite telemetry demonstrates that exchange between IWC stocks does occur (Mikkelsen *et al.* 2007), the geographic range of our samples did not allow for discrimination between the 'Faro- West-Norway' and 'British Isles-Spain-Portugal' stocks. More research is needed to investigate the range and connectivity of fin whales in the North Atlantic, where population structure remains poorly resolved due to recurrent gene flow between subpopulations (Bérubé *et al.* 1998, Palsbøll *et al.* 2004). We propose that stable isotope analysis would complement molecular genetic data towards resolving population structure of fin whales and other rorquals in the North Atlantic (*e.g.* Clegg *et al.* 2003).

2.6. Conclusion

While detailed time-series analysis was restricted here due to temporal discontinuities in the sample, older samples from museums have provided an informative baseline to place samples from more recent times into context. The diverse and discrete isotopic niches occupied by fin, humpback and minke whales appeared to be persistent over the timescale investigated (early twentieth century to the present). The observed isotopic niche partitioning somewhat contradicts recent field observations of fin, humpback and minke whales foraging on common prey in the study area. Instead, diet overlap between these species may only be short-term which shows the importance of considering averaged diet over longer time periods.

Until such time as robust estimates of diet solutions can be derived, most promisingly using Bayesian mixing models (Parnell *et al.* 2011), the isotopic niche concept can progress our understanding of poorly studied ecological communities by helping to formulate pertinent hypotheses on resource partitioning, competition and foraging strategies. We have shown that stable isotope analysis has a role to play in identifying connectivity between fin whales inhabiting adjacent regions in the NEA. Bayesian mixing models complemented by satellite telemetry will be required to clarify resource partitioning and nutrient sources at fine taxonomic and spatial scales among those rorquals occurring sympatrically in the ENA. This would facilitate informed ecosystems-based management decisions towards more effective conservation of marine resources, including rorquals which are still recovering from centuries of over-exploitation.

2.7 Acknowledgments

We are grateful to all those who provided baleen samples, making this study possible: Alex Aguilar, Gary Burrows, Nick Davison, Lucy Hunt, Colin MacLeod, Andrew Malcolm, Nick Massett, Mick O'Connell, Katie O'Dwyer, Paddy O'Dwyer, Kevin O'Hara, Emer Rogan, Andrew Speer, Pádraig Whooley, Jim Wilson and Peter Wilson. Photographs in Figure 7 were kindly provided by Pádraig Whooley and Ronan Cosgrove (Bord Iascaigh Mhara). Thanks also to Mick O'Connell and the membership of the Irish Whale and Dolphin Group for reporting data on stranded whales which is available in real-time online (www.iwdg.ie).

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Chapter 3.

Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of skin and blubber of balaenopterid whales

"Facts do not cease to exist because they are ignored"

Aldous L. Huxley, "Proper Studies" (1927)

This chapter is reproduced from the following published paper:

Ryan C, McHugh B, Trueman, CN, Harrod, C, Berrow SD and O'Connor I (2012) Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of skin and blubber of balaenopterid whales. Rapid Communications in Mass Spectrometry, 26:2745-2754.

3.1 Abstract

RATIONALE: Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of darted skin and blubber biopsies can shed light on habitat use and diet of cetaceans, which are otherwise difficult to study. Non-dietary factors effects isotopic variability, chiefly the depletion of ^{13}C due to presence of ^{12}C rich lipids. The efficacy of *post hoc* lipid-correction models (normalization) must be tested.

METHODS: For tissues with high natural lipid content (e.g., whale skin and blubber), chemical lipid extraction or normalization is necessary. C:N, $\delta^{13}C$ values and $\delta^{15}N$ values were determined for duplicate control and lipid-extracted skin and blubber of fin (*Balaenoptera physalus*), humpback (*Megaptera novaeangliae*) and minke whales (*B. acutorostrata*) by continuous-flow elemental analysis isotope ratio mass spectrometry (CF-EA-IRMS). Six different normalization models were tested to correct $\delta^{13}C$ values for the presence of lipids.

RESULTS: Following lipid extraction, significant increases in $\delta^{13}C$ values were observed for both tissues in the three species. Significant increases were also found for $\delta^{15}N$ values in minke whale skin and fin whale blubber. In fin whale skin, $\delta^{15}N$ values decreased, with no change observed in humpback whale skin. Non-linear models generally out-performed linear models and the suitability of models varied by species and tissue, indicating the need for high model specificity, even among these closely related taxa.

CONCLUSIONS: Given the poor predictive power of the models to estimate lipid-free $\delta^{13}C$ values, and the unpredictable changes in $\delta^{15}N$ values due to lipid-extraction, we recommended against arithmetical normalization in accounting for lipid effects on

$\delta^{13}C$ values for balaenopterid skin or blubber samples. Rather, we recommend that duplicate analysis of lipid-extracted ($\delta^{13}C$ values) and non-treated tissues ($\delta^{15}N$ values) be used.

3.2 Introduction

The stable isotope ratios of carbon and nitrogen in consumer tissues reflect those of the diet in a predictable manner and can thus be used to infer dietary information at the time and location of tissue synthesis.[1, 2] Nitrogen isotopes are relatively strongly fractionated during nitrogen metabolism, and thus increase principally as a function of mean trophic level. The stable isotopes of carbon do not exhibit the same degree of trophic enrichment and tissue carbon isotopes are more indicative of the isotopic composition of primary production fuelling a food web.[3] Baseline $\delta^{13}C$ values vary geographically or between habitats, allowing variation in consumer $\delta^{13}C$ values to be associated with differences in habitat use.[4-6] Stable isotope ratios are thus intrinsic markers from which quantitative information on trophic status, seasonal distribution and foraging area can derived.

Most stable isotope investigations of diet and movement in animal systems explicitly target proteinaceous tissues, as the isotopic composition of a consumer's protein is tightly linked to the protein component of diet.[7] A key consideration before carrying out stable isotope analysis on a tissue that may contain multiple molecular components (e.g., muscle, skin or blood), is the lipid-content of the tissue being analysed. Lipids are enriched in ^{12}C relative to bulk proteins in a given tissue resulting in a decrease in bulk tissue ^{13}C / ^{12}C and hence $\delta^{13}C$ values.[1] Lipid content may be highly variable between ecological samples (both between and within species) and the potential influence of lipid content on bulk tissue $\delta^{13}C$ values must be considered. There are two common approaches used to account for the effect of lipid on $\delta^{13}C$ values; an *a priori* approach using chemical extraction of lipids from tissue samples, and an *a posteriori* approach using mathematical correction (normalization). The latter is based on the carbon:nitrogen elemental ratio (C:N ratio), as tissues enriched

in lipids have a greater relative proportion % C compared to tissues with low lipid concentrations. Both methods have complications and accounting for lipids has been given considerable attention in stable isotope ecology. [8-15] Chemical lipid-extraction definitively removes the influence of lipids on bulk tissue $\delta^{13}C$ values, but may lead to unpredictable changes in tissue $\delta^{15}N$ values due to *inter alia* inadvertent removal of amino acids.[9, 14, 16, 17] This is problematic given that both isotopes are typically recorded simultaneously from the same sample, which reduces the cost of analysis. Furthermore, studies of large marine taxa e.g., whales, often rely on the use of remotely darted biopsies (un-tethered sampling darts fired from a moving boat, at an unrestrained target animal). Using this technique, only a small amount of tissue is available, sometimes preventing duplication of samples for analysis.

Retrospective, arithmetic correction of measured $\delta^{13}C$ values for lipid-content is based on tissue C:N ratios. As lipids do not contain nitrogen, the presence of lipids in bulk tissue will increase tissue C:N ratios and decrease $\delta^{13}C$ values proportionally.[18] Correction of bulk tissue $\delta^{13}C$ values should then be possible through regression. A full regression model should include parameters such as the C:N value of lipid-free tissue and the protein-lipid $\delta^{13}C$ discrimination value. These values are often unknown for the tissues and species in question, and are likely to vary, thus the fundamental assumptions of the models can be difficult to test.[12] Furthermore, one fundamental assumption of most lipid normalization models is that both lipid and protein are derived from the same isotopic source, in order that the lipid-protein discrimination value D is constant. However this assumption is often violated given the differential turnover rates of those tissue components.[19]

Normalization models for specific tissues or whole-body homogenates have been published for terrestrial mammals,[9] fish,[10] invertebrates[12] and cetaceans.[11] However the authors caution against using these models for tissues with high lipid content where the relationship between C:N ratios and bulk tissue $\delta^{13}C$ values becomes non-linear. The use of mathematical correction over lipid extraction is

favourable given the risks posed by exposure to some solvents used in the extraction process (e.g., chloroform is carcinogenic). Another consideration, on which there is no unanimous consensus in the literature,[8, 20] is the effect of lipid extraction on $\delta^{15}N$ values. There are several commonly used lipid extraction techniques using various solvents with a range of polarities. These techniques have the potential to solubilise amino acids to differing degrees, and the lipid-extraction technique employed may thus have an effect on $\delta^{15}N$ values.[21, 22]

The effects of lipid extraction on $\delta^{13}C$ values in skin and blubber of humpback whales have been tested.[23] However the effects of lipid extraction on nitrogen isotope values in these tissues were not investigated. Significant decreases in $\delta^{15}N$ values due to lipid extraction have been reported in skin of Balaenopteridae (although only for fin, humpback and minke whales when pooled together), while significant increases were found in other cetacean taxa.[11] This suggests that changes in tissue $\delta^{15}N$ values caused by lipid-extraction may be species-specific, even among closely related taxa. The study supported the need for species-specific models for lipid normalization of $\delta^{13}C$ values [14] in cetacean skin, and for a greater understanding on the effects of lipid extraction on other isotope ratio values such as $\delta^{15}N$. [11]

3.3 Methods

Sampling, sample preparation and stable isotope analysis

Tissue samples were taken from live fin (*Balaenoptera physalus*) and humpback (*Megaptera novaeangliae*) whales with a *Barnett Panzer V* re-curve crossbow (150lb draw-strength) using modified bolts and sterilised steel 40 mm biopsy tips (designed by Ceta-Dart, Dr. F. Larsen, Copenhagen, Denmark) between winter 2008 and 2011 at two study sites in Ireland (fin and humpback whales) and Boa Vista, Cape Verde (humpback whales only) under permit from the respective national authorities. Skin and blubber were also sampled using a scalpel from dead fin, humpback and minke whales (*B. acutorostrata*) found stranded around the coast of Ireland. Only those carcases exhibiting a code 2 or above on a standardized decomposition scale[24] were

considered to circumvent the possible effects of decay on the integrity of the samples. All samples were initially stored at -20 °C, then transferred to a -80 °C freezer. While still frozen, samples were duplicated (halved longitudinally) and skin and blubber were separated for analysis. As blubber is stratified into biochemically distinct layers in the species concerned,[25-27] only the outer blubber layer was analysed and this stratum was identified by eye for those samples from stranded specimens[28]. Samples were freeze-dried and for duplicates, lipids were extracted by Soxhlet reflux using *n*-hexane and acetone (1:1) for 12 hours.[29] Both lipid-extracted and whole samples were ground to a fine powder, ~0.50 mg weighed into tin capsules and analysed by CF-EA-IRMS in three runs at two different laboratories (University of Southampton and the Chrono Centre, Queen's University, Belfast). In University of Southampton, a EuroVector EA 3000 (EA) combined with a Europa Scientific 20-20 (IRMS) was used. At Queen's University Belfast, Thermo-Scientific Delta V Advantage (EA-IRMS) was used.

Isotope ratios are presented in delta notation as parts per thousand differences from international standards according to the following equation: $\delta^{Y}X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R denotes the heavier:lighter isotope ratio and Y is the atomic mass of the stable isotope X ($\delta^{13}C$ or $\delta^{15}N$). Internal lab standards (L-alanine and ACROS L-glutamic acid for each laboratory respectively), which were calibrated with International Atomic Energy Agency IAEA standards (Vienna, Austria) were used, i.e., Vienna Pee Dee Belemnite (for carbon), atmospheric N_2 (for nitrogen). Internal standards of known carbon and nitrogen composition (nicotinamide and L-glutamic acid) were routinely analysed between samples in order to determine instrument precision. Based on the standard deviation of these internal standards, the lowest analytical precision of all three runs was 0.2 ‰ for nitrogen, and 0.1 ‰ for carbon.

Data Analysis

Changes in $\delta^{13}C$ values after lipid extraction (*i.e.* $\delta^{13}C_{lipid\text{-}free} - \delta^{13}C_{bulk}$) were compared to lipid-free $\delta^{13}C$ values estimated using six published linear and non-linear

normalization models, originally produced to estimate lipid-free $\delta^{13}C$ values in a variety of taxa. The efficacy of models for normalizing $\delta^{13}C$ values was investigated by comparing Akaike information criterion (AIC) values and mean square error (MSE) of the model fits. Furthermore, the percentage of predicted $\delta^{13}C$ values that fell within 0.5 ‰ (> twice the mean instrument error of $\delta^{13}C$ values in most ecological studies) of the lipid-extracted $\delta^{13}C$ value was estimated.[9] The following previously published lipid-normalization models were considered:

Equation 1.[10] A non-linear equation based on McConnaughey and McRoy[18] fitted for whole body marine vertebrates and invertebrates with three variables: Lipid content (L), C:N ratio and an isotopic discrimination factor between pure lipid and protein of the sample, D (6.4 ‰ for cetacean skin).[11] I is a constant, assigned a starting value of -0.02.[18]

Eqn 1

$$\delta^{13}C' = \delta^{13}C + D \times \left\{ I + \frac{3.90}{1 + 287/L} \right\}$$

where

$$L = \frac{93}{1 + \left[0.246 \times (C:N) - 0.775\right]^{-1}}$$

Equation 2.[11] A generalised linear model which estimates $\delta^{13}C_{lipid\text{-}free}$ as a function of $\delta^{13}C_{bulk}$ values, irrespective of the C:N and hence lipid content. This model is deemed to be appropriate for lipid-normalizing skin in Balaenopteridae,[11] however it was not tested for individual species or for blubber. The intercept and slope parameters are denoted by $\beta 0$ and $\beta 1$ respectively.

Eqn 2

$$\delta^{13}C' = \beta 0 + \beta 1 \times \delta^{13}C$$

Equation 3.[8] A non-linear model with two parameters, developed for whole-organisms and muscle for a range of terrestrial and aquatic animal species. This relationship has only been found to be appropriate for tissues with high lipid content (> 15%) and was therefore considered suitable for testing with whale skin and blubber. Terms *a* and *b* are parameters that are estimated from the data while the intercept and slope parameters are denoted by *β0* and *β1* respectively.

Eqn 3

$$\delta^{13}C' - \delta^{13}C = a + b \times (C:N)$$

Equation 4.[12] A generalised log-linear model allowing for the non-linear relationship of the single explanatory variable; bulk C:N.

Eqn 4

$$\delta^{13}C' - \delta^{13}C = \beta 0 + \beta 1 \times \ln(C:N)$$

Equation 5.[12] is a derivation of equation 1 in McConnaughey and McRoy,[18] where the protein-lipid discrimination *D* is replaced by *a*. This three-parameter non-linear model has previously been tested for whole-body homogenates and individual tissues for a range of aquatic vertebrates and invertebrates. Parameters *b* and *c* are estimated from the data.

Eqn 5

$$\delta^{13}C'-\delta^{13}C=\frac{a\times(C:N)+b}{C:N+c}$$

Equation 6.[30] A non-linear equation designed for tissues of freshwater fishes where p and f denote the protein-lipid discrimination and the $C{:}N_{lipid\text{-}free}$ values respectively.

Eqn 6

$$\delta^{13}C'-\delta^{13}C=p-\left(\frac{p\times f}{C:N}\right)$$

Both linear and non-linear models were fitted by least squares, where normally distributed error terms ($\varepsilon \sim N(0,\sigma^2)$ were assumed. Model selection was carried-out based on the lowest AIC and MSE of the estimates. Visual overview of model performance[8, 10, 12, 18, 30] and those specific to skin in balaenopteridae[11] was carried out by comparing predicted values to those values derived from lipid-extracted sample. All statistical analyses were performed in R version 2.12.1.[31]

3.4 Results

Sampling

Biopsy samples obtained from 22 fin and 32 humpback whales were analysed. All biopsies comprised a complete epidermis profile and portion of the outer blubber stratum to a depth of 15-25 mm. Samples from six minke whales which stranded on the Irish and British coasts between 1999 and 2010 were also included in the analysis. In total, skin and blubber samples from 60 specimens were analysed in duplicate, control and lipid-extracted (hereafter referred to as bulk and lipid-free respectively).

Changes in Isotope Ratios Following Lipid Extraction

The $\delta^{13}C$ and $\delta^{15}N$ values stratified by both tissue and species were found to be normally distributed before and after lipid extraction. With the exception of minke whale data, Levene's test for variance indicated that sample variances for $\delta^{13}C$ and $\delta^{15}N$ values did not significantly differ due to lipid-extraction within species or tissues. Two-tailed *t*-tests and Wilcoxon's signed rank tests were thus used to test for significant differences in stable isotope ratios due to lipid extraction. Significant increases in $\delta^{13}C$ values were found for both tissues in all species (Table 3, Figures 11 and 12). The change in $\delta^{13}C$ values due to lipid extraction was found to be higher for those tissue samples with higher C:N ratios, but this relationship was not observed for changes in $\delta^{15}N$ values (Figure 11). Increases in $\delta^{15}N$ values (mean ± SD) were significant for fin whale (1.1 ± 1.5 ‰) but not for humpback whale blubber. Significant increases in $\delta^{15}N$ values, following lipid extraction, were detected in skin for minke whales only (1.6 ± 0.1 ‰). While fin whale skin showed a decrease in $\delta^{15}N$ values after lipid extraction, this was less than analytical precision. A small sample size for minke whale blubber samples precluded their inclusion in the above statistical comparisons.

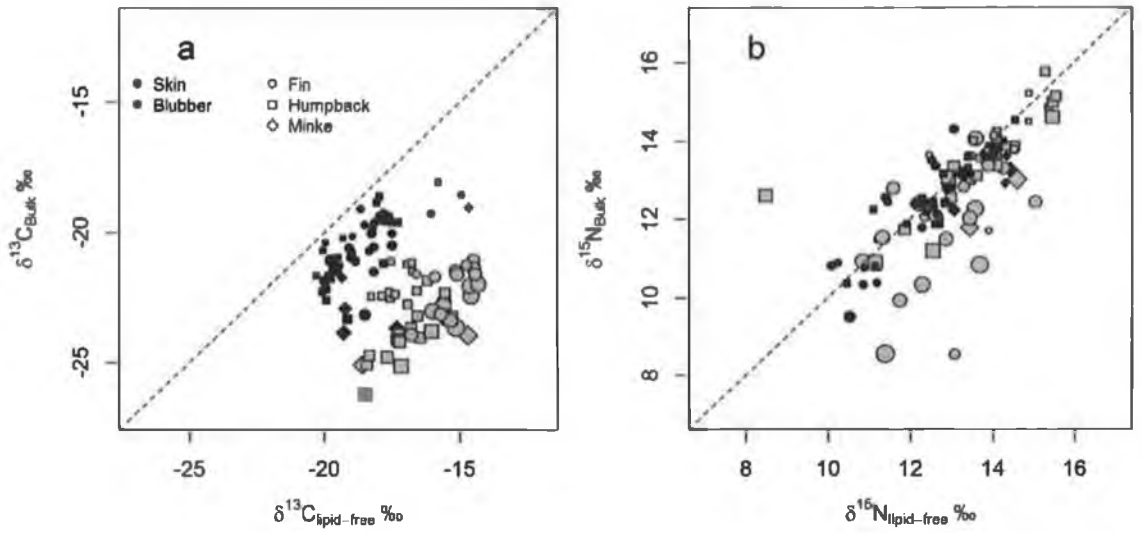

Figure 11. Lipid-extraction leads to enrichment of $\delta^{13}C$ (a) but not $\delta^{15}N$ values (b) for skin. Points are plotted in proportion to log(C:N) values to illustrate that as the lipid-free $\delta^{13}C$ values fall below parity (dashed line); they are more enriched in ^{13}C relative to the untreated (bulk) samples for all species and tissues. No such patterns emerged in $\delta^{15}N$ values.

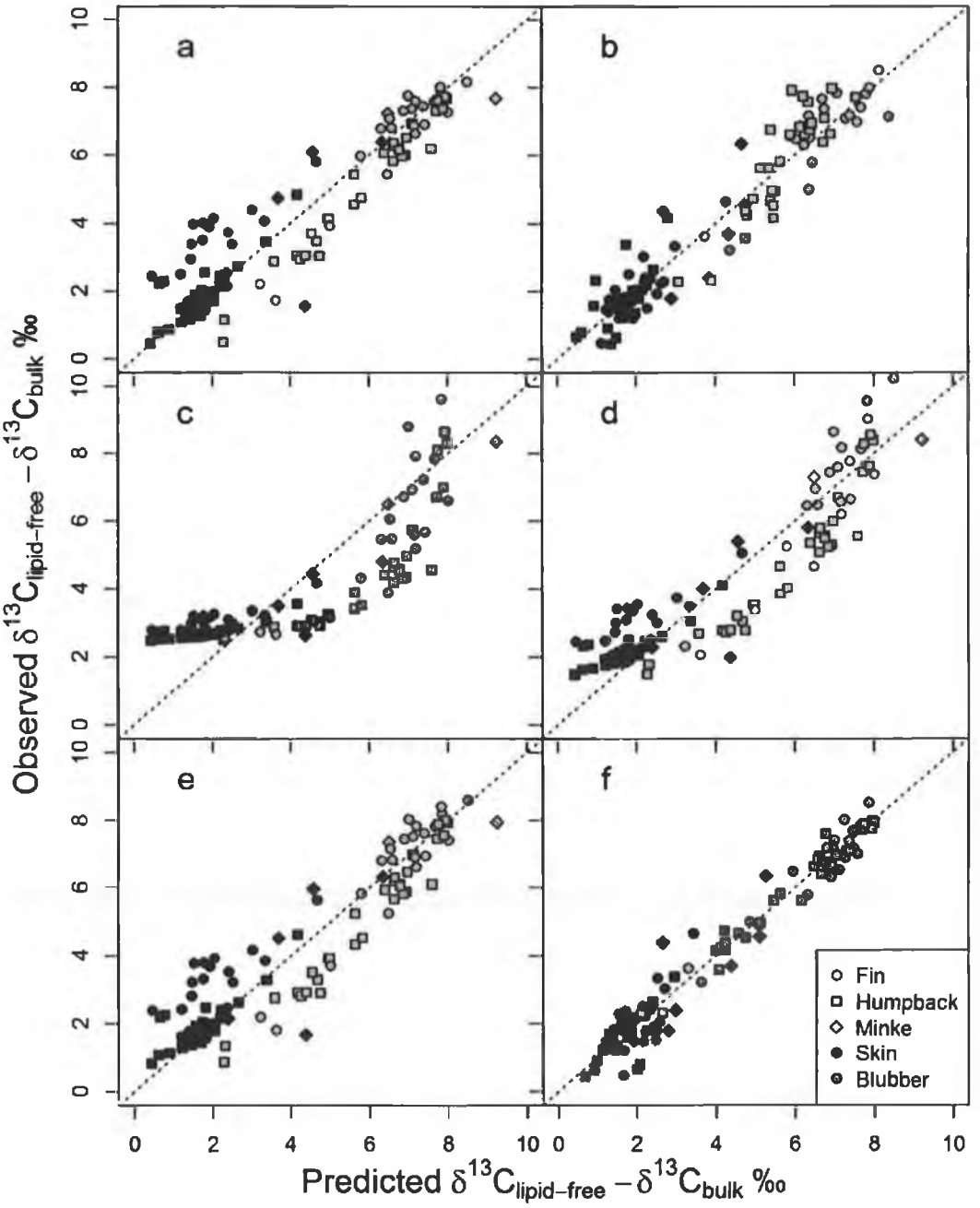

Figure 12. Comparison between observed (lipid-extracted) and predicted (normalized) changes in $\delta^{13}C$. Predicted values were obtained by modeling our data using previously published lipid normalization models: (a) Equation 1,[10] (b) Equation 2,[11] (c) Equation 3,[8] (d) Equation 4 (corresponding to equation 3 in[12]), (e) Equation 5 (corresponding to equation 1a in[12]), (f) Equation 6.[30]

C:N Values for Lipid-Extracted Skin and Blubber

Following lipid extraction, the mean C:N ratio was not consistent between species and tissues (Table 4). These C:N values were higher for skin (fin, 3.67 ± 0.35; humpback, 3.30 ± 0.17; minke, 3.24 ± 0.22): than for blubber (fin, 3.15 ± 0.25; humpback, 2.87 ± 0.06; minke, 3.06 ± 0.10). Fin whale C:N values were higher than those for either humpback or minke whale for both tissues. Our C:N values estimated directly from lipid-extracted skin were similar to the pooled species mean of 3.2 presented in Lesage *et al.*[11] (Table 4). By extrapolation, it was possible to estimate the theoretical C:N value for which the change in $\delta^{13}C$ values was zero, hereafter referred to as $C{:}N_{lipid\text{-}free}$, for some models only (Table 5). The $C{:}N_{lipid\text{-}free}$ values (upper, lower 95% confidence intervals) derived from Equation 6 were within the mean ± one SD of empirical $C{:}N_{lipid\text{-}free}$ values for fin, humpback and minke whale skin (3.2 (2.6, 3.7), 3.3 (2.9, 3.5) and 2.5 (2.1, 3.1)) and for fin and humpback whale blubber (2.6 (2.6, 3.0) and 2.8 (2.6, 3.0)) respectively. $C{:}N_{lipid\text{-}free}$ values derived from Equation 4 were comparable to empirical values for humpback whale skin only (3.1 (1.7, 5.2) (Table 4 and 5).

Table 3. The mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ values of skin and blubber of bulk and lipid extracted samples by species. *P* value pertains to paired *T*-tests for the effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values of skin and blubber, but Wilcoxon's signed rank test in the case of minke whale data, denoted by [v]

		Skin						Blubber					
Isotope	Species	*n*	Bulk	SD	Lipid-Extracted	SD	*P*	*n*	Bulk	SD	Lipid-Extracted	SD	*P*
	Fin	22	-20.2	1	-18.2	0.5	< 0.01	22	-22.1	1.4	-15.4	0.8	< 0.01
$\delta^{13}C$	Humpback	32	-21.3	1.4	-19.6	1.2	< 0.01	32	-23.2	2.3	-17.3	1. 4	< 0.01
	Minke	6	-21.8	2.1	-17.9	1.8	< 0.05[v]	3	-25.0	1.1	-16.9	2.0	-
	Fin	22	12.0	1.2	11.9	1.0	0.43	22	11.6	1.6	12.8	1.1	< 0.01
$\delta^{15}N$	Humpback	32	12.8	1.1	12.9	1.2	0.10	32	13.5	1.2	13.7	1.5	0.39
	Minke	6	13.0	0.6	13.8	0.7	< 0.05[v]	3	12.7	0.8	14.9	1. 7	-

Table 4. Mean (±SD) C:N ratios following lipid extraction for samples used in subsequent analysis.

			Bulk C:N			Lipid-extracted C:N		
Tissue	Species	n	Mean	SD	Range	Mean	SD	Range
Skin	Fin	22	5.57	1.31	4.19 - 10.02	3.67	0.35	3.29 - 4.19
	Humpback	32	4.49	0.72	3.67 - 7.68	3.30	0.17	2.99 - 3.90
	Minke	6	7.35	3.60	4.23 - 12.37	3.24	0.22	2.89 - 3.53
Blubber	Fin	22	18.61	9.80	4.32 - 42.55	3.15	0.25	2.77 -3.50
	Humpback	32	10.98	6.54	3.69 - 26.75	2.87	0.06	2.70 - 2.98
	Minke	3	22.22	4.86	18.78 - 25.66	3.06	0.10	2.99 - 3.13

Table 5. Selected linear and non-linear models with the best fits including parameters (±95% confidence intervals) and modeled C:N ratio for lipid free tissue where computable.

Model	Tissue	Species	Parameters	95% CI (lwr)	95% CI (upr)	Parameters	95% CI (lwr)	95% CI (upr)	MSE	% predicted	AIC	$C:N_{lipid\text{-}free}$
			D			*I*						
Eqn. (1)	Skin	Fin	5.4	2.7	8.2	−0.01	−0.2	0.4	0.44	54.4	48.1	3.2
Kiljunen *et al.*[10]		Humpback	7.7	5.8	9.7	−0.05	−0.1	0.02	0.18	193.5	39.4	3.6
(Non-linear)		Minke	5.6	−1.6	12.8	0.23	−0.15	na	1.56	16.7	23.3	3.9
	Blubber	Fin	7.4	6.2	8.6	0.18	0.05	0.36	0.2	63.6	29.3	3.7
		Humpback	8.4	7.8	9	0.11	0.06	0.16	0.08	90	14.9	3.6
		Minke	na	na	na	na	na	na	na	na	na	na
			β0			β1						
Eqn (2)	Skin	Fin	−13.7	−18.3	−9.1	−0.78	−1	−0.5	0.23	68.2	33.8	na
Lesage *et al.*[11]		Humpback	−8.6	−13.2	−4.1	−0.49	−0.7	−0.3	0.34	77.4	58.8	na
(Linear)		Minke	−5.5	−25.5	14.5	−0.43	−1.3	0.5	2.36	16.7	25.7	na
	Blubber	Fin	−12.3	−17.8	−6.8	−0.86	−1.1	−0.6	0.5	45.5	49.1	na
		Humpback	−6.9	−10.7	−3	−0.55	−0.7	−0.4	1.02	40	89.7	na
		Minke	66.5	na	na	2.39	na	na	na	0	na	na
Eqn. (3)	Skin	Fin	−0.8	−2.2	0.6	0.5	0.3	0.8	0.6	63.6	271.3	2.8
Post *et al.*[8]		Humpback	−2.1	−3.8	−0.5	0.9	0.5	1.2		84.4		3.1
(Non-linear)		Minke	1.2	−0.3	2.7	0.4	0.2	0.5		33.3		1.5
	Blubber	Fin	4.7	4	5.4	0.1	0.1	0.1		36.7		0.6
		Humpback	3.6	3.1	4.1	0.2	0.2	0.2		40		1.1
		Minke	6.8	4.7	8.9	0	0	0.1		33.3		0.2
			β0			β1						
Eqn. (4)	Skin	Fin	−3.1	−5.4	−0.9	3	1.7	4.4	0.4	68.2	45	2.8
Logan *et al.*[12]		Humpback	−5.2	−6.9	−3.5	4.6	3.5	5.8	0.2	75	39.6	3.1
(Linear)		Minke	−1.1	−6.9	4.7	2.6	−0.4	5.6	1.4	33.3	22.4	1.5
	Blubber	Fin	1.2	0	2.3	2	1.6	2.4	0.3	59	38.9	0.6
		Humpback	−0.2	−1.1	0.6	2.7	2.4	3.1	0.3	16.7	53.1	1.1
		Minke	2.7	−96.8	102.2	1.6	−27.7	30.9	2.8	33.3	14.3	0.2
			a			*b*						
Eqn. (5)	skin	Fin	1.9	n/a	n/a	−13.47	n/a	n/a	0.3	68.2	42.6	6.9
Logan *et al.*[12]		Humpback	9.4	4.7	n/a	−29.6	n/a	−16.1	0.2	93.6	40.9	3.2
(Non-linear)		Minke	3.9	n/a	n/a	−34.7	n/a	n/a	2.3	0	25.9	8.9
	blubber	Fin	6.8	n/a	n/a	−64	n/a	n/a	1.4	36.4	71.8	9.5
		Humpback	8.9	8.5	9.37	−25.6	−27.8	−22.7	0.1	86.7	16.8	2.9
		Minke	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
			C									
	skin	Fin	−6.9	n/a	n/a							
		Humpback	2.4	−1.6	n/a							
		Minke	−9.5	n/a	n/a							
	blubber	Fin	−9.5	n/a	n/a							
		Humpback	−0.2	−1.1	0.922							
		Minke	n/a	n/a	n/a							
			p			*f*						
Eqn. (6)	skin	Fin	5.1	3.9	6.298	3.2	2.6	3.7	0.3	50	192.9	3.2
Fry[30]		Humpback	7	5.4	8.641	3.3	2.9	3.5		93.8		3.3
(Non-linear)		Minke	6.6	5.5	7.794	2.5	1.8	3.1		0		2.5
	blubber	Fin	8.4	8	8.787	2.6	2.2	3		68.2		2.6
		Humpback	8.9	8.5	9.389	2.8	2.6	3		90		2.8
		Minke	10.2	8.5	11.971	5.6	1.5	8.5		0		5.6

Lipid Normalization Models for $\delta^{13}C$ values

Most model predictions underestimated the change in $\delta^{13}C$ values due to lipid extraction when compared to observed values for skin. The models tended to overestimate the change in $\delta^{13}C$ values at higher C:N values i.e., for blubber. As indicated by both AIC and MSE values, non-linear models performed better for most species and tissues. In general, the levels of error around the model estimates were high. Most models (Equations 3, 4, 5 and 6) consistently underestimated the change in $\delta^{13}C$ values in skin, while some models (Equations 3, 4 and 5) overestimated that change in blubber (Figure 13). These differentials were species-specific. For example in fin whales, overestimations ranged from means (± SD) of < 0. 1‰ (± 0.5) for Equation 1 to 0.7‰ (± 0.5) in Equation 4 for skin, and ranged from means of < 0. 1‰ (± 0.7) for Equation 1 to 1.2 ‰ (± 1.1) for Equation 3 for blubber. The non-linear model by Kiljunen *et al.*[10] (where $\delta^{13}C'$- $\delta^{13}C$ values are predicted by regressing C:N with the parameters *D* and *I*), gave a robust fit for both skin and blubber (Table 5). Equations 1, 5 and 6 provided the closest and most consistent predictions to parity with observed shifts in $\delta^{13}C$ values following lipid-extraction. Equation 6 gave the highest percentage of predicted values fitted within 0.5‰ of lipid-extracted values: 94 % for humpback skin and 90 % for humpback blubber. Despite being the only model in the literature previously applied to balaenopterid skin,[11] Equation 2 did not perform better than other models for skin (Table 5). There was a significant difference found between the slopes for all species for Equation 2 ($F_{6,105}$=151.6, $p < 0.01$) indicating the need for high model specificity among closely related taxa when using this model (cf Lesage *et al.*).[11]

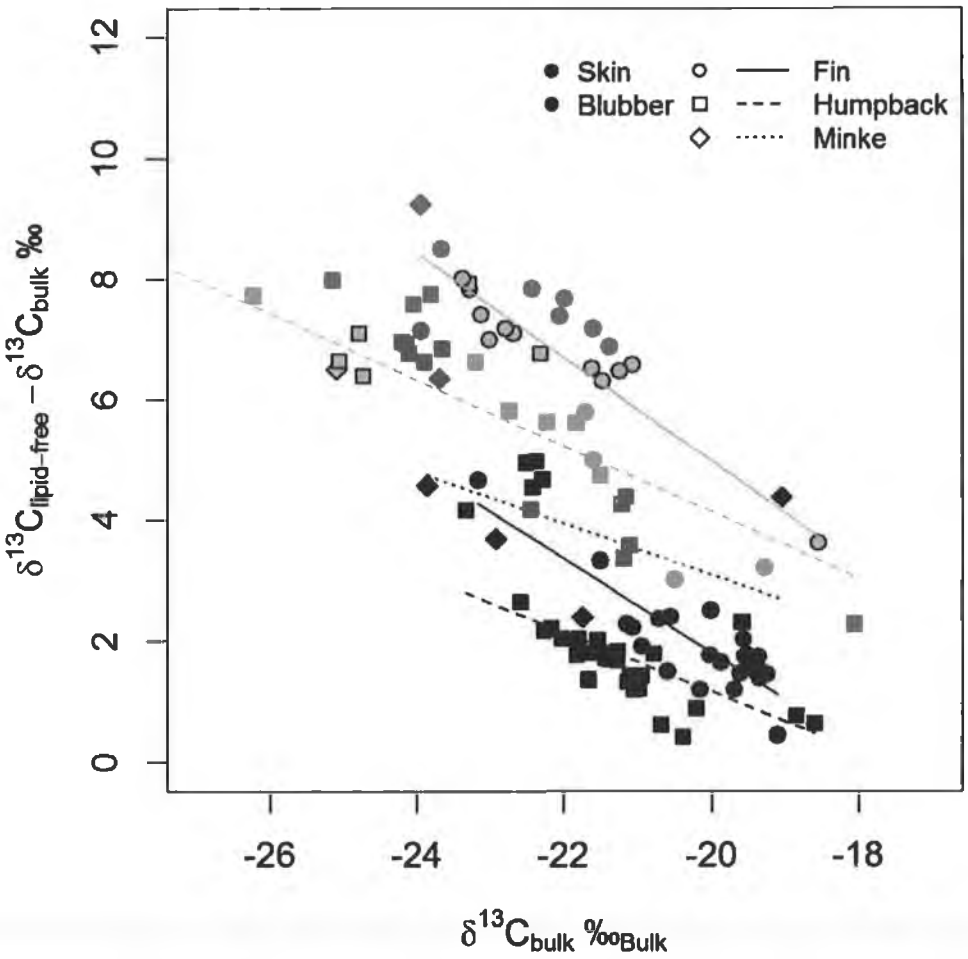

Figure 13. Lipid-normalized $\delta^{13}C$ as predicted by Equation 2:[11] a linear model proposed for lipid normalization of cetacean skin. The model was also tested for blubber and shows species and tissue specific effects. All regression coefficients with 95% confidence intervals are presented in Table 5.

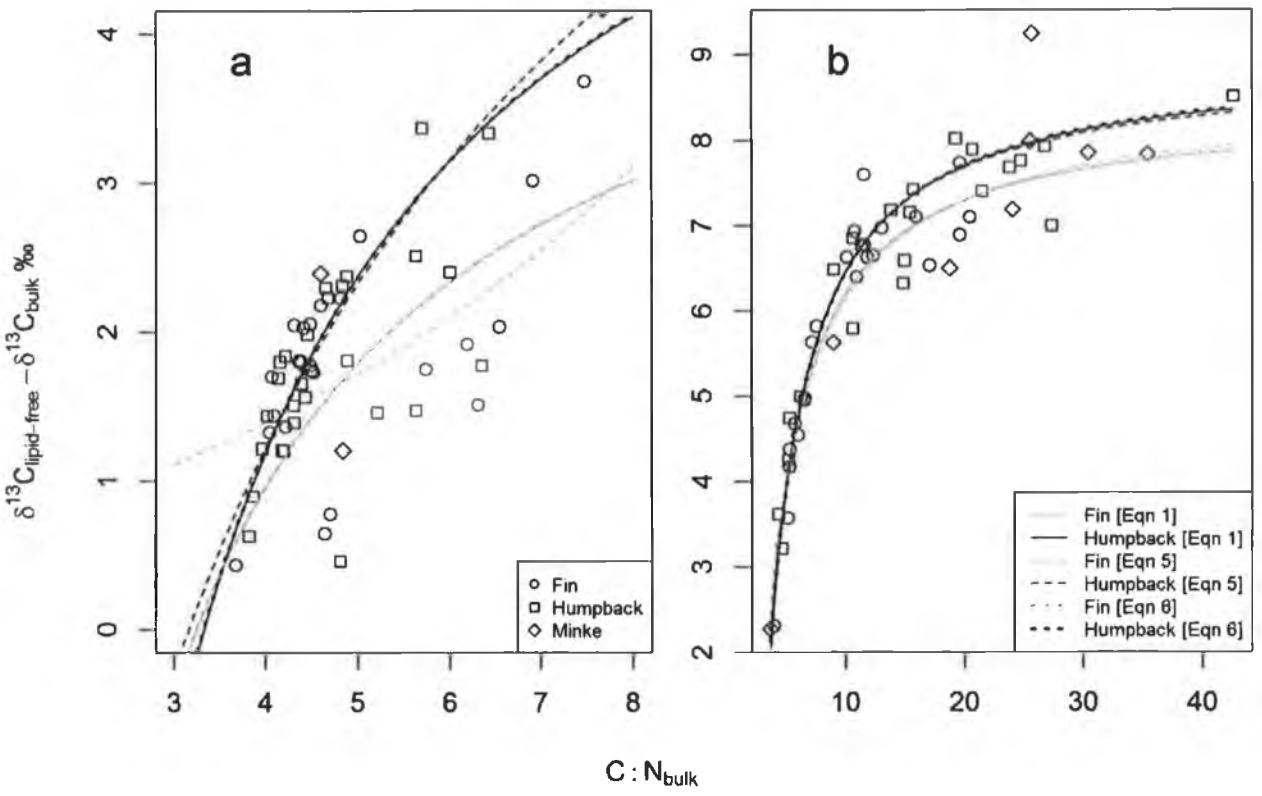

Figure 14. Plots of those three models (Equations 1, 5 and 6) which best described the change in $\delta^{13}C$ values with C:N for fin and humpback skin (a) and blubber (b) based on lowest AIC and MSE (Table 5).

Model Choices

Of the models considered for lipid normalization of skin, Equation 6 was the most appropriate given the overall higher percentage of fitted values (Table 5), and the logical parameters of the model which allows for the derivation of $C:N_{lipid-free}$. For blubber, non-linear models fitted the data better than linear ones (Figure 15). Of the models tested, Equation 6 was again the most appropriate for normalizing $\delta^{13}C$ values in blubber. This two parameter model provided the lowest AIC and MSE values, along with the highest percentage of values predicted to within 0.5 ‰ of observed change in $\delta^{13}C$ values due to lipid-extraction. Furthermore, this model is parameterized to include protein-lipid discrimination values, which allows for

greater model specificity. However, no models satisfactorily fitted the data such that predicted lipid-extracted $\delta^{13}C$ values could be used for e.g., mixing models given the high level of error introduced (Table 5).

Changes in $\delta^{15}N$ values due to lipid-extraction

While the changes in $\delta^{15}N$ values before and after lipid-extraction were found to be significant only for blubber in fin whales and skin in minke whales (Figure 11 and Table 3) those changes for skin were mostly increases. This indicates a loss of solvent-soluble amino acids that are depleted in ^{15}N. While the observed changes in blubber were greater, they were not as unidirectional making it difficult to account for these changes.

When species were pooled, 47 % and 66 % of $\delta^{15}N_{lipid\text{-}free}$ values for skin and blubber respectively were greater than the precision of the instrument (0.2 ‰). The relationships between both C:N and $\delta^{13}C_{lipid\text{-}free} - \delta^{13}C_{bulk}$ values and the change in $\delta^{15}N$ values were examined by least squares regression. In all instances explanatory variables were normally distributed and comparison of residual and fitted values indicated constant variance. The non-linear relationship $\log a(\delta^{13}C_{lipid\text{-}free} - \delta^{13}C_{bulk})$ best explained the change in $\delta^{15}N$ values due to lipid extraction in skin, where a was found to be significantly different to zero (t =13.93, 58 d.f., $p <$ 0.01) and was estimated to be 0.61 (95% CI; 0.52, 0.70) (Figure 15). The change in $\delta^{13}C$ values was poor at explaining the corresponding change in $\delta^{15}N$ values for blubber (intercept = -0.18 (95% CI; -0.60, 0.69), giving a slope (0.12 (95% CI; 0.00, 0.07) which was not significantly different from zero ($F_{1,51}$ =1.20, p = 0.28). The relationships between the change in $\delta^{15}N$ values and both bulk C:N and $C{:}N_{lipid\text{-}free} - C{:}N_{bulk}$ (as proxy for lipid content) of bulk skin and blubber samples were examined, however no significant relationships were found.

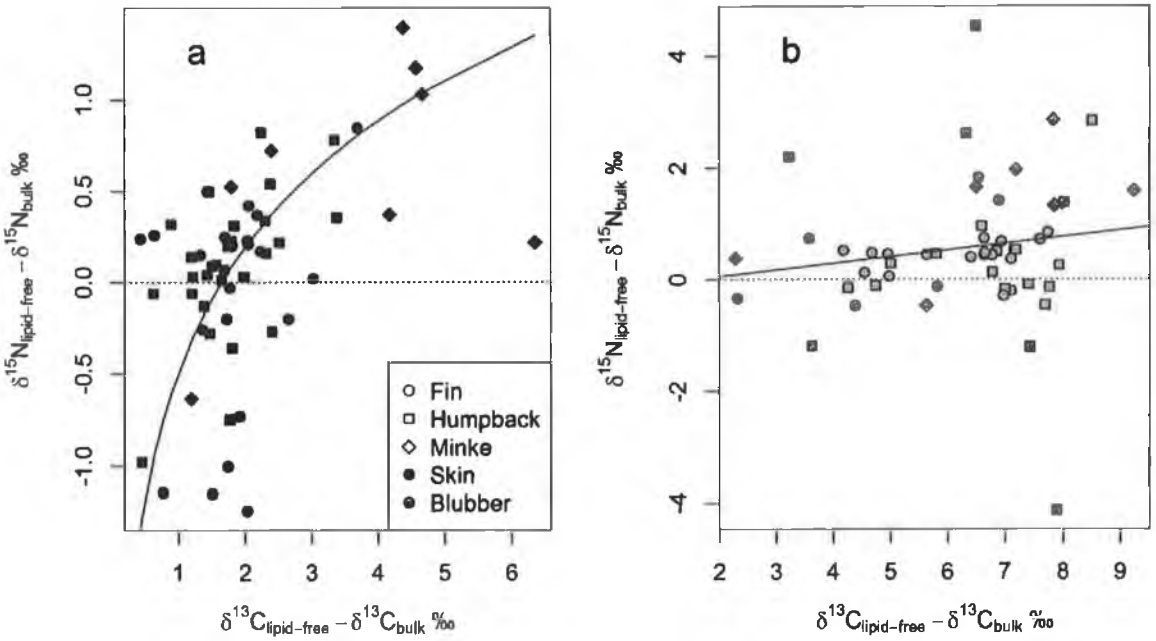

Figure 15. The relationship between the changes in $\delta^{15}N$ and those in $\delta^{13}C$ due to lipid extraction for (a) skin and (b) blubber. Lines were fitted using least squares regression where response variable were normally distributed and residual versus fitted values indicated constant variance. See Results section for model parameters and tests of significance. The broken line denotes no change in $\delta^{15}N$.

3.5 Discussion

Effects of Lipid concentration on bulk tissue $\delta^{13}C$ values

In accordance with other stable isotope studies on animal tissues, chemical lipid extraction resulted in significant increases in $\delta^{13}C$ values of bulk tissue for both skin and blubber (Figure 10). Our findings indicate that despite implementing high model specificity, lipid normalization models introduce high levels of error (range of mean square error for all models considered) in predicted lipid-free $\delta^{13}C$ values for skin (0.10—2.36) and blubber (0.08—2.8). The intended use of stable isotope

data will determine whether or not lipid-normalization is appropriate. Some attention has been paid to the effect of introducing tissue-treatment derived error when estimating prey assignment by mixing models. [10, 11, 32, 33] The probability of erroneous diet assignments arising from the increased error introduced by lipid extraction in cetacean tissues will be dependent on the isotopic distinction between the potential prey items. Lesage *et al.*[11] showed that a 100 % prediction error can arise when sample treatment errors exceed 0.5 ‰. While the non-linear models proposed by Fry[30] and Kiljunen *et al.*[10] provided the best fit out of the six models considered, poor fit corresponding to high C:N values was prevalent for all models (Figures 12 and 15). No model provided a satisfactory fit for $\delta^{13}C$ values to within 0.5 ‰ of observed values (Figures 12, 14 and 15). Where the ultimate use for stable isotope values of balaenopterid skin and blubber is in prey-assignment models, we recommended lipid-extraction rather than normalization of $\delta^{13}C$ values.

C:N Values

The C:N ratio provides a useful and low-cost proxy for lipid-content and has become the standard explanatory variable for normalizing $\delta^{13}C$ values.[18] However, Fagan *et al.*,[34] found no support for the predictive relationship between C:N and percentage lipid in freshwater fishes. Empirical $C:N_{lipid\text{-}free}$ values for fin, humpback and minke whale were 3.7, 3.3 and 3.2 respectively for skin and 3.2, 2.9 and 3.1 for blubber. These values will provide benchmarks when assessing the effectiveness of the lipid-extraction process in future studies. Two non-linear models (Equation 1 and 6) predicted $C:N_{lipid\text{-}free}$ ratios comparable to those empirical values, confirming that bulk C:N can be a useful explanatory variable for lipid-normalization of balaenopterid skin and blubber (cf Fagan *et. al.*) [34] (Table 4 and 5, Figure 14). However, our findings indicate that this relationship breaks down for samples with a high C:N ratio i.e., > 4.5 for skin and > 15 for blubber (Figure 14). Differential tissue and species-specific $C:N_{lipid\text{-}free}$ values presented here (Figure 14) indicate that this value should be measured empirically for

tissues or species not yet investigated. We have shown that even taxonomically similar species can exhibit differences in (mean ± SD) $C:N_{lipid\text{-}free}$ values of tissues, particularly between skin in fin (3.7 ± 0.4) and minke (3.2 ± 0.2) and between blubber in fin (3.2 ± 0.3) and humpback (2.9 ± 0.1) whales.

Changes in $\delta^{15}N$ values following Lipid-Extraction

The observed (mean ± SD) changes in $\delta^{15}N$ values following chemical lipid extraction for fin, humpback and minke whales respectively were 0.1 ‰ ± 0.7, 0.1 ‰ ± 0.3 and 0.8 ‰ ± 0.4 for skin and 1.1 ‰ ± 1.5, 0.1 ‰ ± 0.9 and 1.6 ‰ ± 0.1 and were greater than the level of instrumental precision achieved for $\delta^{15}N$ values (0.2 ‰). Sotiropolis *et al.*[14] proposed that an increase in $\delta^{15}N$ values occurs due to the loss of structural lipids that contain nitrogen-rich amino acids. However, those changes observed for skin in particular in this study were not unidirectional. The mean shifts in $\delta^{15}N$ values observed for skin and blubber were small and were similar to those reported previously for cetacean skin (≤ 1.6 ‰[11]) and for fish and invertebrates (-0.14 to 1.00 ‰ [20]), but were high enough to warrant caution on their use in mixing models.[11]

Similar to Lesage *et al.*,[11] the present study found changes in $\delta^{15}N$ values in skin following lipid extraction. However, we have shown that the magnitude of these changes varies by species within the family balaenopteridae. The unpredictable changes in $\delta^{15}N$ values, will have implications when the ultimate goal is to use stable isotope data from lipid-extracted tissues in a diet mixing model, given the increased error introduced to the model.[10, 11] The tissue-specific difference in changes to the $\delta^{15}N$ value are likely to have arisen from the differential structure and relative abundances of lipid compounds between the tissues.[14, 35] Polar structural lipid compounds such as glycolipids, phospholipids and sphingolipids are closely linked with bound amino acids which are depleted in ^{15}N, and will be removed by polar solvents.[35] An incidental co-extraction of structural lipids and

their associated amino acids has been widely proposed as the most likely explanation for the enrichment in $\delta^{15}N$ values.[14, 15, 35] It has been shown however, that the extraction technique is of little consequence in this regard.[22] Assuming that C:N is a reliable proxy for the amount of lipid being removed, if increases in $\delta^{15}N$ values occurred due to co-extraction of amino acids in structural lipids, then the changes in $\delta^{15}N$ values and C:N would be expected to correlate. However the non-significant relationship between these variables does not support the theory of ^{15}N enrichment due to co-extraction of amino acids and lipids in the present study.

Changes in stable nitrogen isotope values have also been found in egg yolk studies.[13, 32] The effect was attributed to the migratory nature of the birds in question, whereby the nutrients assimilated into the egg yolk were sourced from multiple isotopically distinct environments.[13, 32] Such a scenario is a violation of the fundamental assumption of most lipid normalization models – that lipid and protein are derived from the same source, in order that the lipid-protein discrimination value D is constant. Balaenopterid whales also undertake long distance migrations,[36, 37] and may therefore feed along a broad isotopic cline. If lipids and proteins have differential assimilation rates, lipid-normalization of $\delta^{13}C$ values in the tissues of balaenopterid whales may be inappropriate.

3.6 Conclusion

Analysis of $\delta^{13}C$ and $\delta^{15}N$ values is generally undertaken simultaneously for individual tissue samples. However our findings, which corroborate those of Lesage *et al.*,[11] demonstrate that pre-analysis chemical lipid-extraction treatments can have adverse and unpredictable effects on $\delta^{15}N$ values of blubber (*cf.* Ingram *et al.*).[20] This effect may be related to the lipid content and hence C:N ratio of the sample. Mathematical lipid-normalization of those skin samples with higher C:N values than those analysed here might also be inappropriate. It is recommended, therefore, that sample aliquots are analysed separately: with chemical lipid

extraction performed prior to measurement of $\delta^{13}C$ values, but $\delta^{15}N$ values determined in tissues with no chemical lipid extraction. We advocate caution against retrospective correction for the effects of lipids on $\delta^{13}C$ values in any tissues, before both species and tissue-specific normalization models have been tested. Furthermore, caution must be taken in comparing lipid-normalization models where the lipid-extraction techniques may differ between studies.[21, 22] As such, duplicate analysis, of lipid-extracted and bulk $\delta^{13}C$ and $\delta^{15}N$ values respectively, is recommended for stable isotope analysis of skin and blubber in balaeonopterid whales. While analysis of duplicate samples doubles the cost of the analysis, it is required to maximise the accuracy of the results. This study reaffirms the need for more methodological testing, particularly lipid normalization models, before the underlying assumptions of stable isotope analysis in its application to ecological problems can be met.

3.7 Acknowledgements

CR is a postgraduate student, funded by the Irish Research Council for Science, Engineering and Technology under the Embark Initiative. Funding was provided by the Marine Institute and an Ireland-Newfoundland Partnership Marine Research Grant. Biopsy samples were collected under license from the National Parks and Wildlife Service, Ireland (License Numbers C76/2008, C82/2009 & C130/2010) and Direcção Geral do Ambiente, Cape Verde (Licence Number 03/2011). We are indebted to Cóilín Minto for assistance with R. We are grateful to Pedro Lopez-Suárez, Félix Busto, Colin Barnes, Mick Sheeran, Martin Colfer, Pádraig Whooley, Nick Massett and Andrew Malcolm for assistance in the field. Thanks also to Mick O'Connell (Irish Whale and Dolphin Group), Rob Deaville (Zoological Society of London) Emer Rogan (University College Cork) and Machial Oudejans for providing samples and to Brian Boyle for help with the Soxhlet apparatus.

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Chapter 4.

Prey Preferences of Sympatric Fin (*Balaenoptera physalus*) and Humpback (*Megaptera novaengliae*) Whales Revealed by Stable Isotope Mixing Models

"There is no better high than discovery"

Edward Osborne Wilson, "The Scientist", Volume 18, Issue 1(2004)

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Ryan C, Berrow, SD, McHugh B, O'Donnell, C, Trueman, C and O'Connor I, (In Review) Prey Preference of Sympatric Fin Whales (*Balaenoptera physalus*) and Humpback Whales (*Megaptera novaengliae*) Revealed by Stable Isotope Mixing Models. Marine Mammal Science.

4.1 Abstract

Over-exploitation of top predators and fish stocks has altered ecosystems towards less productive systems with fewer trophic levels. In the Celtic Sea (CS), discards and bycatch levels have prompted concern about some fisheries, while fin and humpback whales are recovering from centuries of over-exploitation. A lack of empirical evidence on the preferred diet of some predators such as whales in the CS has hindered the implementation of effective conservation measures using an ecosystem-based approach to fisheries management. Using a Bayesian framework (SIAR), stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope mixing models were used to assign proportionate diet solutions to fin and humpback whales (skin biopsies) and putative prey items: herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and krill (*Meganyctiphanes norvegica* and *Nyctiphanes couchii*) in the CS. Krill was the single most important prey item in the diet of fin whales, but one of the least important for humpback whales. Age-0 sprat and herring comprised a large proportion of the diet of both species, followed by older sprat (age-1—2) and older herring (age-2—4). An ecosystem based approach to fisheries management will be required in the CS if we seek effective conservation of both fin and humpback whales, and sustainable fisheries.

4.2 Introduction

Ecosystems based management should strive to secure ecosystem functioning, thereby increasing the value of an ecosystem for subsequent generations. For the majority of cases, management of fisheries aims to maximise the yield of target species, which is rarely achieved without detrimental effects to the ecosystem (Pauly et al., 1998; Pinnegar et al., 2002; Pikitch et al., 2004). Recently it has been argued by nations with a whaling interest, that culling of whales could be used as a means to increase fisheries yield given that whales consume large quantities of fish. However this approach is inherently flawed as fisheries do not exert a comparable regulatory force on fish biomass as do top predators (e.g. Gerber et al., 2009). The removal of top predators results in different outcomes for ecosystems that function under predominantly top-down or bottom-up controls (Trites et al., 2006). The anthropogenic alterations of marine ecosystems are such that fish productivity has been reduced, as indicated by prolific lowering of trophic systems; forcing predators to consume food lower down on the food web (Pinnegar et al., 2002) or on sub-optimal quality (Österblom et al., 2008). According to life history theory, for top predators such as marine mammals that require energy-rich prey in high densities such as marine mammals, food shortages will lead to reduced body condition and hence reduced reproductive output (Stearns, 1976; Boeuf, 1994). Thus predation pressure exerted by natural top predators is self-regulating within the ecosystem, whereas predation from fisheries is not. Fisheries management aims to apply similar checks to fisheries pressures, with mixed results (Pauly et al., 2002). Lowered trophic systems, implicit with reduced availability of preferred prey, has exacerbated population declines in already threatened predators such as seabirds (Becker and Beissinger, 2006; Österblom et al., 2008). An understanding of the ecosystem roles and life histories of predators such as cetaceans is key in developing effective conservation measures in ecosystems based management (Hooker and Gerber, 2004).

Direct observation of predation and food consumption of marine predators such as fin (*Blaenoptera physalus*) and humpback whales (*Megaptera novaeangliae*) is

challenging. Conventional foraging studies (*e.g.,* stomach content analysis and direct observations) are subject to biases and are difficult to carry out under ecologically relevant timescales, particularly for wide ranging species such as cetaceans (Pierce et al., 2007). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$)* isotopes are tracers of nutrients and energy through food webs in that they reflect the environment and prey from which tissues of predators are synthesised (DeNiro and Epstein, 1978, 1981). Stable isotope analysis has become a frequently used means for exploring diet, foraging strategies and migration in animal ecology (Hobson, 1999; Newsome et al., 2010). However accurate estimates of absolute values, natural ranges and uncertainty in predator and prey tissue isotopes, tissue-to-source fractionation of stable isotopes as well as turn-over rate of the tissues used must be known before accurate modelling and interpretation of results can be carried out (Focken and Becker, 1998; Martínez del Rio et al., 2009; Caut et al., 2011; Borrell et al., 2012). Stable isotope values of tissues such as skin, which for cetaceans can be sampled remotely by biopsy darting, reflect those of dietary sources as a function of tissue turnover rate. Turnover rates for skin have been reported to be between seven days and one month for humpback whales, although this has never been tested, considering the logistical challenge of controlled experiments on large cetaceans (Todd et al., 1997; Caut et al., 2011; Witteveen et al., 2011). However, we cautiously propose that the turnover rate of skin is more likely to be several months, considering that this rate for skin collagen of other mammals is *ca.* 74 d (Rucklidge et al., 1992).

Using mass balance models (mixing models), it is possible to estimate proportionate contributions of distinct prey (sources) in the ultimate diet of consumers (mixture) using stable isotope values (Phillips and Gregg, 2003). Simple linear mixing models can be used to resolve diet solutions by Euclidean distances between $\delta^{13}C$ and $\delta^{15}N$ in bi-plot space but these models are constrained by the number of isotopes (n) used, whereby the number of sources that can be solved is $n + 1$. Phillips et al. (2001) established theoretical framework for more complex

* Isotope ratios are presented in delta notation as parts per thousand differences from international standards according to the following equation: $\delta^{Y}X = [(R_{sample}/R_{standard}) -1] \times 1000$, where R denotes the heavier:lighter isotope ratio and Y is the atomic mass of the stable isotope X ($\delta^{13}C$ or $\delta^{15}N$)

models that allow for a greater number of sources to be used. This approach, called *IsoSource* thereby allowed for mixing model solutions even for under-determined systems, *i.e.,* where the number of sources exceeds $n + 1$ (Phillips and Gregg, 2003). However, as the number of sources used in a mixture increases, so too does the uncertainty in the source combinations. As such, maximum likelihood approaches are insufficient at resolving unambiguous mixing model solutions. To counter this issue, a Bayesian framework (*MixSIR*) was adopted which permitted any number of sources to be considered (Moore and Semmens, 2008).

Employing stable isotope mixing models using a Bayesian framework has advanced this growing area of ecology, in that models explicitly recognise uncertainty from a number of sources (including diet-tissue fractionation) and incorporate them into model parameter estimates (Parnell et al., 2011). This approach, using Stable Isotope Analysis in R, or *SIAR* (Parnell et al., 2008; R Development Core Team, 2011) has provided robust dietary estimates for humpback whales using skin biopsies as the consumer mixture and putative prey items as sources (Witteveen et al., 2011). Spatial and temporal variation in isotopic baseline of the marine environment (*i.e.,* in phytoplankton tissues) is considerable, ultimately driven by sea temperature, water chemistry, day length, plankton morphometrics and carbon and nitrogen uptake regime (Goericke and Fry, 1994; Hofmann et al., 2000; Jennings and Warr, 2003; Tagliabue and Bopp, 2008). By measuring stable isotope values from consumer and source tissues sampled simultaneously in space and time, confounding effects of both spatial and temporal variation can be minimized. However, the potential bias in deciding on source contributions (*i.e.,* putative prey) in a mixture necessitates careful consideration of sources, based on empirical evidence (Phillips et al., 2005; Ward et al., 2011).

Euphausiidae (hereafter referred to as krill) are key species in marine foodwebs: exerting both bottom-up and top down forcings and supporting biomass of pelagic predators including baleen whales (Verity et al., 2002). The most abundant species found in the Celtic Sea (CS) are *Meganyctiphanes norvegica* and *Nyctiphanes couchii,* whose distributions are generally confined to continental slopes and shelf waters respectively (Lindley, 1982). In the Northeast Atlantic (NEA) stomach

content analysis carried out at whaling stations, supported recently by modelling spatial associations, confirm that fin whales feed chiefly on *M. norvegica* (Fairley, 1981; Aguilar, 2008; Skern-Mauritzen et al., 2011). In the NEA, fin whales are definitive hosts of *Bolbosoma balaenae*, an acanthocephalan parasite for which *N. couchii* is an intermediate host. This host-parasite relationship indicates that fin whales probably also feed on *N. couchii* in the NEA (Gregori et al., 2012). In the CS, fin and humpback whales associate with a seasonal inshore movement of spawning herring (*Clupea harengus*) (Whooley et al., 2011). These herring comprise two stocks targeted by a single fishery. Historically, these stocks have collapsed which has been attributed to a combination of over-exploitation and environmental factors (Lynch et al., 2011; Harma et al., 2012). Sprat (*Sprattus sprattus*) is a major bycatch component of other fisheries in the CS (*e.g.,* for groundfish and herring), but there is also a targeted fishery that is not currently managed or assessed by ICES for which there is an open quota (Enever et al., 2007). Moreover, sprat are recognised as an important diet component for predators in the CS ecosystem (Trenkel et al., 2005; Chivers et al., 2012).

In order to effectively conserve fin and humpback whales in the CS, their basic requirements and roles in the ecosystem must be identified, so that threats to survival and the maintenance of populations can be identified and alleviated. Towards achieving this goal, the present study aims to estimate relative contributions of krill and clupeid fishes in the diet of fin whales and humpback whales that occur sympatrically in the Celtic Sea (CS) using stable isotope Bayesian mixing models. It is hoped that this information may aid the development of ecosystems based approach to fisheries management.

4.3 Methods

Sampling

The study area comprised the CS and coastal waters to the south of Ireland (Fig. 1). A literature review and photographic evidence of surface active feeding were used to identify *a priori* the most likely prey (sources) contributing to the diet of both fin and humpback whales (mixture) it the CS. Herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) were caught by pelagic trawl during dedicated herring fisheries surveys and plankton samples were collected in a ring net (1 m diameter, 360 um mesh) using vertical tows. Plankton samples were collected during February 2010 and fish samples were collected on 18 October 2010 from *R.V. Celtic Explorer*. Species identification of zooplankton was carried out under the microscope.

Skin biopsies were collected from fin and humpback whales from small boats (5—12 m) using modified bolts (CETA-DART) fired from a crossbow (150 lb draw-strength). Steel sampling tips (Specials Engineering, Ireland, 40 mm depth) were fitted to the top of the bolts which have a compressed foam stop-collar to limit penetration, facilitate re-bound and provide buoyancy to the bolt to aid retrieval without the need for a tether. Tips were scrubbed in soapy water, sterilized, solvent-rinsed and foil-wrapped prior to use. Samples were removed from the tips using solvent-rinsed forceps, wrapped in aluminium foil and transported on ice to the laboratory whereupon they were stored in glass vials at -80°C. Photo identification was used to avoid duplicate sampling in the field (see Whooley et al., 2011). Samples were frozen temporarily at -20°C and then at -80°C until analysis.

Photographs of surface-active feeding fin and humpback whales were collected opportunistically during sampling cruises in both coastal and offshore waters. These photographs were examined to provide evidence of some prey species consumed, albeit on a coarse taxonomic level. Together with a literature review, putative prey items (sources) were chosen *a priori* for input into diet mixing models.

Tissue Preparation

Caut et al. (2011) found that for both herring and sprat, stable carbon and nitrogen isotope values of white muscle were an appropriate surrogate for whole-body homogenates, provided that lipids were accounted for. From above the lateral line, white muscle samples from each fish were excised, homogenised and 0.5 g aliquots were freeze-dried for 24h. Zooplankton were also freeze-dried for 24 h followed by removal of carbonates by soaking in a 2 M HCl for 5 min, or until effervescence had ceased (Søreide et al. 2007). After drying in a fume hood, zooplankton samples were homogenised and ground to a fine powder using a pestle and mortar. Fin and humpback whale skin samples were duplicated and diced finely using solvent-washed scalpels on clean glass slides. Entire longitudinal profiles of the skin biopsies to a depth of *ca.* 10 mm were analysed. Lipids were extracted for 24 h (6 h refluxing, 18 h soaking) in Soxhlet washed glassmicrofibre thimbles with 150 mL of 1:1 *n*-hexane and acetone (Ryan et al., 2012). Both lipid-extracted and bulk skin samples were homogenised using a mortar and pestle. *Ca.* 1.5 g of prepared tissues were weighed accurately into tin cups.

Ageing

The age of sprat and herring was considered as an important factor given that stable isotopic composition is likely to change with size and thus age in pelagic fishes (*e.g.*, Overman and Parrish, 2001; Jennings et al., 2002). Furthermore, selective foraging for certain prey age-classes by predators such as baleen whales is possible (Griffiths, 1980). Standard length was recorded for each fish in order to estimate age. Age for herring and sprat was determined using length-at-age regression models that are derived during routine pelagic trawl surveys for stock assessment (Saunders et al., 2010).

Stable Isotope Analysis

Carbon and nitrogen isotope composition of whale skin was determined using continuous flow elemental analysis isotope ratio mass spectrometry (CF-EA-IRMS) at the University of Southampton using a EuroVector EA 3000 (EA) combined with a PDZ Europa Scientific 20-20 (IRMS). Isotope ratios are presented in delta notation as parts per thousand differences from an internal standard (ACROS L-Glutamic Acid) according to the following equation: $\delta^{Y}X = ([R_{sample}/R_{standard}] - 1) \times 10^{-3}$, where R denotes the heavier:lighter isotope ratio and Y is the atomic mass of the stable isotope X ($\delta^{13}C$ or $\delta^{15}N$). Internal standards calibrated with International Atomic Energy Agency IAEA (Vienna, Austria), *i.e.,* Vienna Pee Dee Belemnite (for C), atmospheric N_2 (for N), were routinely analysed between samples in order to determine instrument precision. Based on the standard deviation of these standards, the lowest analytical precision of two runs was 0.2 ‰ for nitrogen, and 0.1 ‰ for carbon. Prey items (fish muscle and homogenised krill) were analysed at University of California, Davis by CF-EA-IRMS using a PDZ Europa ANCA-GSL (EA) combined with a PDZ Europa 20-20 (IRMS). The analytical precision, calculated as the standard deviation of routinely measured bovine liver and glutamic acid standards, was 0.15 ‰ for nitrogen, and 0.06 ‰ for carbon.

Correction of $\delta^{13}C$

In exoskeletons of crustaceans such as krill, carbonates ($CaCO_3$) are derived from isotopically heavy HCO_3- ions from the environment, and are thus a non-dietary fraction and must also be removed as their enriched ^{13}C affects whole-body $\delta^{13}C$ values (Søreide et al. 2006). Lipids are depleted in ^{13}C, thus altering the $\delta^{13}C$ values of tissues. The elemental carbon to nitrogen ratio (C:N) is a useful proxy for lipid content (McConnaughey and McRoy, 1979) and was used to assess for lipid effects on isotopic values in light of those previously published species and tissue specific values for lean tissue. Lipid-free C:N values for whole zooplankton ($\bar{x}$ range) are 3.30—4.03 for marine zooplankton (Kiljunen et al., 2006; Søreide et al., 2007), ($\bar{x}$ ± SD) 3.6 ± 0.1 *M. norvegica* (Bentaleb et al., 2011) and 3.3 ± 0.1 for white muscle in

sprat and herring of (Kiljunen et al. 2006, Caut et al. 2011). These were used as a threshold values, which if exceeded indicated that $\delta^{13}C$ values should be corrected arithmetically (*i.e.*, lipid-normalized) to correct for the presence of isotopically lighter lipid (Table 1). The normalization model used to estimate lipid-free $\delta^{13}C$ values is a non-linear equation from Kiljunen *et al.* (2006) requiring the C:N ratio and the isotopic discrimination factor between lipid and protein (D = 7.018 ± 0.263) of the sample, and a constant (I = 0.048 ± 0.013). After carbonate extraction of krill samples, the sample C:N threshold values were also used to confirm that carbonates had been fully extracted (Søreide et al. 2006). Normalization for the effects of lipid on $\delta^{13}C$ values in fin and humpback whale skin is not currently possible and standard chemical lipid extraction procedures lead to unpredictable changes in $\delta^{15}N$ values (Ryan et al., 2012; Lesage et al., 2010). Therefore $\delta^{13}C$ values from lipid-extracted skin and $\delta^{15}N$ values analysed from non-extracted aliquots of skin were used as end-members (consumers) in mixing models.

Diet Modelling

Diet solutions were estimated by mixing models *via* Bayesian inference using the SIAR package in the statistical programming environment, R (Parnell et al., 2008; R Development Core Team, 2011). SIAR utilises the generalised multivariate equivalent of the Beta distribution, Dirichlet, as a prior which treats each elected dietary source (prey) independently but necessitates a sum to unity (*i.e.,* that diet proportions sum to 1). Models are fitted hierarchically using Markov chain Monte Carlo (MCMC) to produce parameter estimates based on both the data and the prior distribution. Probabilistic density estimates of proportionate dietary contributions of sources (prey) to end members (whale skin) are thus derived. The advantage of this approach over alternative mixing model techniques is the ability to include variability that is unconstrained by the number of sources used (Phillips & Gregg 2003). Unlike other Bayesian mixing models such as *MixSIR* or *Isosource*, SIAR recognises important sources of variability and incorporates them to give diet solutions of consumers that recognise this variability. Thus variability in:

trophic enrichment factors, sources and end members are explicitly accounted for in the model (Parnell et al., 2011). Such variability may be significant in ecological systems and can arise due to unknown dietary sources, baseline isotopic variation and physiological differences between taxa (Caut et al., 2008, 2009).

Using fish muscle and whole zooplankton as sources and whale skin as end members (prey), 500000 iterations (thinned by 20 and with a burn-in discard of 10000) were used to derive parameters of posterior distributions. The following diet-tissue discrimination factors derived for fin whale skin (Borrell et al. 2012) were included in the model for both fin and humpback whales: 1.28 ± 0.38 for $\delta^{13}C$ and 2.82 ± 0.30 for $\delta^{15}N$. No such discrimination factors have been calculated for humpback whales, however closely related cetacean taxa are known to exhibit similar values (Newsome et al., 2010; Caut et al., 2011). Whale species (fin and humpback whale) was used as a grouping factor to investigate resource preferences by species. Model inputs comprised only animal tissues (as opposed to plant tissues) which are likely to exhibit similar elemental concentrations of carbon and nitrogen and are partitioned in a similar manner from food sources. Concentration independent models could therefore used (Phillips and Koch, 2002).

In SIAR mixing models, correlation of posterior distributions of sources implies that the model is poor at differentiating between those sources (given the sum to one constraint in the MCMC draws). Therefore pair-wise correlation of posterior distributions was calculated to interrogate model performance. Probability of model parameters (M) given the prior data (D) is presented in order to investigate differences in diet source contributions, among prey and between fin and humpback whales. These probabilities (Pr) are derived by Bayesian inference whereby lower $\Pr(M|D)$ values imply lower support of the hypothesis.

4.4 Results

Photographs of surface-active feeding whales provided evidence that fin and humpback whales feed on both zooplankton and clupeids (Figure 16). Sufficient evidence from the literature was determined to postulate some of the dietary

sources for fin and humpback whales in the CS (Burfield, 1913; Fairley, 1981; Aguilar, 2008; Whooley et al., 2011; Gregori et al., 2012). The following prey species were therefore used as sources in the mixing models: sprat, herring, *M. norvegica* and *N. couchii* (Figure 17).

Figure 16. Photographs of surface active feeding by fin and humpback whales were collected opportunistically between 2008 and 2011 in coastal and offshore waters during dedicated cetacean and fisheries surveys. Those images showing the strongest evidence for foraging on a diversity of prey are presented in order to provide baseline evidence of some prey taxa. From top left: humpback feeding on clupeids (< 10 km south of Ireland); humpback feeding on zooplankton (unknown spp.) (< 10 km south of Ireland); fin whale feeding in coastal waters on clupeids (< 10 km south of Ireland); orange scats in pelagic waters from fin whales, suggesting zooplanktivorous diet (140 km southwest of Ireland).

According to age-class, sprat exhibited markedly consistent $\delta^{15}N$ values whereas those of herring were more variable (Figure 18, Table 6). After lipid normalization, older fish were less enriched in ^{13}C, although age-4 herring were more enriched than age-2 herring. *M. norvegica* from the 100 m depth sampling station showed less variable $\delta^{15}N$ values compared to those from the 1000 m depth station. However, their respective $\delta^{15}N$ and $\delta^{13}C$ values indicated that they should be pooled into one grouping which was used for the final model. Although only available from the 100 m sampling stations, isotopic values for *N. couchii* were similar to those of *M. norvegica* (Figure 18 and Table 6). After tissue treatments, C:N ratios were similar among all source and consumer tissues, justifying the use of concentration independent models (Table 6). Fin and humpback whale skin samples exhibited indistinguishable mean $\delta^{15}N$ and $\delta^{13}C$ values (Figure 18, Table 6).

Table 6. Mean $\delta^{13}C$, $\delta^{15}N$ and C:N values (standard deviation in parenthesis) for consumer and source values used in the mixing model. Lipid extracted and lipid normalized values are denoted by * and ** respectively. Criteria for normalization of $\delta^{13}C$ for lipid content was C:N > 3.4 for sprat or herring, and C:N > 4.0 for krill (Kiljunen et al., 2006)

Species	$\delta^{13}C$	$\delta^{15}N$	C:N	*n*
Fin Whale	-18.2 (0.5)*	12.1 (1.1)	3.68 (0.35)	21
Humpback Whale	-17.8 (0.3)*	12.9 (0.7)	3.49 (0.45)	4
Sprat (Age-0)	-19.2 (0.5)**	12.2 (0.2)	3.54 (0.33)	4
Sprat (Age-1)	-19.8 (0.6)**	12.3 (0.5)	4.02 (0.51)	20
Sprat (Age-2+)	-20.5 (0.7)**	12.3 (0.6)	4.72 (0.70)	15
Herring (Age-0)	-18.2 (0.1)	13.3 (0.5)	3.30 (0.07)	5
Herring (Age-2)	-19.6 (0.3)**	12.8 (0.6)	3.79 (0.25)	9
Herring (Age-4)	-19.4 (0.5)**	11.5 (0.4)	3.68 (0.31)	5
Meganyctiphanes norvegica (100m)	-21.3 (0.2)	7.9 (0.1)	3.59 (0.21)	4
Meganyctiphanes norvegica (1000m)	-20.6 (1.1)	7.2 (0.4)	3.60 (0.07)	5
Nyctiphanes couchii	-21.9 (0.5)	5.7 (0.5)	3.83 (0.06)	4

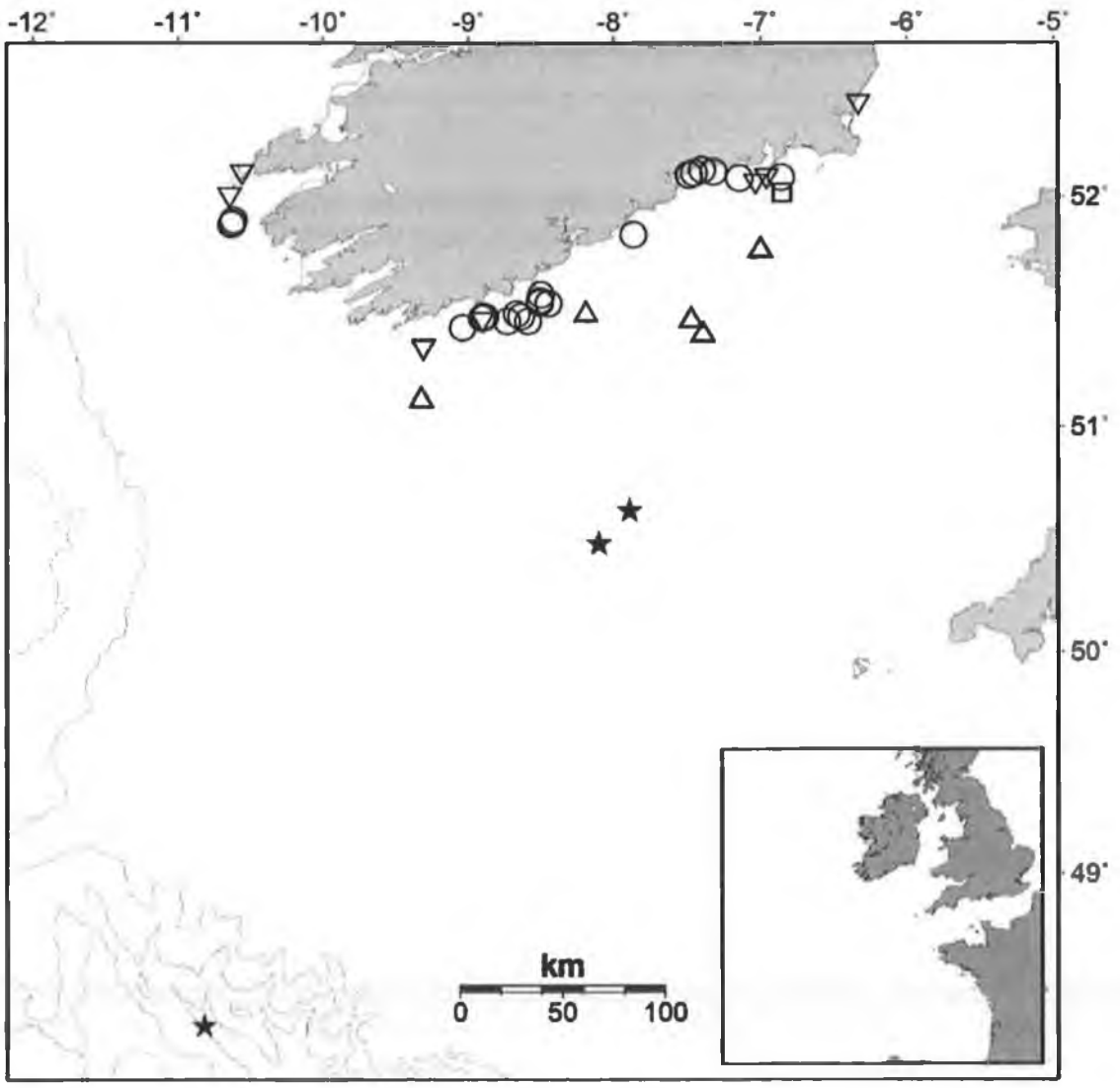

Figure 17. Map of study site showing sampling locations. Circle = fin whale; inverted triangle = humpback whale; triangle = sprat; square = herring; star = krill.

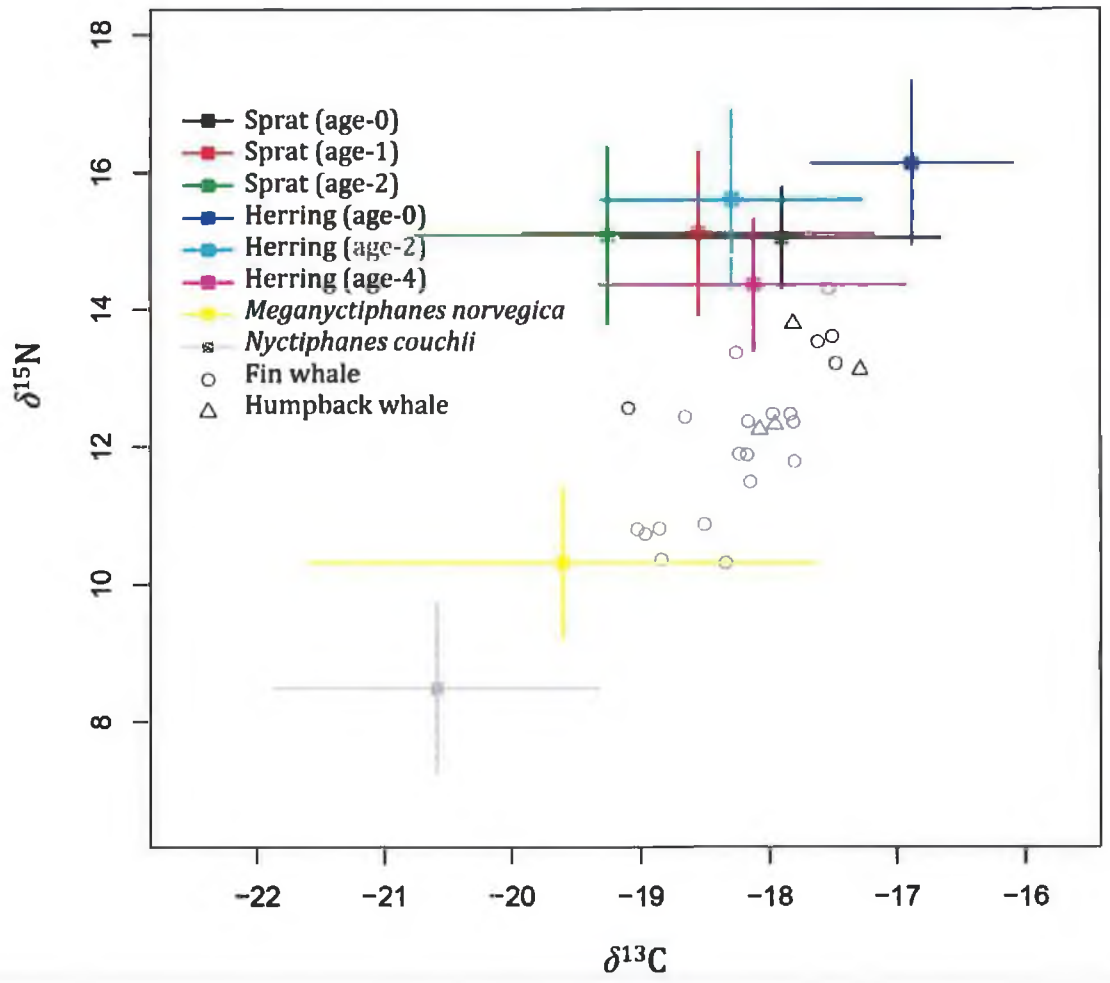

Figure 18. A biplot showing the actual consumer isotopic values and those of putative prey (sources) which have been corrected-for using tissue-diet discrimination factors (Borrell *et al.*, 2012). Fishes are plotted by year class with the age based on total length in parenthesis. This shows the large difference in δ^{15}N between fishes and krill, and similar isotopic values for both carbon and nitrogen in fin and humpback whales.

Mixing Model Diagnostics

The sum-to-one requirement of the Dirichlet distribution results in a potential trade-off between some sources, in order to derive robust mixing model solutions (*i.e.*, where the abundance of one source increases, another necessarily decreases as their total relative abundance must equal 1). Thus correlation between

posterior distribution probability densities of sources indicates poor ability for the model to differentiate these prey contributions to diet solutions. Pair-wise correlations revealed very strong negative correlation between *M. norvegica* and *N. couchii* (-0.71) for fin whales. This indicates that the proportionate contribution of *M. norvegica* and *N. couchii* to the diet of fin whales is inseperable by the model, so both prey items must be considered in collectively. In the final model, of 18 pair-wise correlations for sources, three were greater than -0.30 for fin whales, but none for humpback whales. Correlations of -0.40 were found between age-4 herring and both age-0 herring and *M. norvegica.* Age-0 sprat and age-0 herring showed a similar negative correlation (-0.41) for fin whales only. Mixing models were re-run where sprat and herring age classes were pooled but correlations between source posterior distributions were greater (<-0.50), providing justification for the stratification of fish isotopic data by age.

Mixing Model Solutions

In Bayesian inference, given data (D) and a model (M) the probability from the posterior distribution is presented as $\Pr(D|M)$. Assuming that the model includes all major diet sources, both fin and humpback whale diets included large proportions of both fish and krill species, although fin whales were less piscivorous than humpback whales (Figures 19 and 20). When combined, both krill species comprised a greater proportion of the diet of fin whales than in humpback whales ($\Pr(D|M) = 0.971$). For fin whales, krill was the most dominant diet component and the probability that it comprised a greater proportion than the next most abundant component (age-0 sprat) was 0.955 (Figures 19 and 20). While there was a high probability that age-0 sprat were more abundant in fin whale diet solution than either age-1 (0.696) or age-2 (0.786), the probability that sprat was greater than herring when posterior age class distributions were pooled was very low, $\Pr(D|M) = 0.318$ (Figures 19 and 20). Both krill species exhibited a wide range in $\delta^{13}C$ which is consistent with a high degree of spatio-temporal variability within the sample (Figure 18). Despite this however, the mixing model solutions show unambiguous isotopic separation between fish and krill, leading to reduced uncertainty when partitioning diet sources (Figure 19).

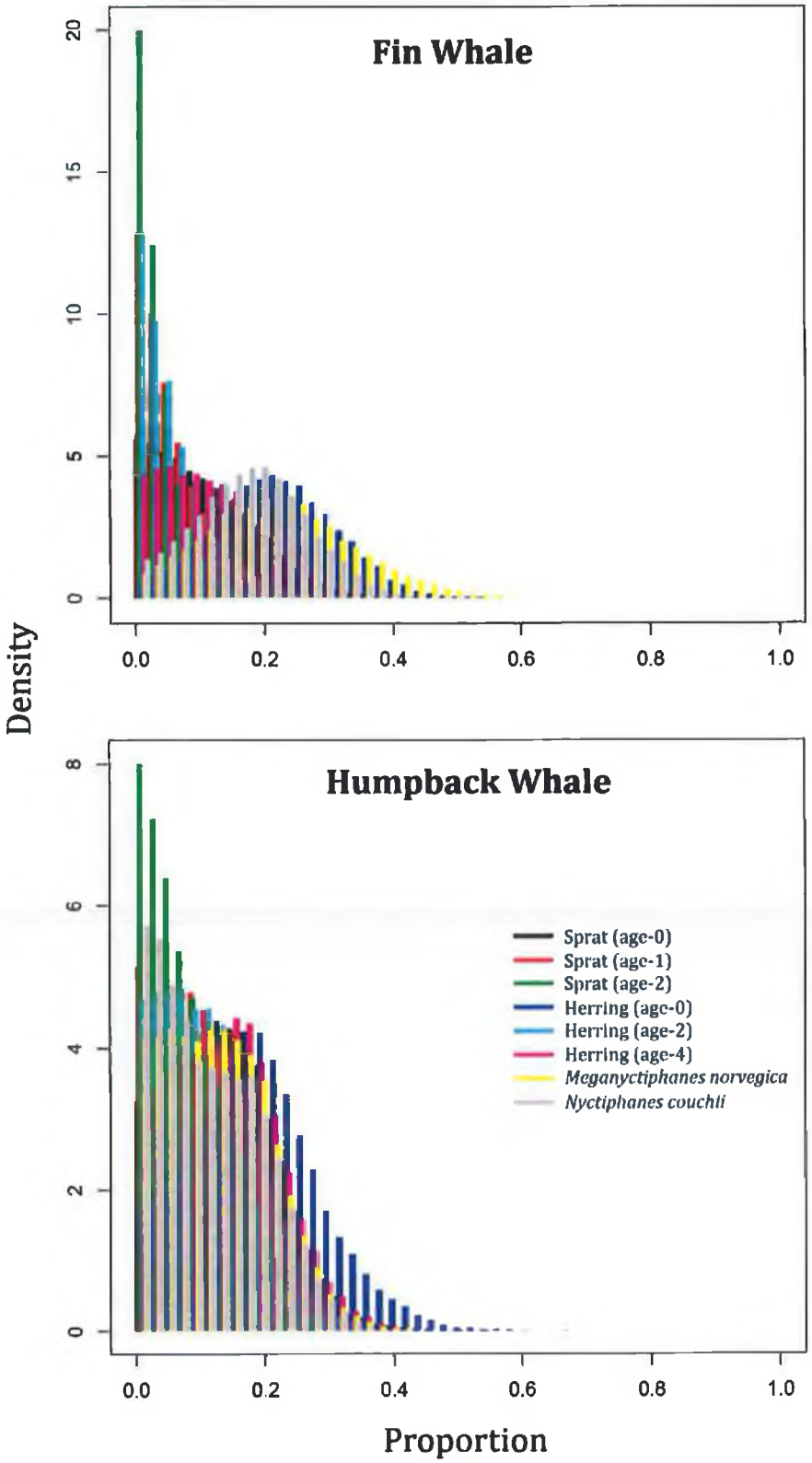

Figure 19. Probability density histograms showing the distribution of posterior parameters from the SIAR model, based on 500000 iterations. The

model estimated that fin whale (left) diet is predominated by krill, whereas humpback whales (right) consumed relatively more fish species. age-0 herring followed by age-0 sprat were the most important fish components in the diets of both whale species.

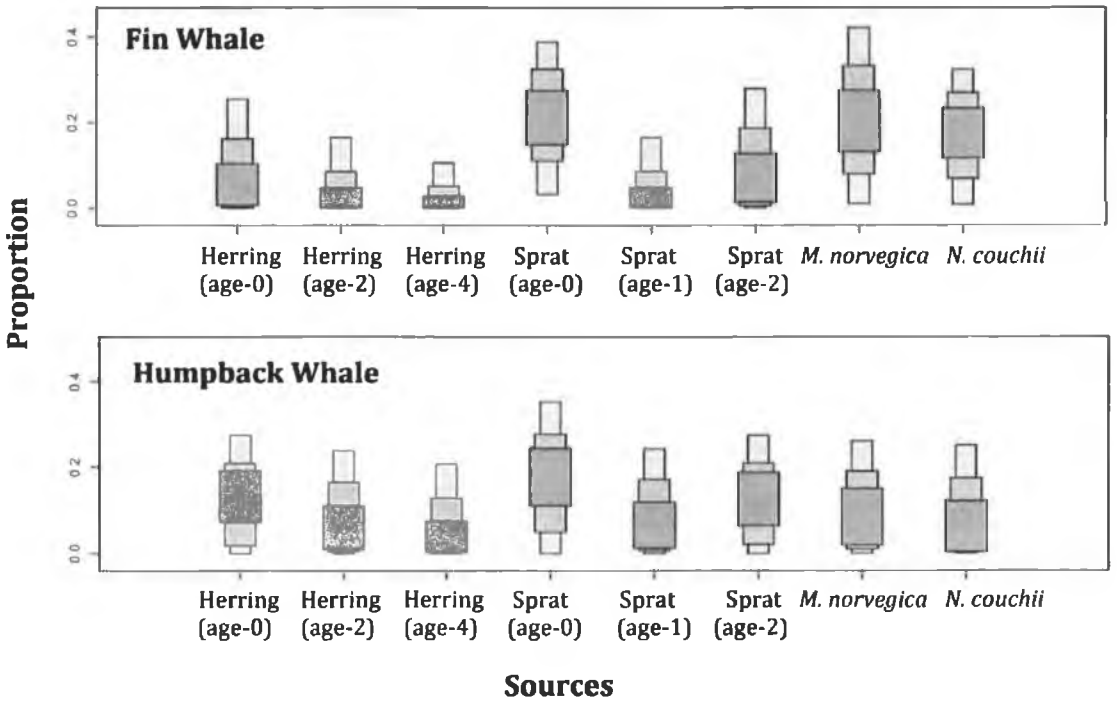

Figure 20. Box plots showing the differential proportionate contribution of sources in modelled diet solutions for fin (above) and humpback (below). Credibility intervals are presented as dark (50%), intermediate (75%) and light (95%) grey boxes. For fishes, the age according to length is given in parenthesis.

4.5 Discussion

A prior assessment of likely diet components of fin and humpback whales in the CS was made, based on the best available evidence from the literature and field observations. This guided the selection of sources for the isotope mixing model. A caveat of this approach was that sources used in the mixing models were unlikely to be an exhaustive representation of the species diversity in the diet of fin and humpback whales which may feed on other fishes *e.g.,* anchovy (*Engraulis encrasicolus*), pilchard (*Sardina pilchardus*), mackerel or blue whiting (*Micromesistius poutassou*), or indeed other species of zooplankton, *e.g,. Calanus* spp. or *Thysanoessa* spp. However there was no evidence from field observations or from the literature that these species are indeed predated upon by fin and humpback whales in the CS or contiguous waters. A potential source of bias in our results is the differential tissue turnover rate between krill and fish muscle. This source of temporal variability may have resulted in an under-representation of krill proportions in the mixing model solutions, given that the entire isotopic range of krill may not have been sampled. However, previous studies have found low spatio-temporal variability in both $\delta^{15}N$ and $\delta^{13}C$ values of *M. norvegica* in the NEA (38° to 45°N, 12° to 13°W) whereby seasonal differences in isotope values were not significantly different (Bentaleb et al., 2011).

Mixing model solutions confirmed that fin and humpback whales are both planktivorous and piscivorous in the CS. While krill (*M. norvegica* and *N. couchii*) also make up a large proportion (maximum *a posteriori* probability estimate, low - high 95% credibility intervals) of their diet, especially for fin whales (0.46, 0.22 – 0.59), both fin and humpback whales were found to have a preference for age-0 sprat (0.22, 0.00-0.37 and 0.30, 0.01-0.38 for fin and humpback whales respectively) and herring (0.17, 0.01-0.35 and 0.22, 0.02-0.36 respectively). Proportions of both age-0 sprat and age-0 herring were not easily differentiated by the mixing models. It is likely that this is ecologically relevant rather than a model artefact, given the propensity for mixed species shoals of age-0 sprat and herring in the CS, collectively known as 'whitebait'. Similarly, sprat and herring fisheries

are unlikely to be selective in catching these shoals. The importance of age-0 sprat and herring in the diet of both fin and humpback whales should be considered in the management of CS fisheries.

Sympatric positive density-dependent foraging by fin and humpback whales has been found at high latitudes in both the eastern and western North Atlantic, owing to the patchiness of prey and suitable foraging conditions (Piatt & Methven 1992, Skern-Mauritzen et al. 2011). Given that these species have evolved to be obligate batch-feeders, prey fields of suitable magnitude and density are critical for optimal foraging leading to threshold foraging behaviour (Piatt & Methven 1992, Goldbogen et al. 2011). Facilitation among these species, as well as seabirds and other marine predators, may be necessary to both locate and contain prey (Rudd et al. 2011). In light of this, the trade-off between positive density-dependent foraging and resource partitioning likely culminates in complex community structure among rorquals which merits further research if management measures are to be implemented using an ecosystems-based approach. Furthermore, spawning herring have been shown to exhibit structurally stable schools that emerge only after threshold population sizes are reached (Vabø and Skaret, 2008). If feeding whales exploit this synchronous behavioural trait in herring, then optimal foraging might will not be met for reduced densities of herring which could occur at a local scale due to disruption caused by trawling, or at the population scale due to over-fishing.

Krill species including both *M. norvegica* and *N. couchii* comprised about half of the diet in fin whales. Humpback whales by comparison consumed significantly lower proportions of krill species indicating a more piscivorous diet. Given that krill comprised one of the smallest dietary components in humpback whales, resource competition between fin and humpbacks whales in the CS might only be short-lived. It remains to be resolved however, the proportions of each krill species that are being consumed. Given that *M. norvegica* and *N. couchii* occur chiefly in offshore and shelf waters respectively, discerning which of these species comprise the preferred prey for fin and humpback whales should be a research priority. This may shed light on feeding strategies when the whales are foraging offshore,

beyond the current reach of researchers. Fatty acid analysis of blubber biopsies may provide more conclusive insights on this issue (Borobia et al., 1995; Grahl-Nielsen, 2009).

Over-exploitation of benthic fishes such as gadoids, has resulted in a reduced trophic system (at a rate of -0.02 to -0.04 TL yr^{-1}) in the CS from which pelagic fishes such as clupeids appear to have benefited by increased biomass (relative to other species in the ecosystem) in spite of fishing intensity (Pauly et al., 1998; Pinnegar et al., 2002). Fisheries may benefit from this lower trophic community structure whereby higher fishery yields are achieved (Pinnegar et al., 2002). Paradoxically, those cetaceans that preferentially feed at lower trophic levels, *e.g.*, baleen whales feeding on krill and clupeids, may benefit from this fisheries-induced ecosystem modification. Chiefly during autumn months, fin whales can be observed in coastal waters of the CS, allowing for shore-based research (Whooley et al., 2011). Whether or not high relative abundance of rorqual whales in the CS (namely fin, humpback and minke (*B. acutorostrata*) whales) has increased in recent years is not currently possible to discern due to a lack of sightings data prior to the 1990s.

The CS herring fishery is unusual from a fisheries management perspective in that commercial exploitation began after routine stock assessment was already in place (Pinnegar et al., 2002). CS herring spawn at the southern-most limit of the species range in the NEA and are therefore particularly vulnerable to changes brought about by climatic change. The two stocks exhibit 'spawning diversity', believed to be a survival strategy, where autumn and winter spawning components are subject to different survival and recruitment driven by environmental factors and fishery mortality (Harma et al., 2012). The relative proportion of autumn-spawning to winter-spawning herring is currently at its lowest since 1959, a trend strongly influenced by the Atlantic Multi-decadal Oscillation (Harma et al., 2012). Such a seasonal shift may have significant implications for rorqual whales, which being capital breeders, are seasonally constrained in their foraging habits: generally feeding at high latitude in summer and breeding in low latitudes during winter (Jonsgård, 1966; Baker et al., 1990). A retraction towards winter-dominated

spawning may lead to mismatch between spawning (and hence coastal aggregations of herring) and the foraging window for whales, given their reproductive requirements to breed at lower latitudes during the winter.

4.6 Conclusions

Stable isotope mixing models showed that fin whales exhibit a preference for krill (*M. norvegica* and *N. couchii*) over clupeid fishes (sprat and herring), whereas humpback whales consume a greater proportion of fish. Thus fin whales are more omnivorous and generally occupy a slightly lower trophic level than humpback whales in the CS. Fin and humpback whales both predate on herring and sprat, species which are intensively fished in the CS. Of the fish consumed, both whale species preferred age-0 sprat and herring, followed by older sprat, and finally age-4 herring. Against a backdrop of historic herring fishery collapse, stock substructure and recruitment that is influenced by complex bio-physical factors, we advocate against management of CS fish stocks from a species-wise maximum fisheries yield perspective. Rather, an ecosystems based approach that implicitly recognises top predators such as rorquals as integral to ecosystem functioning should be adopted. The potential for a mismatch in whale foraging seasonality and winter dominant components of herring spawning has been identified, which may have negative implications for whales in the CS. In light of a declining trophic system and recovery of both fish and whale populations from over-exploitation in the region, it is incumbent on fisheries management that an ecosystems approach is adopted. This will be necessary to effectively conserve top predators including fin and humpback whales, while maintaining secure ecosystem functioning on which sustainable fisheries rely.

4.7 Acknowledgements

Thanks to those who helped in the field: Pádraig Whooley, Collin Barnes, Fien de Raedemaeker, Allesandro Pierini, Nick Massett, Andrew Malcolm, Ann Trimble, Mick Sheeran, Joanne O'Brien, Deirdre Slevin, Paddy Roche, Brian Duffy, Martin Colfer. Thanks to Commodore Mark Mellett and Commanding Officer of L.É. Orla Caoimhín Mac Unfraidh, Irish Naval Service for assistance. Thanks to Brian Boyle and Poppy for help with the Soxlhet apparatus. Thanks to Dave Wall, the other scientists and crew of *R.V. Celtic Explorer* for help with collecting fish and plankton samples. Andrew Jackson is gratefully acknowledged for open-source help with SIAR mixing models. Biopsy samples were collected under permit from the National Parks and Wildlife Service, Ireland (License Numbers C76/2008, C82/2009 & C130/2010).

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Chapter 5.

Lipid Content of Blubber Biopsies is not Representative of Blubber *in situ* for Fin Whales (*Balaenoptera physalus*)

"Luck is what happens when preparation meets opportunity"

Lucius Annaeus Seneca, philosopher (4 BC—65 AD)

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Ryan C, McHugh B, O'Connor I, Berrow S (2012) Lipid content in blubber biopsies is not representative of blubber in situ for fin whales (*Balaenoptera physalus*). Marine Mammal Science 28(4): In Press.

5.1 Introduction

Biopsy darting of free ranging cetaceans is a non-lethal tissue sampling technique where retrievable darts are delivered using a crossbow or modified rifle (Lambertsen 1987). Biopsies permit various analyses of free-ranging cetaceans using a suite of techniques including molecular genetics, fatty acid (diet composition and ageing), immunohistochemical, stable isotope and persistent pollutant analyses (Baker *et al.* 1990, Borobia *et al.* 1995, Gauthier *et al.* 1997*a*, Todd *et al.* 1997, Hooker *et al.* 2008, Herman *et al.* 2009). The findings from such studies have revolutionized our understanding of cetaceans (Borobia *et al.* 1995, Palsbøll *et al.* 1997, Möller *et al.* 2001). This sampling method has been validated for analysis of persistent organochlorine contaminants in the blubber of some species of mysticetes (Gauthier *et al.* 1997*b*). Blubber lipid-content and thickness are of particular importance in the assessment of the life cycle status and health of cetaceans, particularly those capital breeders such as mysticetes where blubber lipid stores govern reproductive success (Pettis *et al.* 2004, Iverson 2008). Lipid content of fin whale blubber for example, has been shown to vary with gender and reproductive status (Lockyer *et al.* 1985). One of the untested assumptions of the remote biopsy technique is that the lipid content of blubber biopsies is representative of that *in situ*, *i.e.*, that there is no sampling effect. However Krahn *et al.* (2007) found surprising differences within the same region and season in percentage lipid content of blubber samples between live (biopsied) and stranded

(excised) whales for both killer (*Orcinus orca*) and beluga (*Delphinapterus leucas*) whales.

Testing how representative blubber biopsies are of the tissue *in situ* with regards to extractable lipid content is pertinent for chemical analyses where the percentage lipid content of the tissue is of great importance. Indeed, several studies have presented lipid content of blubber biopsies for use in subsequent calculations or discussions thus assuming no sampling effect (Varanasi *et al.* 1994, Gauthier *et al.* 1997*b*, Ross *et al.* 2000, Krahn *et al.* 2001, Metcalfe *et al.* 2004, Nino-Torres *et al.* 2010). Field observations of oily slicks in the sea surrounding biopsy darts prior to retrieval lead to us hypothesize that lipids seep out of blubber biopsies while in seawater. Krahn *et al.* (2004) hypothesized three causes for lipid loss from darted biopsy samples: (1) Seepage from the blubber matrix upon rebound of the biopsy dart; (2) Lipid washing away from exposure to sea water; (3) Oblique angle of dart leading to more connective tissue than blubber. The following experiment was carried out to ascertain if biopsy darting leads to lipid loss in blubber samples, and if so whether this effect occurs as a function of time of exposure to sea water (*i.e.*, time for retrieval of dart from the sea in the field).

5.2 Methods

A male fin whale measuring 12 m in length stranded (possibly alive) in Raghly, Co. Sligo, Ireland on 28 November 2011 and was visited the following day. External examination and a subsequent full dissection indicated that the whale was not emaciated. The prevailing weather was cool and damp and the sea temperature offshore was 11.4 °C (Marine Institute M4 weather buoy data). The carcase showed no signs of decay and was considered a Code 2 at the time of sampling on the Smithsonian Institute's scale for carcase decomposition classification (Geraci and Lounsbury 2005). The blubber appeared to be in good condition (*i.e.*, firm, pink and without a sharp odour) and as such, any effects of oxidation on the lipid within blubber was considered negligible.

An area within 2 m of the dorsal fin on the dorsal side of the carcase was demarcated. This is the typical target area for biopsy sampling as it presents during the surfacing sequence and is the most lipid rich area with the greatest depth of integument (Lockyer *et al.* 1985). From this region, control samples were excised using a scalpel to a depth of approximately 40 mm. Using a biopsy bolt and 40 mm Larsen biopsy tips (designed by Ceta-Dart, Dr. F. Larsen, Copenhagen, Denmark), 18 biopsy samples were taken. The use of a crossbow to deploy the bolts was not possible at the time of sampling. Samples were therefore taken by striking the target area with the darts by hand. This method would result in a more conservative loss of lipid as less adipocytes would be ruptured than from a stronger impact by a dart delivered using a crossbow.

To emulate real post-biopsy sampling conditions, samples were placed in containers of seawater, *i.e.*, 'exposure'. Three replicates for five exposures (0, 15, 30, 60, and 180 s) were carried out. Biopsies were then removed from the biopsy tips using clean forceps, and placed in aluminium foil and frozen at -80°C as per standard field procedure in chemical analysis of blubber biopsies. The control samples were preserved in the same way.

In the laboratory, while still frozen, skin was removed from all samples using solvent rinsed scalpels and glass slides as cutting tables. The blubber portion was measured to the nearest mm using a Vernier calliper. Length ($\bar{x}$ ± SD) was found to be 12 ± 2.6 mm, thus control samples were measured and cut to a depth of 12mm from the skin. Samples were accurately weighed ($\bar{x}$ ± SD), controls: 109.9 ± 20.5 mg and biopsies: 92.0 ± 28.7 mg) and placed in glass microfiber thimbles (Whatman, 1.7 mm thickness) in a Soxhlet apparatus with 150 mL of 1:1 *n*-hexane and acetone (Pestican grade). Lipid extraction of tissue samples was carried out in Soxhlet washed thimbles by 6 h refluxing followed by 18 h of soaking. Using the

carbon to nitrogen ratio, measured by isotope ratio mass spectrometry, as proxy for lipid-content this method is considered proficient in thorough extraction of lipid from fin whale blubber (Ryan, unpublished data). Total extractable non-volatiles, referred to as lipids hereafter, were removed from the solvents using a concentration evaporator (TurboVap) under nitrogen stream at 30°C until fully evaporated (approximately 90 min for 150 mL). The lipid was weighed and the percentage lipid thus calculated gravimetrically by dividing total lipid weight by blubber wet weight in mg (Varanasi *et al.* 1994).

5.3 Results

An analysis of variance between percentage lipid content in blubber for all exposures (including control) was carried out. Bartlett's test for homogeneity of variance did not reveal a violation of homoscedasticity and Shaprio-Wilke's test indicated normality of residuals. A one-way ANOVA showed a significant effect of exposure time on percentage lipid content ($F_{5,12} = 47.25$, $P < 0.01$). *Post hoc* Tukey's pair-wise tests indicated that the significant differences were exclusively between control samples and those that were taken using the biopsy darts (all $P < 0.01$). Percentage lipid ($\bar{x} \pm$ SD) was found to be 81 ± 1.2% for controls ($n = 3$) and 37 ± 6.6% for pooled exposures ($n = 15$). Variability was higher within the biopsy samples in comparison to controls (Figure 21). Linear regression analysis indicated a non-significant ($r^2 = 0.29$, $P = 0.09$) negative relationship between length of exposure in sea water and percentage lipid in the blubber.

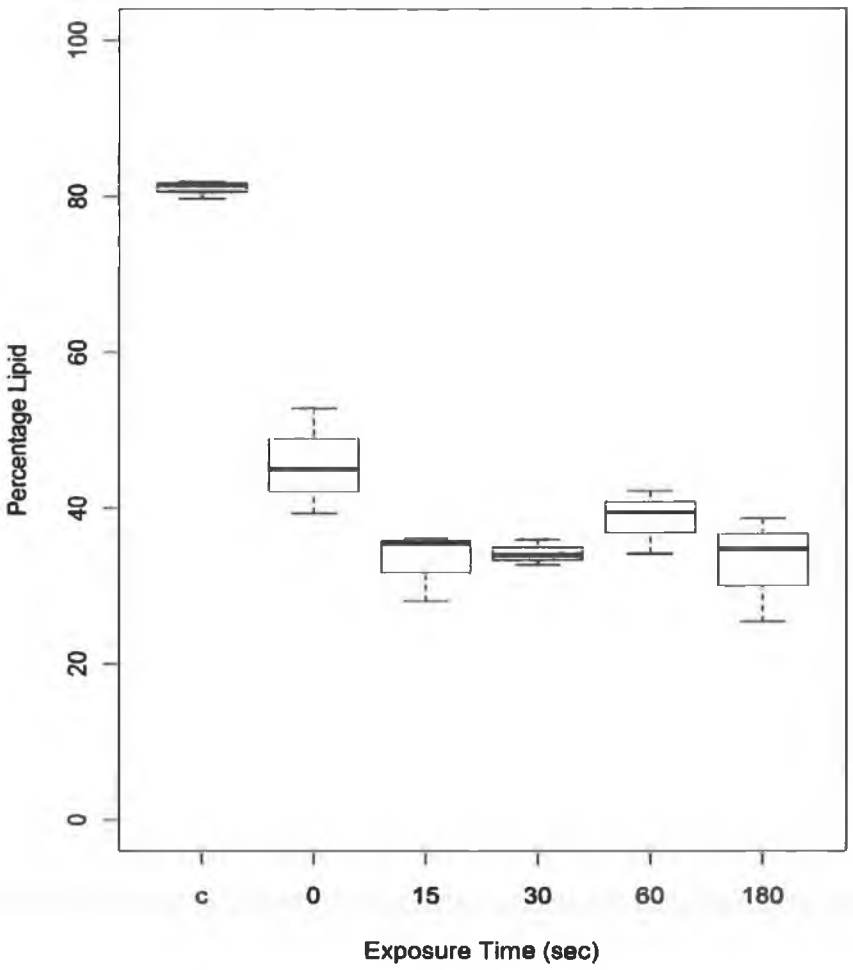

Figure 21. Lipid content (percentage total non-volatiles) of controls and biopsies for each seawater exposure.

5.4 Discussion

The percentage lipid content of blubber is an informative measure of fattening and thus body condition in cetaceans (Aguilar and Borrell 1990, Krahn *et al.* 2001). Blubber lipid content is also an important factor in interpreting *e.g.*, concentrations of persistent organic pollutants within and between species. Some biopsy-based studies have factored this into calculations (Metcalfe *et al.* 2004, Walton *et al.* 2008), however our results indicate that lipid content of blubber biopsies is never

representative of blubber *in situ* due to a sampling effect leading to a loss of lipid. As a result of unexpectedly low lipid content values from killer whale biopsies, Krahn *et al.* (2007) alluded to the possibility of such a sampling effect. Gauthier *et al.* (1997*b*) examined the differences between blubber lipid content of samples taken from stranded minke (*B. acutorostrata*) and blue (*B. musculus*) whales and those from live (biopsied) animals. It was concluded that percentage lipid from minke and blue whale biopsies were representative; however some of the stranded whales to which these were compared were at an advanced state of decay (> 1 mo). Furthermore, it was not possible to test for sampling effect in that study as biopsies and excised samples came from different specimens.

In the present study there was a high variability in lipid content of biopsies from the same individual, where sampling location on the body was consistent. While lipid content does vary with location on fin whales, the variation observed here would not be expected considering the lipid content of anterior dorsal blubber is known to be largely homogenous in fin whales (Lockyer *et al.* 1985). The very high lipid content of the control samples was in line with previous findings for male fin whales (Aguilar and Borrell 1990). Highly variable and low concentrations of lipid in cetacean blubber samples have been reported elsewhere (Krahn *et al.* 2007, Walton *et al.* 2008), *e.g.*, Krahn *et al.* (2007) report a range of 9.6 % to 40.9 % in killer whale biopsies attributed to factors other than a sampling effect. A non-significant, weak negative relationship between soak time in seawater and lipid content suggests that lipid loss may continue as a function of soak time, however a greater number of replicates and exposures is required to test this.

5.5 Conclusion

The present study found that the lipid content of the blubber portion of darted biopsies from fin whales is not representative of the tissue *in situ*. We propose that lipid loss occurs as adipocytes are burst due to the force of the biopsy cutting tip. It is probable that this finding holds for other similar species. As such, careful

consideration should be given to the use of remotely sampled blubber biopsies of cetaceans in quantitative analysis of lipid. In light of these findings we urge caution when interpreting results regarding lipid content from remotely sampled blubber biopsies.

5.6 Acknowledgements

This work was carried out as part of CR's PhD which is funded by the Irish Research Council for Science, Engineering and Technology. Thanks to Brian Boyle for help in the laboratory and to both Mareike Volkenandt and Teresa Martin for assistance in the field. We are grateful to Mick O'Connell, the Irish Whale and Dolphin Group Strandings Scheme for reporting and providing information about the stranded whale. Constructive criticism from anonymous reviewers was greatly appreciated and helped to improve this note. The authors declare no conflict of interest.

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Chapter 6

Levels of Persistent Organic Pollutants in Northeast Atlantic Humpback Whales (*Megaptera novaengliae*)

"When we destroy something created by man we call it vandalism, but when we destroy something by nature we call it progress"

Ed Begley Jr., actor and environmentalist (1949—)

This chapter is reproduced from the following paper which is currently in submission:

Ryan C, McHugh B, Boyle, B, Bérubé, M, Lopez-Suárez, P, Elfes, C, Boyd, DT, Ylitalo, G, Van Blaricom, GR, Clapham, PJ, Robbins, J, Palsbøll, PJ, Berrow SD and O'Connor I (In Submission) Levels of persistent organic pollutants in Northeast Atlantic humpback whales *Megaptera novaengliae*. Endangered Species Research.

6.1 Abstract

Concentrations of 14 organochlorine compounds (OCCs) and 10 polychlorinated biphenyls (PCB) were measured by gas chromatography with electron-capture detection (GC-ECD) in lipid from humpback whale (*Megaptera noveaengliae*) blubber biopsies sampled in the Northeast Atlantic (NEA; Cape Verde and Ireland) Concentrations of dichlorodiphenyltrichloroethane (DDT), PCB and Chlordanes were found to be an order of magnitude lower than in western North Atlantic humpbacks (Gulf of Maine, GOM), but more elevated than those from the Pacific. Concentrations (mean ng g^{-1} ± SD Cape Verde and Ireland respectively) of ΣDDTs (505 ± 375 and 251 ± 202) and ΣPCBs (299 ± 198, 174 ± 128) for males were less than those reported for fin whales (*Balaenoptera physalus*) in the region (1981 and 2026 ng g^{-1} respectively). A male bias was found within the Cape Verde sample and males exhibited higher POP burdens than females. Lower chlorinated PCB congeners (28, 31 and 52) HCHs and hexachlorobenzene (HCB) were elevated in NEA compared to GOM whales, probably reflecting the higher latitudes of feeding grounds in the NEA. DDT congener ratios of %*p'p'*-DDE / Σ DDT, Σ PCB / Σ DDT and DDT / Σ DDT suggest that Cape Verdean and Irish whales harbour more recent inputs of DDT and proportionately greater sources of agricultural rather than industrial pollutants than those from GOM. Principal components analysis on lipid based concentration-independent POP profiles (including data published from GOM) found that more recalcitrant PCBs and HCHs could be discriminated

between geographic regions, providing further evidence of population structuring between the eastern and western North Atlantic.

6.2 Introduction

Following evidence of increases in the size of many populations, the status of humpback whales (*Megaptera novaengliae)* was recently revised to "Least Concern" from "Vulnerable" on the IUCN Red List (Reilly *et al.* 2008). While some infra-specific assessments have been made by IUCN for humpback whales (*i.e.* the Oceania and Arabian Sea subpopulations are listed as Endangered), current knowledge on other humpback whale populations is insufficient to assess their conservation status. Humpback whales breeding in the Northeast Atlantic (NEA) are a case in point. Until recently it was thought that humpback whales in the NEA comprised a panmictic population that breeds in the West Indies (Palsbøll, Judith Allen, *et al.* 1997). Confirmation that a second breeding ground still exists at an old whaling ground, Cape Verde Islands, has only recently been made (Palsboll *et al.* 2001, Jann *et al.* 2003, Wenzel *et al.* 2009). There are two known breeding grounds in the North Atlantic Ocean: the West Indies (WI) and Cape Verde (CV). An estimated 11,600 humpback whales breed in the WI, while just 200 are thought to breed around CV (Smith *et al.* 1999, Punt *et al.* 2007). Photo-identification has provided strong evidence for isolation between the known breeding grounds in the North Atlantic, however some high-latitude feeding grounds in the NEA are shared, namely Iceland and the Barents Sea (Jann *et al.* 2003, Bérubé *et al.* 2004, Wenzel *et al.* 2009).

Humpback whale song is thought to function as a breeding advertisement and possible fitness indicator for males, and is known to vary among populations (Winn & Winn 1978, Tyack 1981). Song recorded in both the WI and CV was found by Winn *et al.* (1981) to be essentially identical, and this has been used as evidence for the existence of a single panmictic population in the North Atlantic (Smith *et al.* 1999). However, whales from both CV and WI share some common feeding

grounds, where they likely sing on occasion during summer (and are known to sing *en route)*. Consequently, exchange of songs may occur in higher latitudes, irrespective of isolation between breeding grounds (Charif *et al.* 2001, Jann *et al.* 2003, Wenzel *et al.* 2009). It remains uncertain, however, whether the Cape Verde Islands constitutes the only breeding location for humpback whales in the NEA (Punt *et al.* 2007, Wenzel *et al.* 2009). To date, two feeding grounds have been assigned to whales breeding in CV: Iceland and the Barents Sea (Jann *et al.* 2003, Wenzel *et al.* 2009). Genetic evidence has indicated that some of those whales summering in the Barents Sea belong to a breeding population other than the WI, but a lack of genetic samples from CV precluded assignment of this breeding ground to those whales (Palsbøll *et al.* 2001). Furthermore, of those whales that breed in the WI, photographic matches to the Barents Sea were less numerous than those from other feeding grounds (Stevick *et al.* 2003). This suggests fidelity to a breeding ground other than WI, such as CV, for whales feeding in the Barents Sea. However, the number of humpback whales that use the CV today appears to be too small to account for the presumed large population feeding in the NEA, suggesting that a third, as yet unidentified, breeding area exists in the North Atlantic.

Photographic identification of individual humpback whales confirms that those feeding in the Gulf of Maine (GOM) comprise a spatially discrete population that breeds in the WI (Stevick *et al.* 2006). In the NEA, at a similar latitude to the GOM, Irish waters host small numbers of humpback whales. Passive acoustic monitoring indicates that waters to the west of Ireland function as a migratory corridor for south-bound humpback whales, and observations along the southern coast of Ireland indicate that some whales also feed there in summer (Charif *et al.* 2001, Whooley *et al.* 2011). It is believed that humpback whales in this region have not yet recovered from intensive whaling in the nineteenth century (Went 1968, Fairley 1981). Despite a recent increase in research effort (O'Brien *et al.* 2009), none of the 20 photo-identified whales have been matched outside of Ireland, except for a round-trip documented between the Netherlands and Ireland by a juvenile whale (Berrow *et al.* 2012). The migratory destination and population

affinity of humpback whales feeding in waters around Ireland and the British Isles remains unknown.

The present study uses quantitative and qualitative analyses of a suite of halogenated aromatic compounds known as persistent organochlorine pollutants (POPs) in the blubber lipids of humpback whales from two feeding locations (Ireland and the GOM) and one breeding ground (CV) to infer population connectivity. POPs are almost exclusively synthetic and highly resistant to biodegradation, which partly accounts for their many uses in electronic components, as pesticides, lubricants and flame retardants. A wide number of POPs have been subject to bans since the 1970s in developed nations due to health concerns following links with immune, endocrine, reproductive and nervous system disruption in both humans and wildlife. Despite regulation and bans on their usage, they are ubiquitous and remain prolific in the environment due to their high transportability in the sea and atmosphere, coupled with their propensity for bioaccumulation due to their lipophilicity. Regional differential inputs of various classes and congeners of organic pollutants has culminated in geographic patterns (Iwata *et al.* 1993) throughout the marine environment. For example high latitudes are net sinks for POPs, despite low usage there, leading to a latitudinal increase in, *inter alia*, Hexachlorocyclohexanes (HCHs) and PCBs. As such, POPs can be used as tracers to infer coarse geographic range. Differential recalcitrance, residence and concentrations of various POPs in the environment can be investigated by long-term monitoring of long-lived, lipid-rich and top trophic level animals such as cetaceans (Aguilar *et al.* 2002). Furthermore, cetaceans exhibit a poor ability to metabolise persistent organochlorine compounds, leading to a higher pollutant loadings in their tissues than is generally observed in terrestrial mammals (Tanabe *et al.* 1988).

Patterns in POP concentrations have been used to delineate population structuring in cetaceans occurring sympatrically or in adjacent regions (Hobbs *et al.* 2001, Borrell *et al.* 2006). Elfes *et al.* (2010) demonstrated that concentrations of different classes of POPs in lipid from humpback whale blubber biopsies were specific to feeding grounds. Feeding by humpback whales on breeding grounds is

exceptionally rare and may be limited to juveniles (Baraff *et al.* 1991, Gendron & Urban 1993, De Sá Alves *et al.* 2009). Humpback whales additionally exhibit strong fidelity to spatially discrete feeding grounds which can thus be expected to result in POP loadings characteristic of these regions (Stevick *et al.* 2006). As such, profiles of POPs can potentially be used as a tracer in examining geographic patterns and ecological parameters for humpback whales.

Notwithstanding their use in estimating ecological parameters, knowledge of the concentration of POP residues in tissues is pertinent in identifying anthropogenic risks to threatened populations. POPs may contribute to immunosuppression, developmental abnormalities, reduced survival and impaired reproductive output in some marine mammals, which may impede the maintenance of populations (reviewed in Aguilar *et al.* 2002). Humpback whales are particularly at risk to potentially high levels of POPs given their high-latitude feeding grounds, great longevity, high trophic status and large body size. In light of this and considering their small estimated population size, information on the levels of contaminants is important in assessing impacts of long-term industrial activities on the health and reproductive potential of NEA humpback whales.

6.3 Materials and Methods

Sample Collection and Preparation

Sampling was carried out at two sites (Figure 22): the southern coast of Ireland (from September 2009 to January 2011) and Boa Vista, Cape Verde (April and May 2011) within 10 km of the coast. Photo identification was used to avoid duplicate sampling in the field. Humpback whales were biopsied from small boats (5—12 m) using a crossbow (Barnett Panzer V ®, 150lb draw-strength) with modified (CETA-DART) bolts and 40mm steel cutting tips (Specials Engineering, Ireland) under permit from the respective governments. Sampling tips were fitted to the top of the

bolts which have a compressed foam stop-collar to limit penetration, facilitate re-bound and provide buoyancy to the bolt to aid retrieval without the need for a tether. Tips were scrubbed in soapy water, sterilized, solvent-rinsed and foil-wrapped prior to use. Bolts containing samples were collected from the sea after darting. Samples were removed from the tips using solvent-rinsed forceps, wrapped in solvent rinsed aluminium foil and transported on ice to the lab whereupon they were stored in glass vials at -80°C. The blubber portion of each biopsy was removed from the skin and sliced thinly on a glass cutting table using a solvent-washed scalpel while still frozen to prevent lipid-loss. The skin portion was preserved in 20% salt-saturated dimethyl-sulfoxide for sex determination via molecular genetic analyses (Amos & Hoelzel 1991). The entire longitudinal blubber profile available (*circa* 30mm) was used. While the blubber depth varied between samples due to angle and distance from which the samples were obtained, none exceeded the depth of outer blubber stratum. Biopsies were halved longitudinally and *circa*75mg of blubber was used for POP analysis. The biopsy sampling methodology employed was consistent with that of a similar study in GOM (Elfes *et al.* 2010). While lipid composition in baleen whales may be stratified with respect to contaminant loads according to depth, mean (± SD) depth of biopsy blubber profiles using 40 mm tips was the same between laboratories: 1.20 (0.26) cm (Ryan *et al.* 2012) and 1.38 cm (SD not reported) (Elfes *et al.* 2010).

Extraction and Analysis

Total extractable non-volatiles (hereafter referred to as lipids), were extracted using a Soxhlet apparatus with 150ml of pestican grade *n*-hexane and acetone in a ratio of 1:1 (Ryan *et al.* 2012). Tissue samples were extracted in glass microfiber thimbles (Whatman®) for 24 hours (6 hours reflux and 18 hours soak). Lipid weights were determined gravimetrically to the nearest mg. Lipids were re-suspended in *n*-hexane prior to clean-up on a glass chromatography column filled with 6g aluminium oxide and 0.5g silica gel (both 5 % (w/w) deactivated , mesh size 0.063—0.200 mm) 2 ml of lipid-solvent solution were added to the column and eluted with 60 ml of *n*-hexane. 2,2,4-Trimethylpentane (1 ml) was added to

the eluant which was concentrated under a stream of nitrogen at 30°C using a TurboVap ®.

Samples were analysed by dual column Gas Chromatography with Electron Capture Detection (GC-ECD) using a Varian 3800 with a Varian CP8400 auto-injector on HT8 (SGE Analytical Science) and Rtx-PCB (Restek) fused silica columns.

Quality Control

Analysis was carried out in laboratory with a track record of successful participation in QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency exercises for the analysis of POPs. While no reference material was available for marine mammals, a full quality control programme was incorporated into the analysis batches including: procedural blanks, replicate samples and determination of contaminants in other reference marine tissues and successful participation in international proficiency studies for the analysis of POPs. Limits of quantification (LOQs) ranged from 0.013 to 0.017 ng g^{-1}(lipid based) while recovery of a spiked recovery standard (PCB 112) ranged from 93 % to 106 % with a mean of 101 % (±0.03 SD). Concentrations from reference samples were within two standard deviations of an inter-laboratory mean for all compounds measured; therefore analytical procedures were deemed fit for purpose.

Concentrations are reported of PCB congeners (31, 28, 52, 101, 105, 118, 138, 153, 156, and 180), in addition to organochlorine pesticides (OCCs) HCB, ⍰HCH, ⍰HCH, γ-HCH (Lindane), Oxychlordane, Heptachlor, *trans*-Chlordane, *cis*-Chlordane, *trans*-Nonachlor, Dieldrin, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT. All concentrations were derived on a lipid-weight basis as a sampling effect of biopsy darting leads to lipid-loss in blubber biopsies (Ryan *et al.,* in press). Sums of (10)

PCBs and OCC classes (Chlordanes, DDTs and HCHs) were considered in order to compare pollutant profiles between sampling regions, and especially to those reported by Elfes *et al.* (2010).

Statistical Analysis

Individual parameter concentration data measured in a different laboratory by Elfes *et al.* (2010) were obtained, allowing comparison of contaminant profiles between humpback whales from the eastern and western North Atlantic. A number of statistical analyses were then completed on lipid normalised contaminant data to partially account for potential concentration effects. Principal components analysis (PCA) was thus carried out on a concentration-independent basis. ΣPCBs was calculated as the sum of 10 congeners; ΣHCH as the sum of □-HCH, □-HCH, γ-HCH, Σchlordanes as the sum of oxychlordane, heptachlor, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor; and ΣDDT as the sum of *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT.

A PCA was used to investigate for structuring within the CV sample, and between potential feeding grounds (Ireland and the Gulf of Maine). *A priori* zero-centring and scaling were carried out to give comparable unit variance using the *prcomp* package and resulting ordinations were plotted using the *vegan* package in R (R Development Core Team 2011). Scree-plots were used to assess the number of principal components that should be considered. Following Kolmogorov-Smirnov test for normality and Levene's test for homogeneity of variance, Student's t-tests were employed to test if differences between principal component factor scores between regions were significant ($P < 0.05$).

Toxicokinetics of PCBs and OCCs in Marine Mammals

Due to their varying structures and planarities, some PCBs such as mono-ortho congeners are considered to be more resistant to metabolisation than others and to exhibit dioxin-like properties, particularly in marine mammals where they are found in higher abundances than polychlorinated dibenzodioxins (PCDDs) (Tanabe 2002). For the purpose of detoxification within the animal, activation of the

mixed-function oxidase system (MFO) is the most important enzymatic system in the metabolism of PCBs with those organisms inhabiting polluted environments will exhibit greater MFO enzyme activity (Fossi *et al.* 1995). Where the relative abundance of recalcitrant congeners is greater than labile congeners (at baseline *i.e.* in prey), it has been reported that this may indicate greater MFO activity and hence long-term exposure to toxic compounds (Fossi *et al.* 1988, Borrell *et al.* 1997).

It is possible to track the potential for such metabolic activity by the use of congener profiling techniques. Three different methods were employed as follows: 1) determination of the relative proportion of individual congeners as a percentage of the summed total for that compound grouping (*e.g.* %PCB31 / ∑10PCBs); 2) calculation of the percentage contribution of each congener relative to that of a fixed recalcitrant reference congener (*e.g.* PCB153), to reduce the potential for effects of other co-factors (such as age and nutritive condition) on observed patterns; 3) the relative proportions of metabolized to non-metabolized forms of other OCCs are of interest, *e.g.* ppDDE/∑DDT, and may provide information about degradation of compounds in the ecosystem (Aguilar 1984).

Sex determination

DNA was extracted from the skin portion of each biopsy using commercial extraction kits (QIAGEN DNeasy® blood and tissue kit). Sex was determined genetically following the protocol of Bérubé & Palsbøll (1996) using primers designed for mysticetes. Briefly, the ZFX/ ZFY complex at the sex chromosomes were sequenced with two sets of primers where forward primers anneal to both and reverse primers anneal to either ZFX or ZFY. Gel electrophoresis of the PCR products in 2% Agarose gives sufficient separation of fragments to allow for scoring by eye. Quality control of determinations was ensured by running positive and negative controls in each analytical batch.

6.4 Results

Sample Collection and Sex determination

A total of 35 samples were collected: 28 from Cape Verde and 7 from Ireland. Photo-identification confirmed that two individuals had been sampled twice (both from Cape Verde), leaving 33 individual samples for sex determination. All samples were sexed unambiguously by molecular genetic analysis as follows: Cape Verde, 9 female, 17 male; Ireland, one female, six male. A significant sex biased distribution was found where males were significantly more abundant than females (1.9 : 1) in the samples from Cape Verde (X^2 = 1.08, 1 d.f.; $P < 0.05$). The small sample size from Ireland precluded a reliable sex ratio determination. Biopsy samples had sufficient tissue for POP analysis of blubber lipid from 24 samples: 20 (12 male, 8 female) from Cape Verde and 4 from Ireland (3 male, 1 female).

Concentrations of POP compounds

The following PCBs were found at quantifiable levels (>LOQ) for all samples: 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. The following OCCs were also detectable for all individuals: HCB, □-HCH, □-HCH, γ-HCH, Oxychlordane, Heptachlor, *trans*-Chlordane, *cis*-Chlordane, *trans*-Nonachlor, Dieldrin, Endrin, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT,. All results are reported on a lipid as extracted, lipid content (percentages) of blubber biopsies is not presented as this has previously been demonstrated to be unrepresentative of the tissue *in situ* due to lipid-loss arising from the biopsy sampling technique (Ryan *et al.* 2012). While Elfes *et al* (2010) utilised methylene chloride extraction as against *n*-hexane/acetone followed by size-exclusion high-performance liquid chromatography (SEC-HPLC) with subsequent gas chromatography/mass spectrometry (GC/MS) detection, we argue that analytical specificity and limits of quantification are comparable between studies.

Differences in POP Concentrations

According to Sex

Differences according to sex were found in the concentrations of several compounds, where males exclusively exhibited significantly higher burdens than females (Table 7). This was particularly apparent for PCBs where the Σ 10PCB was very highly significantly different ($P < 0.005$) between the sexes, although not every individual congener exhibited significant differences (*i.e.* PCBs 28, 31, 138, 153, 156 and 180) (Table 7). Conversely, while only a single female was available from the Irish sample, this individual exhibited concentrations higher than the mean for Irish males for all compounds measured with the exception of three compounds namely (PCB28, Lindane and *cis*-chlordane). Male humpbacks from CV had significantly greater concentrations both of HCB ($P < 0.01$) and α-HCH ($P < 0.05$) than did those in Ireland; however concentrations of all other analytes measured were not statistically different between the two regions (Table 7). Four of the ten PCB congeners measured (PCBs 52, 101, 105 and 118) were present in significantly lower concentrations in female than in male humpbacks from Cape Verde (Table 7).

Table 7 Mean ± standard deviation (lower-upper range) concentrations ng g^{-1} lipid wt. of POP analytes measured in the present study (Cape Verde and Ireland) and that of Elfes *et al.* (2010) (Gulf of Maine). ^ Mann-Whitney U test between sexes from Cape Verde. # Mann-Whitney U test between males from Cape Verde and Ireland. Significance is denoted as follows: * = <0.05, **=<0.01, *=<0.005**

Congener	Cape Verde		Ireland		Gulf of Maine
	Female^	Male	Female	Male#	Male
PCB31	2 ± 1 (1-4)	9 ± 13 (1-44)	6	2 ± 2 (1-5)	1 ± 0 (1-2)
PCB28	4 ± 1 (3-5)	6 ± 3 (1-14)	3	5 ± 4 (2-10)	2 ± 1 (1-3)
PCB52	13 ± 13 (0-34) *	40 ± 25 (10-86)	54	16 ± 8 (7-22)	86 ± 40 (34-140)
PCB101	6 ± 8 (1-20) **	22 ± 14 (6-50)	23	13 ± 11 (1-21)	80 ± 46 (28-170)
PCB118	21 ± 20 (6-57) **	55 ± 37 (16-127)	45	20 ± 15 (3-33)	202 ± 104 (79-410)
PCB153	35 ± 31 (9-89)	77 ± 54 (16-182)	100	63 ± 53 (9-115)	635 ± 644 (210-2400)
PCB105	2 ± 1 (1-4) ***	8 ± 4 (2-16)	9	4 ± 1 (3-5)	29 ± 15 (11-52)
PCB138	28 ± 21 (9-65)	63 ± 43 (17-144)	75	36 ± 26 (8-60)	477 ± 453 (160-1700)
PCB156	2 ± 1 (1-4)	5 ± 4 (1-12)	4	3 ± 2 (1-6)	17 ± 10 (6-38)
PCB180	6 ± 4 (1-12)	14 ± 11 (2-32)	18	12 ± 11 (1-23)	146 ± 177 (33-630)
α-HCH	6 ± 2 (3-9)	6 ± 3 (1-13)	3	2 ± 1 (1-2) *	5 ± 3 (2-10)
β-HCH	1 ± 0 (0-2)	2 ± 1 (0-4)	NA	7 ± 11 (0-20)	2 ± 2 (1-8)
γ-HCH (Lindane)	11 ± 3 (8-18)	14 ± 13 (4-51)	4	5 ± 2 (3-7)	2 ± 1 (1-3)
Cischlordane	10 ± 7 (4-22)	11 ± 9 (2-37)	2	4 ± 4 (0-8)	8 ± 4 (3-14)
Heptachlor	20 ± 21 (2-57)	35 ± 29 (4-107)	21	13 ± 11 (1-22)	1± 1 (1-3)
Oxychlordane	20 ± 21 (4-56) *	40 ± 24 (11-92)	35	20 ± 13 (5-29)	58 ± 44 (13-150)
Transnonachlor	74 ± 71 (16-196)	142 ± 96 (33-356)	134	82 ± 67 (7-132)	306 ± 219 (99-830)
o'p'-DDD	16 ± 17 (4-46)	22 ± 17 (0-54)	29	8 ± 4 (4-11)	20 ± 13 (5-51)
o'p'-DDT	29 ± 34 (3-98)	69 ± 62 (8-210)	77	22 ± 9 (14-31)	88 ± 158 (14-530)
p'p'-DDD	46 ± 52 (8-138) *	85 ± 54 (21-202)	93	38 ± 33 (1-66)	274 ± 238 (77-900)
p'p'-DDE	143 ± 152 (28-405)	302 ± 228 (54-789)	305	164 ± 142 (4-275)	856 ± 873 (240-3200)
p'p'-DDT	21 ± 22 (2-60)	27 ± 25 (4-94)	42	19 ± 19 (1-39)	50 ± 39 (16-140)
HCB	74 ± 55 (28-166) *	137 ± 81 (68-343)	71	19 ± 5 (16-25) **	49 ± 25 (18-99)
Dieldrin	1 ± 1 (0-3) ***	41 ± 89 (0-279)	201	59 ± 95 (1-169)	197 ± 104 (63-400)
Σ PCBs	120 ± 96 (46-285) ***	299 ± 198 (86-690)	337	174 ± 128 (40-295)	1672 ± 1411 (563-5393)
Σ Chlordanes	129 ± 122 (28-342) *	236 ± 153 (60-622)	192	123 ± 96 (14-192)	372 ± 262 (117-984)
Σ HCHs	18 ± 3 (13-23)	22 ± 16 (8-67)	NA	14 ± 14 (5-30)	7 ± 6 (0-20)
Σ DDTs	256 ± 276 (48-747)	505 ± 375 (95-1350)	547	251 ± 202 (24-411)	1247 ± 1274 (347-4681)

According to Sampling Location

Concentrations by sums of classes of congeners (Σ10PCBs, Σ5Chlordanes, Σ3HCHs and Σ5DDTs) were compared among three regions (Figure 22). Concentrations of summed compound classes were found to be significantly different for GOM whales, whereas CV and Irish whales (collectively North-eastern Atlantic, or NEA) were statistically indistinguishable (Table 7). The Σ10 PCB level was an order of magnitude greater in whales from GOM compared to those from the NEA. The Σ 3HCHs however, showed a higher concentration in NEA than in GOM whales.

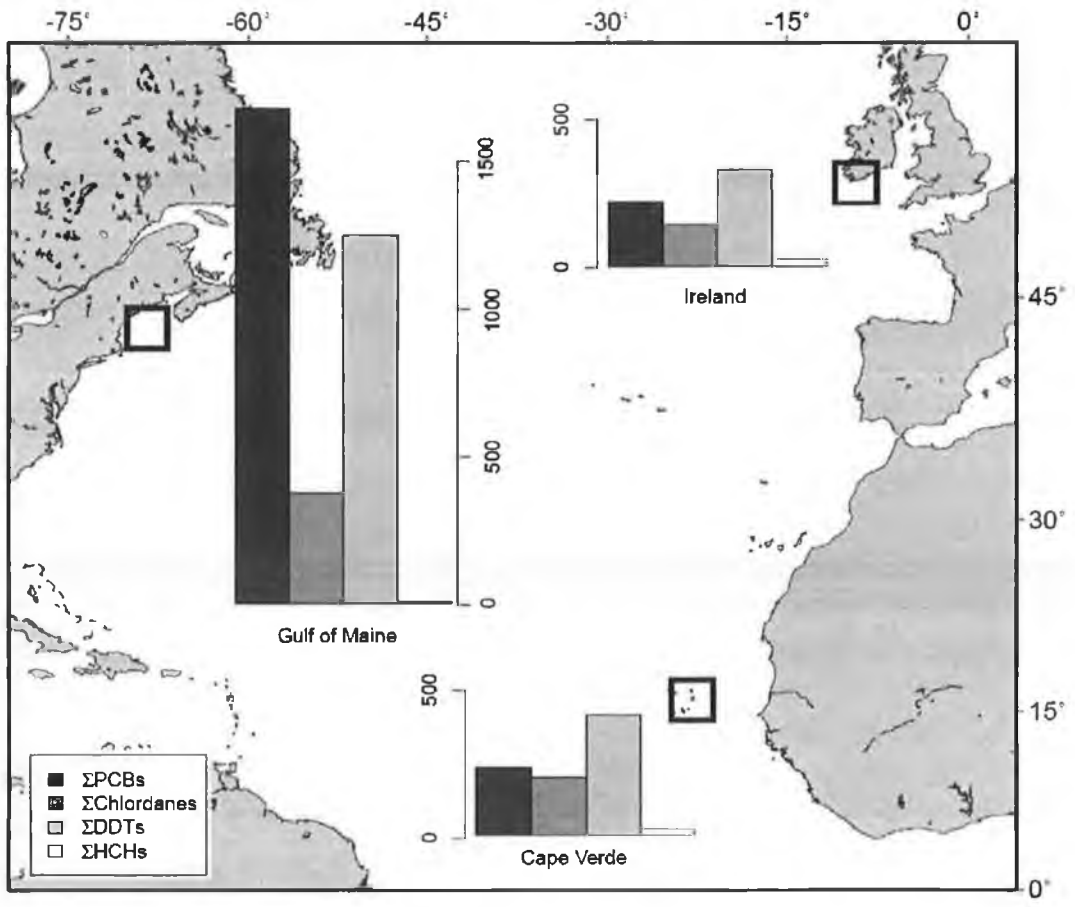


Figure 22. Map illustrating three sampling regions in the study including one breeding ground (Cape Verde) and two feeding grounds (Ireland and Gulf of Maine). Concentrations of persistent organic pollutant classes (sums of congeners) are in ng g^{-1} lipid wt.

Patterns and Geographical Distribution of POP Compounds

A scree-plot for the PCA of all compounds (normalized on the sum of each class, see methods) indicated that the first three principal components, which cumulatively explained 76.5 % of the variance, should be considered. No females were available from GOM, so given the sex-bias in POP profiles (Table 7), females were omitted from the analysis giving a sample of n=25 (n=3 from Ireland, n=10 from GOM and n=12 from CV).

Congener Profiling

Normalised relative to the sum of congeners

A concentration-independent PCA analysis for all four classes of POPs (normalized on the sum for each class) classified eastern and western North Atlantic humpback whales into distinct clusters (Figure 23). Eastern and western North Atlantic whales were significantly different with respect to the first factor (t = -7.09, d.f.=19, p <0.01), and with the second factor (t = -2.13, d.f.=21, p <0.05). The loadings (both PC1 and PC2) indicated that PCB 138, 153 and 180 were highly influential eigen-values for GOM whales, whereas eigen-values for NEA samples were characterized by less recalcitrant PCBs (28, 31, 52, 105, 118 and 156). Loadings from Transnonachlor strongly influenced the biplot distribution of GOM whales, whereas the remaining chlordanes (Heptachlor, cis-Chlordane and Oxychlordane) were more influential on Irish and CV whales (Figure 23). Whether variables have a negative or positive projection on the principal components can inform about correlations within the data. Negative loadings for *p'p'*-DDE and *p'p'*-DDD characterised GOM whales, whereas positive *o'p'*-DDD and *o'p'*-DDT influenced Irish and CV whales. With regard to HCHs; α-HCH exerted greater influence on GOM whales, β-HCH on both GOM and Irish whales, but positive loadings for Lindane were found for CV whales (Figure 23).

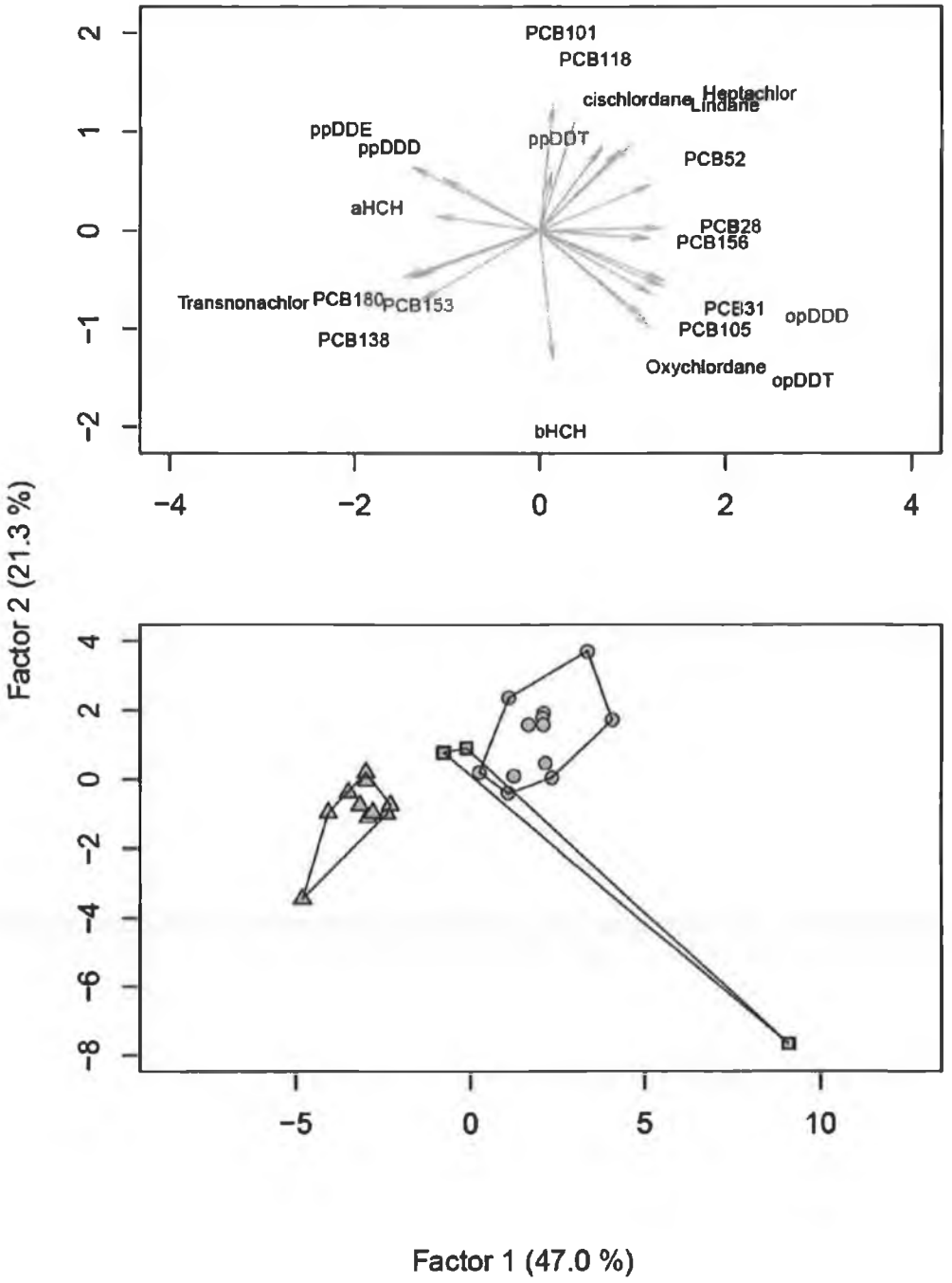
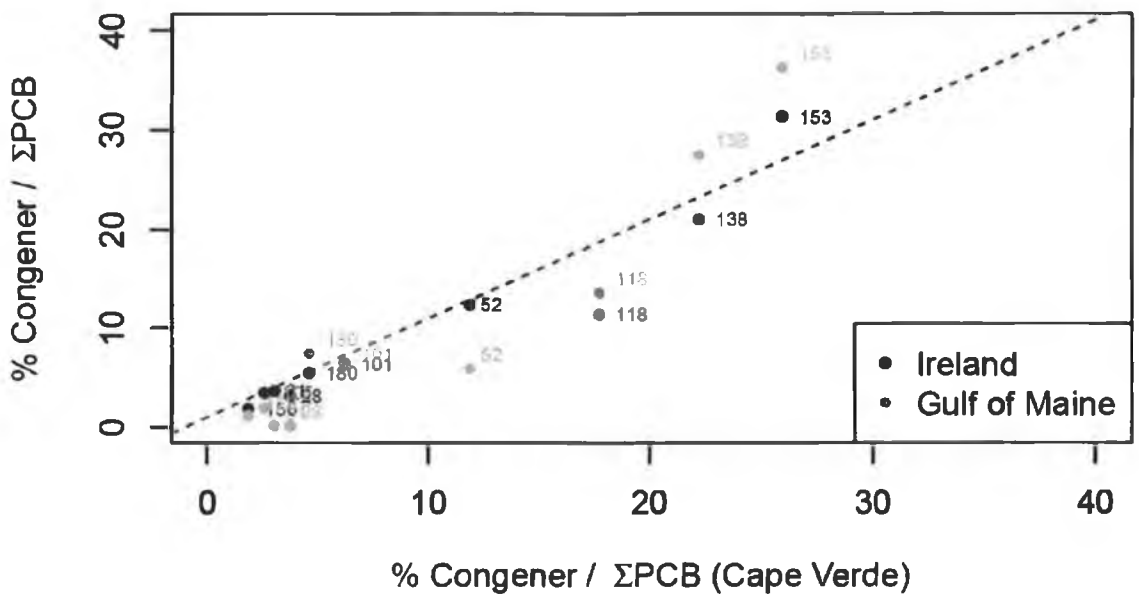


Figure 23. Principal components analysis of concentration-independent POPs (normalized by Σ10 PCB, Σ 3HCH, Σ 5DDT and Σ 5Chlordanes) in blubber lipid of male humpback whales. A: PCA loadings according to analyte. B: PCA scores where lines are convex hulls according to sampling region: CV = circle, Ireland = square and GOM = triangle (Elfes *et al.* 2010). Percentages denote the proportion of variance described by respective principal components.

Normalised relative to proportions of recalcitrant PCB congener 153

This normalisation differed for the previous methodology in that the concentrations of individual congeners were normalised relative to that of PCB 153. The primary purpose of this approach was to investigate evidence of MFO activity caused by long-term exposure to more toxic compounds in a manner that accounts for the potential bias of co-factors including reproductive and nutritive condition of the whales (which is unknown). The concentrations of most organochlorines in whale blubber lipid considered here were lower in NEA compared to the western North Atlantic. Furthermore, the pollution profile of the latter was characterised by higher proportion of more recalcitrant PCB forms while lower condensed forms were less prolific in western compared to eastern whales. Congener proportions normalized to Σ10PCBs indicated that di- and mono-ortho congeners (138, 153 and 180), followed by mono-ortho congeners (105, 118 and 156) accounted for a higher proportion of total PCB burden in GOM whales, while more labile congeners dominated in CV whales (Figure 24). Compared with CV samples, Irish samples exhibited higher proportions of just one recalcitrant congener; PCB 153 (Figure 24).



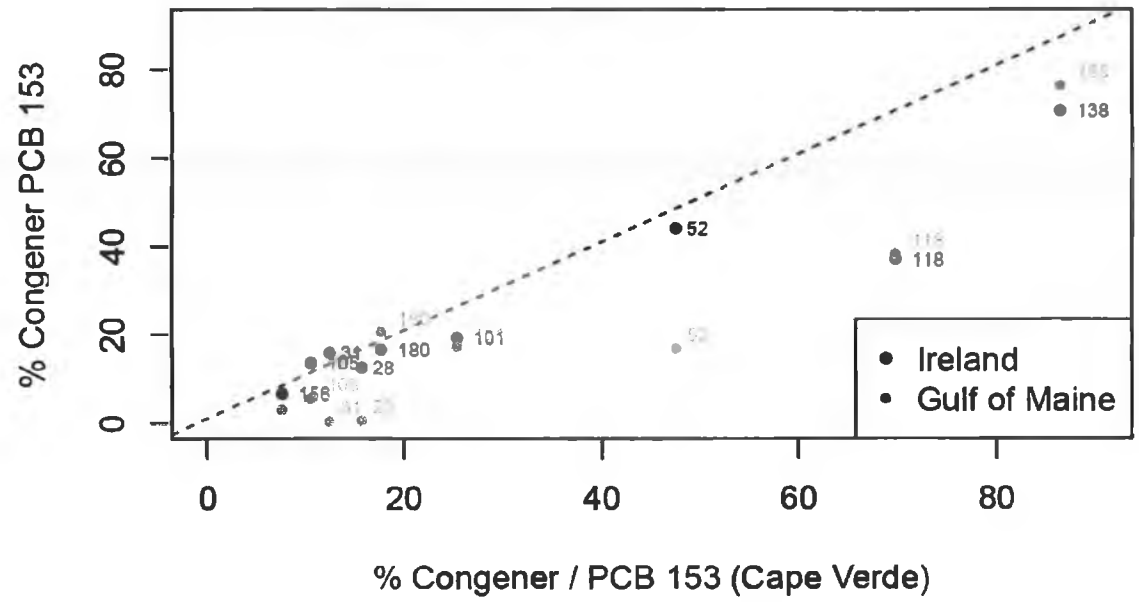


Figure 24. Top: Mean percentage of PCB congeners plotted relative to ΣPCB (*i.e.* sum-normalized). Bottom: Mean percentage of PCB congeners plotted relative to the most recalcitrant PCB: 153 (*i.e.* PCB 153-normalized). The broken line indicates parity between regions, such that any points lying above the line have a greater percentage contribution to overall PCB profile than those CV profiles.

The relationship between concentration and the proportion of PCB congeners normalised to PCB 153, was not consistent for each congener (Figure 25). For all mono-ortho congeners (105, 118 and 156) as concentration increased, there was a significant decline in the proportion to PCB 153. Concentrations of PCB 180 however were found to increase in proportion to those of PCB 153, both sharing di-ortho planarity. These relationships were consistent across all regions.

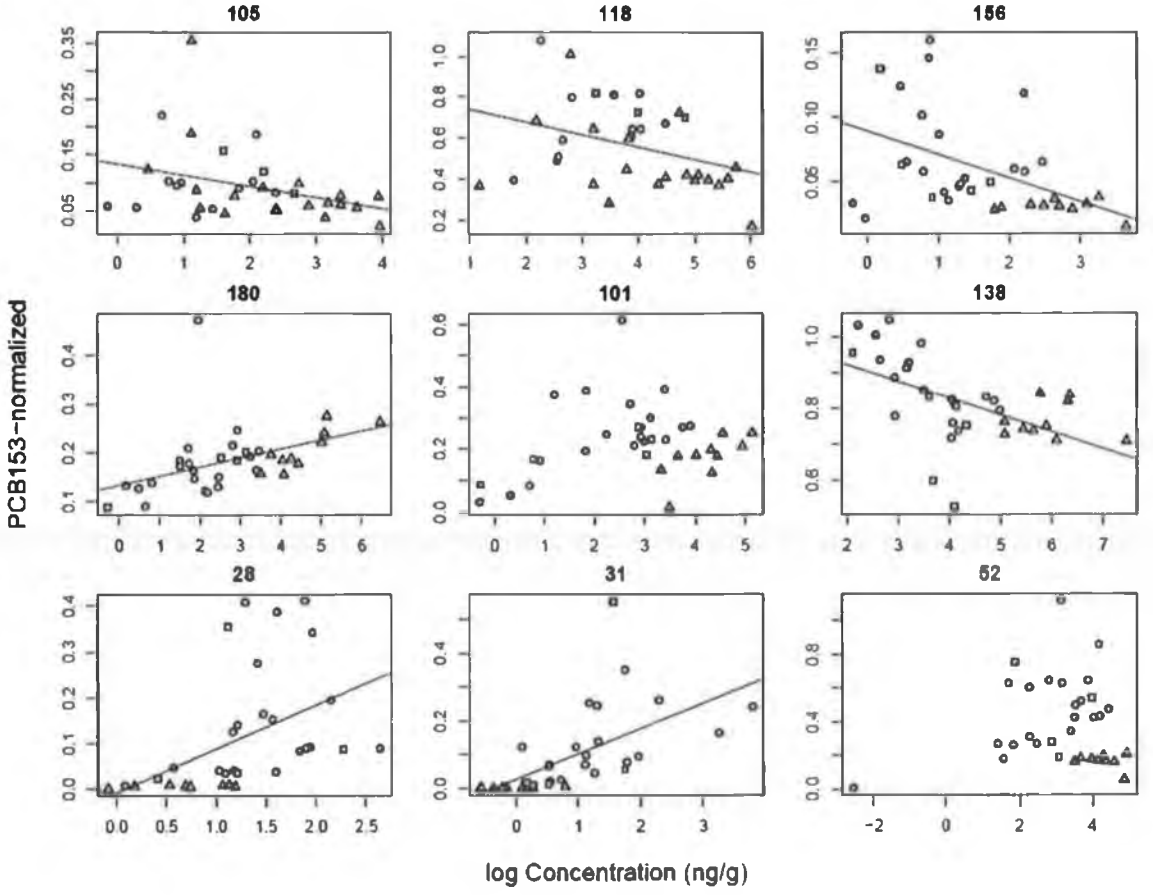


Figure 25. PCB congener proportions (relative to recalcitrant PCB 153) plotted against their concentrations (log ng g^{-1}). Lines were included when Studentized residuals were normally distributed and linear fit was found to be significant ($P < 0.05$). Rows are ordered primarily by planarity such that row 1= mono-ortho; row 2= di-ortho; row 3 = most volatile congeners. Circle=CV; square=Ireland; triangle=GOM.

Inferring Biogeographical Patterns using Ratios of DDTs and PCBs

Widespread bans on the usage of DDT have lead its decline in the environment, with a simultaneous relative increase in the metabolised forms such as *p'p'*-DDT and *p'p'*-DDE. Therefore DDT ratios in animal tissues can shed light on biogeographic patterns inferred by regional differential persistence or metabolisation. The percent (± standard deviation) *p'p'*-DDT / Σ DDT was found to be 51.8% for Irish and 57.7% for CV whales. This compares with 69.2% for GOM and 62.7% for humpback whales in the Gulf of St. Lawrence (calculated from Gauthier *et al.* 1997). The mean ratio of *p'p'*-DDT to the degraded form (*p'p'*-DDE) was found to be 0.13 for Irish; 0.13 for CV and 0.07 for GOM whales. Similarly, given their differential persistence and useage, the ratio of PCB to DDT can be informative when investigating spatially explicit patterns in ecological samples. The mean (± standard deviation) ratio of Σ PCB / Σ DDT was lowest in CV whales (0.63), followed by Irish (0.89) and then GOM whales (1.47).

6.5 Discussion

This the first published study investigating the ecology of NEA humpback whales from their Cape Verde breeding ground using techniques other than whaling records or photo-identification. Although based on a small sample (n = 26), the male-biased sample of 1.9 from CV was similar to that found on other breeding grounds, and may arise due to longer winter residency in tropical waters by males or overwintering on feeding grounds by some females (Brown *et al.* 1995, Rosenbaum *et al.* 2009). At most feeding locations the sex ratio of humpback whales is in parity (Clapham *et al.* 1995, Palsboll *et al.* 1995). The highly skewed sex ratio from the Irish sample was therefore unexpected, but is likely an artefact of a small sample size.

Levels of Persistent Organic Pollutants

Total DDT concentrations in blubber lipid were similar to those found in fin whales in the NEA for females (mean 483 ng g^{-1}) but less than half of those found in males (mean 1981 ng g^{-1}), while PCB concentrations were lower by a factor of four (832 and 2026 ng g^{-1} for males and females respectively) (Aguilar & Borrell 1988). The POP burden in humpback whales was one to two orders of magnitude lower than some odontocete and pinniped species in the NEA (Law *et al.* 1989, Borrell 1993, Aguilar *et al.* 2002). Concentrations of POP compounds were generally (notable exceptions being HCHs, PCB 31 and PCB 28) an order of magnitude lower for eastern (Cape Verdean (CV) and Irish) than western North Atlantic (Gulf of Maine (GOM) and Gulf of Saint Lawrence (GOSL)) humpback whales (Gauthier *et al.* 1997, Elfes *et al.* 2010). However, again with the exception of HCHs levels of all classes of POPs in NEA whales were higher than those from the Pacific (Alaska, Aleutian Islands, Bering Sea, and California) (Elfes *et al.* 2010). Elfes *et al.* (2010) have reported that HCH concentrations in Pacific humpback whales are higher than those in the western North Atlantic generally.

Environmental and Biological Factors Effecting Observed Concentrations

Differential transportability and regional inputs has contributed to latitudinal patterns in various classes of OCs (Iwata *et al.* 1993). HCHs may be found in high concentrations at high latitude even if other toxic residues are not abundant (Simonich & Hites 1995). Being relatively volatile, HCHs are distilled to high latitudes culminating in a global cline where concentrations in the environment increase towards the poles, thus concentrations correlate strongly with latitude (Simonich & Hites 1995). This may account for the observed differences in HCH (namely β-HCH and Lindane) in the present study where CV and Irish whales had greater mean concentrations than GOM whales, considering that the former are known to feed only at higher latitudes (Jann *et al.* 2003, Wenzel *et al.* 2009). Similarly, HCB exhibits a strong latitudinal cline (Simonich & Hites 1995).

Assuming that humpback whales in the NEA feed at a consistent trophic level, the significantly lower ($P < 0.01$) HCB concentration in Irish versus CV male whales suggests that those whales sampled in Irish waters may integrate a POP signature from latitudes lower than the Arctic, *i.e.* evidence in favour of Ireland and contiguous waters being a primary feeding ground for at least some whales, and not just a migratory stop-over point.

The sex-biased distribution of POP concentrations observed herein is consistent with most mammalian studies and may be explained by the potential for reproductive transfer exhibited by females, where lipophilic contaminants are transferred to offspring directly across placental membranes during gestation and thereafter by lactation (Aguilar & Borrell 1994). Due consideration must given to myriad other sources of variation in xenobiotic residues in tissues. Variation may arise from differences in diet (trophic enrichment), geographic range, nutritive condition, body mass, metabolic rate and age (Aguilar & Borrell 1988, Tanabe *et al.* 1988, Aguilar *et al.* 1999, Elfes *et al.* 2010). In this study, concentrations were normalized on both PCB and the sum of PCBs to dampen the effects of some of these cofactors. There are, however, several interactions between some factors (*e.g.* body size and metabolic rate) and there is evidence of potential confounding mechanisms effecting tissue concentrations. Mysticetes undergo prolonged fasting with changes in both blubber lipid content and composition, whereby body mass may be reduced by one half (Lockyer 1987, Aguilar & Borrell 1990). Concentration or partitioning of lipophilic xenobiotics such as POPs is possible whereby lipid stores are metabolised, but not the residues in question, culminating in possible seasonal increased tissue concentrations. However, the influence of nutritive condition on organochlorine concentrations in humpback whales is not known due to a lack of studies investigating this process. We propose that blubber biopsies from individuals taken on both feeding and breeding grounds, where identity is determined (*e.g.* Palsbøll, Allen, *et al.* 1997) could be used to address this shortfall in our knowledge.

Population Structuring

On their own, POP concentrations are generally ineffective at discriminating between populations due to their high variability and over-dispersion in individuals. However, ratios between concentrations of various compounds may be more informative and robust to different sampling approaches (Aguilar 1987). The %DDE/ΣDDT ratio is indicative of DDT degradation (Aguilar 1984, Aguilar & Borrell 2005) and was found to be highest in the western North Atlantic (GOM = 69%; GOSL = 63% (calculated from Gauthier *et al.* 1997)) than eastern (CV = 58%; Ireland = 52%). This is likely to reflect the differential levels in the environment between regions, or perhaps a trophic effect. Qualitatively, according to ratios of %DDT / Σ PCB and DDE / Σ DDT, whales sampled on either side of the North Atlantic exhibit distinct pollutant loads, reflecting geographical differences. The importance of considering relative concentrations and proportions of PCB congeners in light of their differential recalcitrance was demonstrated by this study. Significant negative relationships between the relative proportion of PCB 153 with concentration for mono-ortho- congeners shows that humpback whales may preferentially metabolise more labile congeners relative to other more recalcitrant ones (such as 153 and 180). Such processes were evident irrespective of sampling region, indicating that they are universal, and occur due to metabolic activity rather than provenance.

In cetaceans, the POP burden arises chiefly *via* diet, *i.e.* from prey (Aguilar *et al.* 1999). Diet may vary among individual whales and POP sources may vary due to 'import' and 'export' if prey is migratory. However it has been shown that localised prey preferences gives rise to marked spatial patterns of contaminant levels in humpback whales (Elfes *et al.* 2010). By extension, inferences can be made regarding the geographical range of humpback whales, assuming that GOM and NEA whales have integrated their POP profiles primarily from prey. Trophic transfer (bioaccumulation) is expected to be consistent; thereby differences in profile should be reflective of regional and/or aerial contaminant inputs. Indeed,

humpback whales throughout the North Atlantic are believed to share a similar lower trophic level diet of euphausiids and small forage fishes such as clupeids (Piatt & Methven 1992, Borobia *et al.* 1995, Skern-Mauritzen *et al.* 2011). Some PCB congeners such as 105, 118 and 156 have dioxin-like properties and have thus been assigned toxic equivalency factors by the World Health Organisation to facilitate risk assessment (Van den Berg *et al.* 2006). Cape Verdean and Irish whales exhibit a pattern consistent with lower metabolic response to these dioxin-like contaminants than those from GOM, a highly industrialised region of the United States. POP profiles of GOM whales were characterised by more recalcitrant PCBs. In contrast with GOM whales, higher proportions of lower condensed (*i.e.* relatively more volatile) PCB congeners in CV and Irish whales suggest that atmospheric transport by diffusion, rather than point-source contamination, may have an influence on observed PCB residue concentrations in these whales. This provides further evidence that CV and Irish whales feed in, and hence integrate POP profiles from, regions far from the direct effects of industrial point-source pollution. This agrees with current knowledge of their arctic feeding grounds given the susceptibility of arctic mammals to high concentrations of volatile POPs far exceeding those expected given the lack of industry there (Muir *et al.* 1988). While seals have the capacity to metabolise HCB compounds, resulting in lower tissue burdens than those of their prey, this does not appear to be the case for baleen whales (Muir *et al.* 1988, Ruus *et al.* 2002, Goerke *et al.* 2004, Barber *et al.* 2005). As such, HCB levels in humpback whales will be expected to reflect the sources of origin, *i.e.* prey species, subject to bioaccumulation.

Both quantitative (concentrations) and qualitative (concentration-independent PCAs) analyses indicated that GOM and CV whales are distinct with regards to their POP burden, with CV and Irish whales indistinguishable with respect to the majority of parameters measured. While POP profiles of Irish whales are statistically indistinguishable to those from CV, this is not direct evidence that they are part of a CV breeding population; rather it supports the belief that humpback whales breeding in CV are unlikely to feed in the western North Atlantic. Further POP analysis, including samples from the West Indies breeding ground, or indeed

molecular genetic analysis, may allow assignment of CV whales to specific feeding grounds. This would help to resolve spatial structuring of populations for this species, which remains at least partly unresolved in the North Atlantic (Palsboll *et al.* 2001).

6.6 Conclusion

According to observed POP profiles of residues in blubber lipid, CV whales constitute a population that may feed in a discrete geographic range, most likely confined to the high-latitude NEA. Furthermore, humpback whales sampled in CV and the GOM (and probably the GOSL) constitute separate populations with respect to both patterns and concentrations of POPs, confirming evidence from photo-identification studies of spatially explicit breeding populations within the NEA (Jann *et al.* 2003, Stevick *et al.* 2006, Wenzel *et al.* 2009). While the contaminant profiles exhibited by humpback whales from Irish waters were statistically indistinguishable to those from CV for most compounds considered, based on these data alone it is still equally probable that these whales breed in either the West Indies, Cape Verde, or both. None of the individuals analysed presented concentrations or patterns consistent with those of the western North Atlantic. This finding supports the belief that humpback whales breeding in Cape Verde migrate exclusively to feeding grounds in the NEA, including the Barents Sea and Iceland (Jann *et al.* 2003, Wenzel *et al.* 2009). In light of their small estimated population size and isolated breeding ground, we urge further research to facilitate a more thorough assessment of the conservation status of the putative NEA humpback whale population.

6.7 Acknowledgements

CR is a PhD candidate funded by the Irish Research Council for Science, Engineering and Technology. We are particularly grateful to Island Foundation for funding fieldwork in Cape Verde. The Heritage Council, National Parks and Wildlife

Service and Irish Environmental Network are acknowledged for sponsorship towards fieldwork in Ireland. CR was awarded a Marine Institute Networking Travel Grant to visit University of Groningen for molecular genetics lab work. An Ireland-Newfoundland Partnership grant supported the purchase of lab consumables. We are indebted to Fred Wenzel and Beatrice Jann for encouragement and guidance. We are also grateful to our Cape Verdean partners for assistance in acquiring research permits INDP (Institute for the Development of Fisheries). Biopsy samples were collected under license from the National Parks and Wildlife Service, Ireland (License Numbers C76/2008, C82/2009 & C130/2010) and Direcção Geral do Ambiente, Cape Verde (Licence Number 03/2011). Humpback whale tissues were imported to Ireland under CITES permit (ECR113/11).

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Chapter 7.

Conclusions

"No lobbyist can bribe nature. In the end, all politicians and everyone else must accept nature's mandates and the consequences of violating them. In that is my optimism"

Perry Mann, teacher and lawyer (1921—)

7.1 Intrinsic Markers: Uses and Caveats

7.1.1 Stable Isotope Analysis

For highly migratory species, effective conservation measures are difficult to implement given that they move across international boundaries, and information on population connectivity and spatial linkages is often inadequate (Burke 1984, Martin *et al.* 2007). While the deployment of electronic tracking devices such as satellite tags are often logistically and financially impractical, intrinsic markers in tissues such as stable isotopes present an attractive alternative (Ramos *et al.* 2009, Ramos & González-Solís 2012). One of the major benefits of intrinsic markers over electronic devices is that representative sampling is practicable, *i.e.* all individuals will have an intrinsic marker. Tagging may be subject to biases including sex, age or nutritive condition; however intrinsic markers are also subject to similar constraints. The Irish government has on several occasions refused to licence the deployment of satellite telemetry devices on whales, although biopsy sampling is permitted. As such, analysis of intrinsic markers from biopsy samples was a feasible means of addressing key questions regarding population connectivity, movement and foraging ecology in rorquals.

A lack of experimental evidence as a mechanistic foundation for the use of stable isotopes in ecology once inhibited the use of this tool in ecological applications (Gannes *et al.* 1997). The technique has great potential in studying poorly understood, highly migratory species that are difficult to access, such as cetaceans. However carrying out controlled experiments to measure fundamental parameters (*e.g.* fractionation and diet-tissue-discrimination) for large marine vertebrates over relevant timescales is exceptionally difficult (Newsome *et al.* 2010, Caut *et al.* 2011). The most important factors to consider when investigating diet and movements using stable isotope analysis are: diet-tissue discrimination, isotopic routing, tissue composition, isotopic turnover rate and baseline isotopic variability. In the present study, tissues with known isotopic turnover rates and diet-tissue discrimination factors were chosen (Borrell *et al.* 2012) so that inferences on diet

could be made. Consumers and putative prey were sampled simultaneously, thus dampening the effects of baseline variability (Chapter 4). Skin biopsies from whales were used to derive feasible diet solutions using mixing models which incorporated uncertainty in putative sources (prey) and diet-tissue discrimination factors (Chapter 4). In the analysis of baleen, over protracted spatiotemporal scales, it was not possible to account for isotopic baseline variation. Instead, the isotopic niche concept was used to delineate resource partitioning (while recognising isotopic baseline) among sympatrically occurring species over a long timescale (Chapter 2).

7.1.2 Niche Theory: A Framework for Identifying Resource Partitioning

The ecological niche is a conceptually robust framework for investigating resource partitioning and competition among members of an ecological community (Hutchinson 1957). However, niche parameters are difficult to quantify. Therein lies the benefit of stable isotope values – they can be used to estimate isotopic niche width and niche area which are proxy to those realised ecological niche counterparts (Bearhop *et al.* 2004, Layman *et al.* 2007). The isotopic baseline variability assimilated in fin, humpback, minke and blue whale baleen over space and time may be significant. In analysing samples over the course of 100 years (for fin and minke whales)or several decades (for humpback whales), it was shown that in spite of this variability, isotopic niches were discrete among all four species considered. Although fin and humpback whales (and probably minke whales) share the same resources while foraging inshore (Chapter 4), the long term diet as indicated by isotopic niches from baleen, suggest that foraging on shared resources is temporary. This reaffirms that long-term diet should be considered, as niche overlap and hence sharing of resources may only be short lived, as illustrated for fin and humpback whales (Chapters 2 and 4).

7.1.3 Biopsy Samples: Uses and Untested Assumptions

When employing an invasive research method on endangered or indeed poorly understood species, the benefits to conservation must be carefully assessed to see if they out-weigh the risks to the population which is being studied. Here, skin and blubber biopsies were collected after risk assessment and under strict adherence to best practice and licensing guidelines (Wenzel *et al.* 2010). Given the reliance on biopsy samples for such a large proportion of research on cetaceans (Noren & Mocklin 2012), it is critical that the underlying assumptions of the technique are tested. About 0.5g of tissue (skin and blubber) was used for stable isotope, persistent organic pollutant and molecular genetic analyses (sexing). The methodological study into sampling effects of the biopsy technique indicated that lipid content of blubber biopsies is never representative of blubber *in situ* for fin whales, and probably all cetaceans (Chapter 5). It was important to test for this effect as it has an overwhelming outcome on how the POP concentrations are interpreted, namely that concentrations should never be presented by tissue weight, rather by lipid weight. This finding also has implications for studies that aim to use lipid content of blubber biopsies as a metric to infer nutritive condition of marine mammals.

There are multiple uses for each tissue in a biopsy sample, however most analytical techniques are destructive in nature. Developing techniques whereby the amount of tissue required is reduced is therefore a research priority in cetacean research. By using less tissue per sample, multi-faceted studies using a range of analytical techniques can be employed, as demonstrated by this study. In continuous flow isotope ratio mass spectrometry, $\delta^{13}C$ and $\delta^{15}N$ values are routinely measured simultaneously from the same sample. Unfortunately this is not possible for rorqual skin or blubber, given the effects that lipids have on $\delta^{13}C$ values and the effects that lipid extraction has on $\delta^{15}N$ values (Chapter 3). Stable isotope analysis thus requires two aliquots of each tissue where mixing models are

to be employed. One of the benefits of biopsy sampling in cetaceans is the retrieval of two tissue types which can theoretically be used to identify diet shifts given that tissue with differential isotopic turnover rates will provide information about diet of different timescales (MacNeil *et al.* 2005). However, the diet-tissue discrimination of blubber is not known for any cetacean and remains untested due to the challenges faced by accounting for lipid-effects in such a lipid-rich tissue (Assumcío Borrell, pers. comm.). Determining the diet-tissue discrimination factor for blubber should be a research priority, in order to facilitate a multi-tissue approach to stable isotope analysis using biopsy samples of cetaceans.

7.2 Migration and Population Structure

Migration routes and destinations of rorquals occurring in Irish waters remain unknown. This study has shed light however, on population structuring on fin and humpback whales in the eastern North Atlantic (Chapters 2 and 6). There is evidence from seasonal occurrences, into late spring (pers. obs.) that some fin, humpback and minke whales might not undertake migratory movements. Whether these comprise juvenile, sexually immature or resting female whales remains unknown. It is possible that Irish waters, particularly the Celtic Sea, comprise a winter feeding ground for those whales that do not migrate south to breed.

7.2.1 Fin Whales

Isotopic niches have provided information on the population structure of fin whales in Irish and contiguous waters, suggesting that they may overlap spatially with those of the Bay of Biscay (Chapter 2). Of those fin whales that stranded around Ireland and the UK (from which baleen samples were analysed), a diet rich in clupeids most likely accounts for their observed unique isotopic niche among fin whales from adjacent areas (Bay of Biscay and Mediterranean Sea) (Chapters 2 and 4). While these piscivorous fin whales may overlap spatially with other fin whales,

their unique foraging strategy indicates that they will require different conservation management plans.

Static acoustic monitoring off the west coast of Ireland and the UK (SOSUS) detected seasonal directional migration of humpback whales but not fin or blue whales (Clark & Charif 1998). Furthermore, photo-identification of individual fin whales in coastal waters of the Celtic Sea over 10 months of the year indicate that if migratory movements do occur at all, they are likely to be short-lived (Whooley *et al.* 2011). Indeed, one fin whale was tracked during a brief (3 weeks) movement southwest from Ireland towards the Azores, where fin whales are most abundant during spring (Mikkelsen *et al.* 2007). This seasonal occurrence in the Azores corresponds to the lowest abundances observed in Irish waters (Visser *et al.* 2011, Whooley *et al.* 2011). However the movements of fin whales that show high site fidelity to the Celtic Sea (CS) remain unknown (Whooley *et al.* 2011). Continued research effort using photo-identification, satellite telemetry coupled with the techniques used herein will be required to clarify these movements: to determine their destinations and seasonality.

7.2.2 Minke Whales

While the occurrence of minke whales appears to exhibit a more marked seasonal trend compared to other rorqual species in Irish waters (Berrow *et al.* 2010), the location of their breeding grounds remains unknown (Anderwald *et al.* 2011). The isotopic niche area of minke whales was smaller than that of fin whales, suggesting a narrower trophic range of preferred prey (reflected by $\delta^{15}N$), but also a more geographically discrete feeding range (reflected by $\delta^{13}C$), similar to humpback whales (Chapter 2).

7.2.3 Humpback Whales

The present study showed for the first time, that the humpback whales breeding in Cape Verde constitute a population whose range appears to be restricted to the eastern North Atlantic (Chapter 6). This is supported by the small number of photo identification matches which have tracked whales between Cape Verde and both Iceland and The Barents Sea, but not to the large feeding aggregations in the western North Atlantic (Chapter 1, fig. 2) (Jann *et al.* 2003, Wenzel *et al.* 2009). Samples collected during the present study are facilitating a North Atlantic-wide molecular genetic analysis of humpback whale populations. Preliminary results indicate that Cape Verde and West Indies breeding populations are highly isolated at both maternal (mitochondrial) and bi-parentally inherited DNA markers (microsatellites) (Per Palsbøll, pers. comm.). However, the breeding population affinity of Irish whales remains uncertain due to a small sample size.

Blubber lipid POP profiles of humpback whales sampled in Irish waters were more similar to those of Cape Verde than the Gulf of Main (GOM) or Gulf of Saint Lawrence (GOSL) (Chapter 6). This may reflect geographically similar feeding grounds among Cape Verde and Irish whales, but not necessarily a shared breeding ground. Humpback whales monitored by SOSUS in offshore waters to the west of Ireland were found to exhibit a seasonal migration in the direction of West Indies, and not Cape Verde (Charif *et al.* 2001). With the best available evidence, it remains equally probable that Irish humpback whales comprise a mix of both West Indies and Cape Verde breeding populations (Chapter 6). Sightings of humpback whales in Irish waters peak from autumn into early winter (September—November) (Berrow *et al.* 2010) rather than in summer as in most feeding grounds. This is consistent with a movement close to Ireland during the southerly migration of humpbacks from high latitude feeding grounds in the Northeast Atlantic (NEA), whose migratory destinations are known to be either Cape Verde or the West Indies (Stevick *et al.* 1999, Charif *et al.* 2001, Wenzel *et al.* 2009).

South-bound migrating humpback whales may be using the CS as a staging post or stop-over during autumn and early winter when herring is locally abundant while spawning (Brophy *et al.* 2006). In the western North Atlantic for example, humpback whales are abundant for two months of the year in Bermuda where they stop-over on their north-bound migration in order to feed on the high biomass afforded by the local deep-scattering layer (Stone *et al.* 1987). To the west of Ireland, SOSUS arrays detected a southerly migration but corresponding northerly movement was not detected. This may be due to less vocal activity, or a different trajectory during the north-bound migration (Charif *et al.* 2001). It is possible that the Azores serves as a stage post for the north-bound migrating whales during April and May to take advantage of the early spring plankton bloom (Visser *et al.* 2011). This may result in a north-bound migratory route further offshore (out of the range of SOSUS) than the south-bound migration route. Results from the analysis of whaling ship log-books, used to track humpback whale occurrence agrees with this hypothesis (Reeves *et al.* 2004). High densities of whaling ships targeting humpback whales were found due north of the Azores to the west of Ireland (the so called 'Commodore Morris' whaling ground) during June and July, but not in autumn or winter (Reeves *et al.* 2004). As with fin whales, satellite telemetry and continuation of photo-identification projects will aid the interpretation of the findings from the present study. Genetic tagging of whales using microsatellite loci or single-nucleotide polymorphisms (SNPs) is especially promising to this end, given the already considerable investment of research effort using this technique for tracking humpback whales in the North Atlantic (Palsbøll *et al.* 1997).

7.3 Rorquals in the Celtic Sea Ecosystem

The CS herring fishery is seemingly well managed and carefully assessed on an annual basis since 1989 (Saunders *et al.* 2010). Herring, as well as sprat are likely to be keystone species in the CS ecosystem, supporting biomass of many subsequent trophic levels including predatory fishes, marine mammals and seabirds (Trenkel *et al.* 2005, Chivers *et al.* 2012) Herring stock components in the CS have a history of stock collapse and are subject to a dynamic shifts in biomass and body size at age which are strongly linked to climatic regimes (Harma *et al.* 2012). Conversely, the sprat fishery is not currently managed, and knowledge on the life history and stock structure is poorly known. The benthic predatory fishes, such as gadoids, have been intensively fished to the extent that the CS has become a trophically reduced system (Pinnegar *et al.* 2002, Trenkel *et al.* 2005). Against this backdrop, rorquals forage in the CS for many months of the year, often close to shore. Both the fate of clupeid biomass and the roll of top predators are key concerns for resource management within the CS ecosystem.

Image Caption: A fin whale alongside an inshore sprat/herring boat on 30 January 2011, south of Hook Head, Co. Wexford. Photograph courtesy of John Coveney.

7.3.1 Foraging Strategies

The large isotopic niche area of fin whales is consistent with foraging over large spatial scales and across trophic levels (Chapter 2). In the Azores fin, blue and humpback whales are most abundant between April and May (with a rapid decline thereafter), where they feed mostly on *M. norvegica,* (Visser *et al.* 2011). The peak in abundance coincides with the minimum abundance of fin whales in the CS (March to May). This is consistent with a southerly movement of fin whales in the springtime to exploit the earlier spring bloom there, where they may shift from a diet of clupeids to euphausiids (Chapter 4). Indeed, the only satellite telemetry data from fin whales in Irish waters show a southerly movement towards the Azores, although during summer rather than spring (Mikkelsen *et al.* 2007). However, it cannot be discounted that fin whales occurring in the CS may simply move offshore or into the Bay of Biscay to forage on *M. norvegica* during spring, remaining close to Irish waters. Indeed, mother-calf pairs have been observed during the spring in Irish waters (pers. obs.).

Both fin and humpback whales exhibited a preferred diet of year-0 herring and sprat (Chapter 4). Correlation between these two sources in the mixing model posterior distributions indicates that the model was unable to decipher the relative contribution of each with certainty. This uncertainty is likely to have arisen from an ecological effect where year-0 sprat and herring may shoal and hence forage together, thus exhibiting a similar isotopic values (assuming comparable isotopic routing and metabolic fractionation). Humpback whales fed on much lower proportions of euphausiids than fin whales did, *i.e.* a more piscivorous diet (Chapter 4, fig. 5). In both species, sprat comprised a greater proportion of the diet than herring did and there was a tendency for a decrease in the diet with age for both fish species. This indicates that humpback whales, exhibiting a narrower isotopic niche and greater reliance on fish, are more at risk of direct competition with fisheries (Chapters 2 and 4). Fin whales could perhaps counter the effects of reduced fish biomass in the CS by shifting to a more plankton-rich diet, however the seasonal availability of euphausiids to fin whales

may not permit this at certain times of the year. It is interesting to note that in the Gulf of Saint Lawrence (western North Atlantic), the relative trophic positions of fin and humpback whales is reversed, whereby fin whales are more likely than humpback whales to feed on fishes (Borobia *et al.* 1995).

Although isotopic niches are only proximal to realised trophic niches (given baseline effects and biological fractionation), the isotopic niches of minke whales suggest a higher trophic diet compared to fin, humpback or blue whales (Chapter 2). Minke whales are one of the most abundant rorquals in Irish waters (Berrow *et al.* 2010), but their inconspicuous nature and avoidance of boats did not allow for biopsy sampling and hence mixing model diet analysis during this study. Minke whales can be observed foraging in association with fin and humpback whales, often at the periphery of major feeding aggregations (pers. obs.). Future work should focus on obtaining biopsy samples and putative prey for minke whales to obtain a more complete picture of the role of rorquals in the CS ecosystem using the stable isotope mixing models approach.

7.3.2 Predator-Prey Dynamics: Rorquals and Clupeids in the Celtic Sea

It has been widely established that a more holistic approach to management of fisheries must be adopted in order to estimate truly sustainable quotas that recognise inherent uncertainty as well as the roll and requirements of non-target species (Pauly *et al.* 2002, Pikitch *et al.* 2004). An ecosystem approach to fisheries management recognises the importance of predators, such as rorquals in maintaining community structure and hence resilience to perturbations (Folke *et al.* 2004). To achieve sustainable fisheries using an ecosystems based approach, assessments and management reference points must explicitly recognise the consequences of predation (Tjelmeland & Lindstrøm 2005, Moore 2012). Multiple levels of predation should be considered, given that counter-intuitive effects may

emerge in trophic systems, *e.g.* predator-predator interactions may actually reduce the risk of predation mortality for some prey (Sih *et al.* 1998).

The population dynamics of preferred prey may have a consequence on optimal foraging and hence life history traits in predators, such as rorquals (Krivan & others 1996, van Baalen *et al.* 2001). The proportion of winter-spawning to autumn-spawning herring, an important prey for fin and humpback whales (Chapter 4)increases in an easterly direction on coastal spawning beds in the CS (Brophy & Danilowicz 2002, Saunders *et al.* 2010). The winter-spawning component is undergoing an increase in biomass with a simultaneous decrease in autumn-spawners and understanding these dynamics should be a priority towards more effective management and conservation (Harma *et al.* 2012). Humpback whales rely greatly on clupeids including herring (Chapter 4), and are particularly constrained in their seasonal occurrence at high latitudes by protracted southerly migrations in late autumn or winter. Therefore there is a risk of a mismatch between the optimal foraging conditions for preferred prey, and the timing of their migration if the timing of herring spawning continues delays further into the winter. Considering the lack of information on the life-history traits of sprat, it is difficult to determine if a similar risk of predator-prey mismatch may occur between rorquals and sprat.

Another important characteristic of CS herring, both to fisheries and foraging whales, is the decline in herring size at age (Harma *et al.* 2012). Considering that both fin and humpback whales have a marked preference for smaller fish (Chapter 4), the increased biomass of smaller herring in the CS stocks may be beneficial. As such a paradoxical management challenge arises – maintaining sustainable ecosystem functioning usually necessitates the preservation of trophic diversity (Folke *et al.* 2004, Trites *et al.* 2006), yet the trend towards a trophically depauperate ecosystem in the CS appears to be benefiting both fisheries and rorquals in terms of available herring stock biomass (Trenkel *et al.* 2005). A similar scenario arose in the North Sea where sandeel (*Ammodytes* spp.) populations

increased due to competitive release. Discards provisioned seabirds, which now rely heavily on the altered sandeel populations (Furness 2003). This is an example where indirect effects of fisheries (discard practices) had profound consequences on the community structure of the ecosystem (Votier *et al.* 2004). In light of ecological regime shifts affecting top predators elsewhere (*e.g.*in the North Sea), the results of the present study show that age-class specific predation by rorquals on both managed (herring) and unmanaged (sprat) fish stocks should be included in assessments for fishery quotas in the CS. This should be a conservation priority both for cetaceans and exploited fish stocks.

7.4 Research Priorities for the Future

Prior to this study, the majority of information on rorqual movements and seasonality in Irish waters has come from three sources using (Clark & Charif 1998, Mikkelsen *et al.* 2007, Whooley *et al.* 2011). These studies which employed acoustic monitoring, photo identification and satellite telemetry have been instrumental in interpreting the results of the current research. Further research effort using these techniques will undoubtedly complement the findings of the present study.

An understanding of the population dynamics, fisheries-induced mortality and stock structure should be a research priority, considering the importance of sprat in the diet of both fin and humpback whales. This may also apply to minke whales although this remains untested. The effects of schooling and spawning behaviour in dictating optimal foraging conditions for rorquals would be of great importance. This may shed light on possible threshold foraging and also the effects that physical disruption due to trawling may have on the ability of rorquals to forage efficiently. Information from such studies may prove useful in identifying both temporal and spatial fishery closures as a conservation tool.

The use of intrinsic markers from multiple tissues presents the opportunity to investigate ecological parameters over different time scales. For example, stable isotope analysis of liver and cartilage in mako sharks (*Isurus oxyrinchus*) detected prey switching that would otherwise have been undetected in muscle alone (MacNeil *et al.* 2005). Considering that multiple tissues are routinely collected by biopsy sampling cetaceans (*i.e.* skin and blubber), the potential for similar studies on diet switching in cetaceans exists. The lack of a diet-tissue discrimination value coupled with a poor understanding of isotopic routing in cetacean blubber however, currently prevents this line of investigation. Thus investigating routing and diet-tissue discrimination of stable isotopes in blubber should be priority areas of research in the future.

In addition to the blubber lipid-loss trials conducted herein (Chapter 5) other methodological studies ought to be carried out in such as testing the premise that whales are biopsied in a truly random manner (*i.e.* that age, nutritive condition, gender or behavioural state do not influence the chance of successful sampling). Another key consideration should be the use of samples from stranded carcases for stable isotope studies. By using samples from those carcases that had been subject to full conclusive pathological examinations, it may be possible to ascertain if cause of death has an effect on the isotopic values of tissues. For example, one might expect elevated $\delta^{15}N$ values where malnourishment was contributory (Hobson *et al.* 1993).

Finally, continued biopsy sampling should be prioritised in order to facilitate molecular genetic analysis of humpback and fin whales in the eastern North Atlantic. An ocean-basin scale genetic analysis is currently underway for humpback whales, including samples from both Cape Verde and Ireland collected in this study. A geographic extension of sampling to include the waters of mainland West Africa may provide additional insights into the population structure and breeding ground affinities of humpback whales occurring in Irish waters (Reeves *et al.* 2004, Punt *et al.* 2007). A similar molecular genetic study is planned for fin whales; however this is awaiting funding (Mikkelsen pers. com.).

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Appendix A. Glossary

Allozyme— Forms of the same enzyme that are encoded for by different alleles. They are useful markers in genetic analysis of populations.

Autolysis— The 'self-digestion' of tissue by cells following the release of enzymes into the cytoplasm, leading to gas-build-up in carcasses.

Bayesian— A form of statistical inference whose interpretation is based on 'degree-of-belief' rather than frequency distribution (frequentist) or maximum likelihood estimation. It is based on a theorem by Thomas Bayes (1701—1761):

$$P(A|B) = \frac{P(B|A)\,P(A)}{P(B)}$$

Where *P(A|B)* is the probability of event *A* given *B* and *P(B|A)* is the conditional probability of *B* given *A*. The proposition *A* known as the 'prior' is the degree of belief in *A*. *P(B|A)* is called the 'posterior' and is the degree of belief once *B* has been considered. The above theorem therefore provides the probability of support that *B* provides for *A*.

Bubble-netting— A means of corralling prey using a curtain of bubbles emitted from the blowhole, or indeed the mouth.

Buccal Cavity— The oral cavity bounded by the jaws, which in balaenopterid whales is grossly enlarged.

Capital Breeder— Species that allocate stored energy reserves to reproduction, as opposed to income breeders, which instead rely on recently assimilated food energy.

Chirality— Refers to left or right handed bias in animal behaviour. Also refers to geometric structure of compounds in chemistry where mirrored structured compounds (enantiomorphs) may exhibit very different properties.

Exaptation— A trait that evolved for one function, but subsequently serves a different function.

Fluking— Raising of the tail fluke above the water's surface before diving

Food-web— A consumer-resource system encompassing all of the trophic interactions in an ecological community.

Fractionation— In stable isotope ecology this refers to the partitioning of heavy and light isotopes, often due to biological (metabolic) processes.

Fronto-mandibular stay— A fibrous appendage associated with the temporalis muscle (the temple). It ensures that the mandibles are re-articulated with the cranium after lunge-feeding and stores elastic energy during that process.

Highly-derived— A phylogenetic term referring to a trait in one taxon that has evolved to be very different to that of closely related taxa. It does not necessarily infer greater complexity.

Isoscape— An isotopic landscape or a map depicting geographical patterns in baseline stable isotopic compositions.

Isotope— An atom of an element with a variable atomic mass due to varying numbers of neutrons.

Lob-tailing— Propelling the tail (flukes and caudal peduncle) out of the water, causing a loud sound and large splash. Used primarily for communication.

Lek— A gathering of males for the purpose of hierarchical selection for mating, often based on display or combat.

Monotypic— A taxonomic term that indicates one member per taxon.

Optimal foraging— A theory which supposes that animals will forage in a manner that maximizes the rate of net energy income. This dictates behavioural states and foraging strategies.

Philopatry— Natal homing, or returning to birthplace.

Polygyny— A social order where males mate with multiple females.

Routing— When used with respect to stable isotopes, routing refers to the differential partitioning of various stable isotopes, such as $\delta^{13}C$ versus $\delta^{15}N$, into different tissues during assimilation.

Stenotypic— Narrow in one's preference, *i.e.* a specialist.

Suborbital— Part of the cranium underlying the eyes (eye-socket).

Xenobiotic— A compound that is alien to a living organism, and is often resistant to metabolic degradation. Xenobiotics include organochlorines, heavy metals and antibiotics.

Appendix B. Photographic Identification Numbers of Biopsied Whales

Biopsies analysed in the present study were collected by licensed arbalesters denoted by initials under the 'sampler' column below (Conor Ryan, Simon Berrow, Pedro Lopez Suárez and Frederick Wenzel). Corresponding photo-identification catalogue codes are also presented for the North Atlantic Humpback Whale Catalogue, Allied Whale ('NAHW ID') and the Irish Whale and Dolphin Group ('IWDG ID'). Sex was determined genetically (see Chapter 6 for methods).

Sample ID	Species	Date	Latitude	Longitude	Licence	Sampler	NAHC ID	IWDG ID	Sex
IRMn03001	*Megaptera novaengliae*	20/09/2003	51.35000	-9.31000	C/2003	SB	4532	HBIRL3	M
IRMn08001	*Megaptera novaengliae*	26/11/2008	51.47100	-8.90000	C76/2008	SB	4770	HBIRL9	M
IRMn09001	*Megaptera novaengliae*	30/09/2009	52.10700	-10.55800	C82/2009	SB	4752	HBIRL10	M
IRMn10001	*Megaptera novaengliae*	22/01/2010	52.09000	-6.96000	C82/2009	SB	4753	HBIRL11	M
IRMn11001	*Megaptera novaengliae*	11/01/2011	52.06625	-7.03606	C130/2010	CR	4743	HBIRL13	F
IRMn11002	*Megaptera novaengliae*	18/01/2011	52.40509	-6.33280	C130/2010	CR	na	HBIRL14	M
IRMn11003	*Megaptera novaengliae*	22/07/2011	52.00943	-10.64295	C130/2010	CR	4742	HBIRL17	F
CVMn11001	*Megaptera novaengliae*	11/04/2011	16.11863	-22.95016	03/11	PLS	na	na	F
CVMn11002	*Megaptera novaengliae*	12/04/2011	16.13166	-22.93490	03/11	CR	4977	na	M
CVMn11003	*Megaptera novaengliae*	12/04/2011	16.17623	-22.95301	03/11	CR	na	na	F
CVMn11004	*Megaptera novaengliae*	12/04/2011	16.18481	-22.96604	03/11	CR	na	na	M
CVMn11005	*Megaptera novaengliae*	12/04/2011	16.13078	-22.95561	03/11	CR	na	na	F
CVMn11006	*Megaptera novaengliae*	13/04/2011	16.10024	-22.97289	03/11	CR	4977	na	M
CVMn11007	*Megaptera novaengliae*	16/04/2011	16.21961	-22.93090	03/11	CR	4756	na	M

CVMn11008	*Megaptera novaengliae*	16/04/2011	16.18899	-22.98090	03/11	CR	na	na	F
CVMn11009	*Megaptera novaengliae*	16/04/2011	16.15442	-22.94827	03/11	CR	na	na	M
CVMn11010	*Megaptera novaengliae*	16/04/2011	16.10845	-22.94905	03/11	CR	na	na	F
CVMn11011	*Megaptera novaengliae*	16/04/2011	16.25059	-22.90984	03/11	CR	na	na	M
CVMn11012	*Megaptera novaengliae*	17/04/2011	16.05506	-22.99675	03/11	CR	4810	na	F
CVMn11013	*Megaptera novaengliae*	19/04/2011	16.25353	-22.94270	03/11	CR	na	na	M
CVMn11014	*Megaptera novaengliae*	30/04/2011	16.08474	-22.96834	03/11	CR	4950	na	M
CVMn11015	*Megaptera novaengliae*	01/05/2011	16.12718	-22.94818	03/11	CR	na	na	F
CVMn11016	*Megaptera novaengliae*	03/05/2011	16.08821	-22.96771	03/11	CR	na	na	F
CVMn11017	*Megaptera novaengliae*	03/05/2011	16.09191	-22.96378	03/11	CR	na	na	F
CVMn11018	*Megaptera novaengliae*	03/05/2011	16.10716	-22.95464	03/11	CR	4817	na	M
CVMn11019	*Megaptera novaengliae*	08/05/2011	16.13615	-22.92739	03/11	CR	na	na	M
CVMn11020	*Megaptera novaengliae*	09/05/2011	16.14440	-22.93472	03/11	CR	na	na	M
CVMn11021	*Megaptera novaengliae*	10/05/2011	16.14934	-22.94428	03/11	CR	na	na	M
CVMn11022	*Megaptera novaengliae*	13/05/2011	16.18257	-22.97041	03/11	CR	na	na	M
CVMn11023	*Megaptera novaengliae*	14/05/2011	16.04324	-22.99058	03/11	CR	na	na	M
CVMn11024	*Megaptera novaengliae*	14/05/2011	16.01951	-22.97430	03/11	CR	4960	na	M
CVMn11025	*Megaptera novaengliae*	15/05/2011	16.11053	-22.93183	03/11	CR	na	na	M
CVMn11026	*Megaptera novaengliae*	15/05/2011	16.10290	-22.94108	03/11	CR	4820	na	F
CVMn11027	*Megaptera novaengliae*	15/05/2011	16.07047	-22.99780	03/11	CR	4960	na	M
CVMn11028	*Megaptera novaengliae*	15/05/2011	16.05911	-23.01688	03/11	CR	na	na	M
CVMn95001	*Megaptera novaengliae*	20/03/1995	16.58000	-22.90000	na	FW	4821	na	M
IRBp08001	*Balaenoptera physalus*	12/11/2008	51.54100	-8.50600	C76/2008	SB	na	FWIRL49	F

IRBp08002	*Balaenoptera physalus*	12/11/2008	51.55100	-8.50600	C76/2008	SB	na	FWIRL50	M
IRBp08003	*Balaenoptera physalus*	12/11/2008	51.53900	-8.45000	C76/2008	SB	na	FWIRL51	F
IRBp08004	*Balaenoptera physalus*	12/11/2008	51.53900	-8.45000	C76/2008	SB	na	FWIRL52	F
IRBp08005	*Balaenoptera physalus*	12/11/2008	51.57600	-8.49900	C76/2008	SB	na	na	M
IRBp08006	*Balaenoptera physalus*	26/11/2008	51.47400	-8.90500	C76/2008	SB	na	FWIRL56	F
IRBp08007	*Balaenoptera physalus*	26/11/2008	51.48000	-8.89200	C76/2008	SB	na	FWIRL7	M
IRBp08008	*Balaenoptera physalus*	26/11/2008	51.47900	-8.88800	C76/2008	SB	na	FWIRL14	M
IRBp08009	*Balaenoptera physalus*	26/11/2008	51.47800	-8.88300	C76/2008	SB	na	FWIRL10	M
IRBp08010	*Balaenoptera physalus*	03/12/2008	51.43200	-9.03800	C76/2008	SB	na	FWIRL61	F
IRBp08011	*Balaenoptera physalus*	03/12/2008	51.43200	-9.04100	C76/2008	SB	na	FWIRL62	F
IRBp10001	*Balaenoptera physalus*	26/11/2010	52.10450	-7.31850	C130/2010	CR	na	na	F
IRBp10002	*Balaenoptera physalus*	26/11/2010	52.07633	-7.14217	C130/2010	CR	na	na	F
IRBp10003	*Balaenoptera physalus*	11/12/2010	52.11293	-7.39551	C130/2010	CR	na	na	F
IRBp10004	*Balaenoptera physalus*	11/12/2010	52.09132	-7.48487	C130/2010	CR	na	FWIRL30	F
IRBp10005	*Balaenoptera physalus*	14/12/2010	52.09977	-7.45130	C130/2010	CR	na	FWIRL21	M
IRBp10006	*Balaenoptera physalus*	15/12/2010	52.09147	-7.48650	C130/2010	CR	na	FWIRL63	M
IRBp10007	*Balaenoptera physalus*	15/12/2010	52.08277	-6.85807	C130/2010	CR	na	FWIRL7	M
IRBp11001	*Balaenoptera physalus*	22/01/2011	51.83422	-7.86402	C130/2010	CR	na	na	F
IRBp11002	*Balaenoptera physalus*	13/07/2011	51.49228	-8.66515	C130/2010	CR	na	na	M
IRBp11003	*Balaenoptera physalus*	13/07/2011	51.48214	-8.63651	C130/2010	CR	na	na	F
IRBp11004	*Balaenoptera physalus*	13/07/2011	51.45984	-8.58749	C130/2010	CR	na	na	M
IRBp11005	*Balaenoptera physalus*	13/07/2011	51.45922	-8.73232	C130/2010	CR	na	na	F
IRBp11006	*Balaenoptera physalus*	09/08/2011	51.90001	-10.61478	C130/2010	CR	na	na	F

IRBp11007	*Balaenoptera physalus*	09/08/2011	51.88467	-10.62282	C130/2010	CR	na	na	F
IRBp11008	*Balaenoptera physalus*	09/08/2011	51.88054	-10.62896	C130/2010	CR	na	na	M