Resolving the role of jellyfish in marine food webs

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Abstract

Jellyfish populations in the Irish Sea have been increasing. This has caused a variety of economic problems, such as the destruction of aquaculture installations, and new opportunities, such as the establishment of a jellyfish fishery. However, interactions between jellyfish and other biota in the ecosystem is poorly characterised and ecological consequences of an increasing jellyfish population remains unknown. Molecular gut content analysis methodologies were developed to address this data gap. Cnidarian specific primers were developed and showed using more than 2500 stomachs that, during February and March, moon and mauve-stinger jellyfish were consumed by common fish species including herring, whiting, and lesser-spotted dogfish. Revisiting the ecosystem in October with 375 additional samples, the primers indicated jellyfish predation varied temporally: small jellyfish were still targeted by mackerel, however moon jellyfish adults were not preyed upon. To understand the context in which jellyfish consumption occurred a high throughput sequencing (HTS) approach using two universal primers was developed. A meta-analysis of HTS studies suggested results contained a quantitative signal, and the methodology could be used to move beyond a presence/absence approach. Using 188 samples from nine fish species, it was shown that jellyfish were consumed as part of a generalised diet during summer months. Finally, the approaches used to model jellyfish in the ecosystem model Ecopath with Ecosim (EwE) were reviewed. Jellyfish were included more frequently over time, however approaches remained relatively crude in the absence of high quality data in many ecosystems. Together, these approaches have gone some way towards addressing the data gap: jellyfish interactions with other biota have been recorded, and new approaches for studying these interactions have been developed. This has established a baseline for novel research opportunities such as mechanistic modelling of jellyfish, exploration of quantitative HTS approaches, and the generation of dietary time-series data to be conducted.

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Author contributions

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M.I.T., E.H. and J.K.P. designed the study. E.H. participated in sample collection. P.D.L. conducted molecular lab work, data analysis, and wrote the manuscript. S.C., E.H., J.K.P., R.G.D. and M.I.T. contributed to writing and manuscript revision.

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P.D.L conceived the study, conducted review and meta-analysis, and wrote the first draft of the manuscript. The supervisory team (S.C., E.H., J.K.P., R.G.D. and M.I.T.) critiqued and commented on the analyses and edited the manuscript.

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Chapter 1: General introduction

1.1 Our embattled seas

A legacy of anthropogenic activities has profoundly altered marine environments[1]. Marine ecosystems are now experiencing adverse effects from climate change[2], ocean acidification[3], eutrophication[4], marine litter (including microplastics) [5], dredging, offshore construction, and waste disposal[6]. Alarmingly, these pressures appear to be adversely affecting marine ecosystems synergistically[4]. Multi-disciplinary research, and holistic policy solutions are urgently required to tackle these issues. However, of all these issues affecting the marine environment unsustainable fishing practices remains the most damaging[7].

Fisheries are an important global food source: 15.7% of animal protein comes from fisheries, and at least 3 billion people depend on fisheries for at least 1/6th of animal protein[8]. Historically, the impact of fishing was thought to be negligible. In 1883 Huxley, denouncing contemporaries concerned about the state of fisheries and proclaimed "I believe then, that the cod fishery, the herring fishery, the pilchard fishery, the mackerel fishery, and probably all the great sea fisheries, are inexhaustible, that is to say nothing we do seriously affects the number of fish. And any attempt to regulate these fisheries seems consequently, from the nature of the case, to be useless". With time, this viewpoint fell out of favour: in 1914 Hjort, when studying fish populations in the Norwegian Sea, stated "scientists interested in the subject have become more and more inclined to the opinion that the hauls made by the fishermen really represent a very considerable portion of the actual stock of the sea"[9]. Hjort's assertion proved to be correct: paleoecological, archaeological, and historical records suggest long before Huxley's address, many marine species were in a state of decline[10]. Overfishing continued unabated, and in many cases accelerated, over the 20th century resulting in precipitous declines[11], and crashes[12], for many exploited species.

Globally the trend of over exploitation continues: the UN's Food and Agriculture Organisation conservatively lists 32% of marine fish stocks as currently being

overexploited[8]. However, the problem of overexploitation may be far more serious: Worm *et al.*[13] described 63% of global fish stocks as requiring rebuilding, Cullis-Suzuki and Pauly[14] in a separate analysis estimated 66% of stocks on the high seas were either overexploited or depleted. Briefly putting aside the serious food security implications, biologically speaking this is not just a simple case of 'less fish in the sea': selective removal of the most commercially attractive species, usually predators, collapses the structure and complexity of marine ecosystems; this leaves a much simplified, often fundamentally different, ecosystem remaining[15]. Under the strong selective pressure of fisheries, many exploited species are undergoing evolutionary changes such as maturation at small size, more rapid development, and reduced annual growth[16]; further modifying the relationships within, and consequently the overall functioning, of the ecosystem. Fisheries sometimes switch to exploit smaller, less commercially attractive, fish that are able to survive in the altered ecosystem in a process known as 'fishing down the foodweb'[17]. However, it should be noted that targeted species do not always change: economic incentives can cause the removal of predatory species to increase[18]. Furthermore, 'ratchet-like' processes such as the investment in fisheries equipment can lead to an acceleration of fishing down the foodwebs, as overexploitation of remaining stocks becomes a necessity to generate economic profit[19].

Even though it is doubtful if we will ever understand how most marine environments looked and behaved before human intervention, there exists a real issue in getting stakeholders to accept the relative productivity of the marine ecosystem just a generation or two ago. People normalise the current state of the marine ecosystem, and are ignorant of the fact that species assemblages used to be richer and individuals within a species often larger. These 'shifting baselines' contribute to a lack of political will and a continuation of decline[20].

1.2 Ecosystem approach to fisheries management

Some of the declines in fisheries may be attributable to the focus of fisheries' managers on maximising economic revenue from individual species: although such decisions may make short-term economic sense, it may be to the detriment of the other species

in the ecosystem. Consequently, calls for an ecosystem approach to fisheries management (EAFM), also called Ecosystem based management, are widespread in both academic publications[20–24] and policy documents. It is also a central requirement of both the EU's marine strategy framework directive and Common Fisheries Policy[25]. Although many definitions for EAFM exist, they all broadly encompass four key ideas: the linkages between biota are fundamental to the health and functioning of the ecosystem and must be maintained; for effective ecosystem management there is need to act at multiple spatial and temporal scales; no ecosystem exists in isolation, so the effects of management on other ecosystems must be considered; and stakeholder engagement is vital in creating management goals[26]. By shifting the focus away from the commercial management of individual species to the ecosystem it should be possible to stabilise, or maybe increase, the populations of exploited fish species. For effective EAFM to take place there needs to be both a thorough understanding of the biology of major taxa of the ecosystem, and an understanding of their relationship with other species. While good progress is being made in implementing EAFM[22], certain taxa, such as jellyfish have not been the focus of research efforts and still require further invesitagion before we can be confident of their interactions with other organisms, and the functional role they are playing in the ecosystem.

1.3 Jellyfish

1.3.1 Taxonomy & basic biology

Jellyfish are an polyphyletic assemblage of organisms[27] and there remain many unknowns, even in the most basic areas of their ecology[28]. The definition of jellyfish itself is somewhat loose: high water content (95% or higher) and a planktonic lifestyle[29] are the main criteria for being classed as a jellyfish, or gelatinous zooplankton. As such, jellyfish species are found within three phyla: Cnidaria, Ctenophora, and Chordata.

Pelagic tunicates, sometimes referred to as salps or doliolids, are herbivorous chordates that feed on phytoplankton[30]. Despite sharing a gelatinous body plan, they are distantly related to other jellyfish, and lack many of the population dynamics

or life history characteristics of other jellyfish[31]. For this reason, they are often not included in jellyfish studies as they occupy a different ecological role.

Ctenophores, otherwise known as comb jellies, are the second group of organisms to be considered jellyfish. Although, deep-history phylogenies are notoriously difficult to construct, there is compelling evidence to suggest Ctenophores are the earliest of all animal lineages: it is thought that their, admittedly somewhat limited, neural systems evolved independently to other animals[32]. Comb jellyfish are hermaphrodites, releasing male and female gametes into the water. Upon fertilisation, they begin life in the plankton, maturing directly into adults while feeding on zooplankton (and in some instances other jellyfish)[33]. Unlike Cnidarians, Ctenophores lack the ability to sting[34]. Instead they capture prey through the presence of a sticky layer, secreted from specialised cells known as colloblasts[35]. Despite the lack of noxious effects, Ctenophores can still impact human industry and ecosystems: owing to their ability to survive in a hypoxic conditions[36], and the ability to rapidly reproduce to create blooms they can overwhelm ecosystems. Taking advantage of eutrophic conditions, the invasive Ctenophore sea walnut (*Mnemiopsis leidyi*) overran the Black Sea during the 1980s[37] before cooler temperatures[15], and the invasion of the predatory jellyfishes *Beroe* sp.[38] returned populations to more manageable levels.

Cnidarian jellyfish, are another ancient lineage and have existed since the Cambrian[39]. Composed of classes Cubozoa, Hydrozoa, and Scyphozoa these organisms share stinging structures known as cnidae[35], which are used for hunting or grazing on a range of zooplankton including copepods, amphipods, cladocerans, icthyoplankton, and the larval stages of many invertebrates [35,40–43]. Some cnidarian jellyfish, such as the Atlantic sea nettle (*Chrysaora quinquecirrha*), also feed upon jellyfish[44]. Populations of jellyfish and zooplankton are tightly coupled[45], and large aggregations of jellyfish, known as blooms, can deplete local zooplankton populations[46].Cnidarian jellyfish have a worldwide distribution with some species, such as the cosmopolitan moon jellyfish (*Aurelia aurita*), displaying a remarkable ability to thrive in a range of environments including a range of different temperature[47], dissolved oxygen content[48], and salinity[49] regimes. This ability to survive in heavily modified ecosystems, coupled with a unique life-history (detailed below), has facilitated

jellyfish to prosper in some ecosystems that are no longer able to support large fish populations[50].

1.3.2 Cnidarian jellyfish life history

Although variability exists within jellyfish life cycles[51,52], (hydrozoans' life cycle is dominated by the polyp stage, in contrast to cubozoans and scyphozoans, in which the medusae stage is most important), a generalised life cycle shown in figure 1.1.

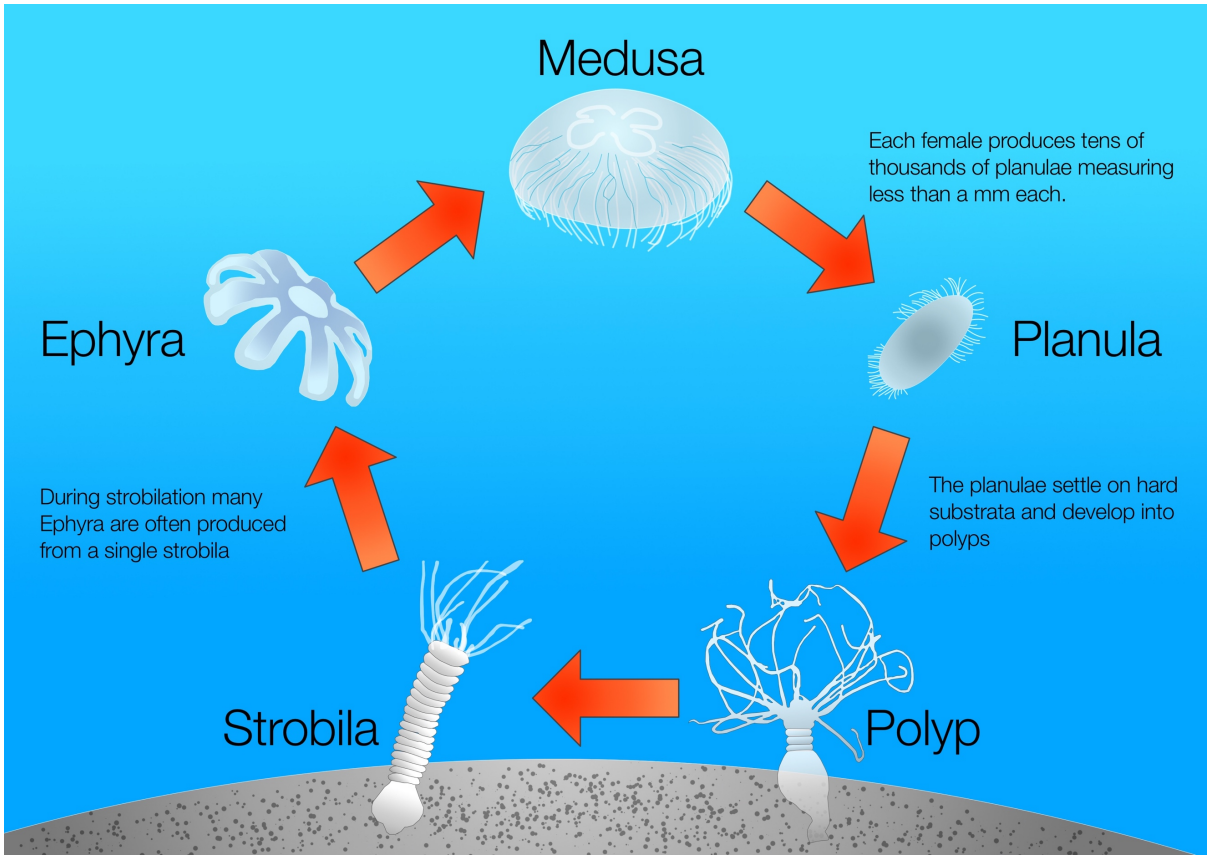

Figure 1.1. Generalised life history of cnidarian jellyfish.

In the northern hemisphere medusae reproduce sexually in the summer and autumn and the fertilised zygotes develop into planulae[53]. These free-swimming planulae are released, and spend some time in the water column (approximately 2 days for moon jellyfish[53]) before settling on hard-substrata to form sessile polyps. It is worth noting that some species, notably mauve-stinger jellyfish (*Pelagia noctiluca*) and *Stephanoscyphiostoma* do not form polyps, instead developing directly into ephyrae[40,52]. The number of planulae produced from a single jellyfish can be vast: *Aurelia aurita* typically produce approximately 58,000 planulae per female; some females have been documented with as many as 414,000 planulae[53]. Scyphozoan polyps form colonies on hard substrata, reproducing asexually before the production

of ephyrae (pre-medusa jellyfish), is stimulated by the onset of winter temperatures[47]. Many different processes for strobilation exist[54], producing one (monodisk) or many (polydisk) ephyrae[55]. Cubozoan polyps do not reproduce asexually, and always undergo monodisk strobilation[35]. Some Scyphozoan species, such as *Atolla vanhoeffeni*, have been documented producing as many as 4000 ephyrae from a single strobilation event[56]. The conspicuous UK species moon jellyfish, lion's mane jellyfish (*Cyanea capillata*), and blue jellyfish (*Cyanea* lamarckii) typically produce between 5 and 10 ephyrae during strobilation[57]. Polydisk strobilation, in combination with the high fecundity of medusae, is thought to be one of the life history characteristics responsible for the rapid development of blooms[55]. Ephyrae typically appear in late winter or early spring (February-April)[58]. They continue to grow in size, and develop their characteristic tentacles, becoming medusae in around 4 weeks[59]. Mortality often occurs in the same year (September is typical for moon jellyfish *aurita* and *Cyanea* spp. in the North Sea)[58], although multi-year individuals have been observed in other regions[51].

1.3.3 Blooms & Jellyfish Population Increases

A notable feature of jellyfish populations is their ability to form localised, super dense, aggregations known as 'blooms'. Much like the actual definition for what constitutes a jellyfish, no official definition exists. Broadly blooms are broken down into 'true' blooms, from an increase in the number of jellyfish in the ecosystem, and apparent blooms where jellyfish aggregate due to factors such ocean currents or other chemical cues but no net increase in jellyfish numbers across the entire ecosystem is seen[60].
Although most cnidarian and ctenophore species are known to bloom, some species have a greater propensity than others. Scyphozoans bloom more than cubozoans or hydrozoans, pelagic species bloom more than deep-sea varieties, and smaller jellyfish bloom more frequently than large species[61].

Blooms are undoubtedly a natural part of jellyfish ecology[29], however there is fierce debate about whether or not the frequency of blooms is increasing. There is a a lot of evidence[43,62,63] suggesting that jellyfish prevalence and blooms have increased due to the development of artificial marine structures[64], eutrophication[48], overfishing[65], and climate change[66]. However, in many systems there is a lack of long-term data-sets on jellyfish abundance [67] so evidence for global increases is

somewhat lacking[68]. Sanz-Martin *et al.*[69] conducted a network analysis of 159 papers that had cited papers detailing jellyfish increases. However, 48.9% of the publications had not drawn the correct conclusions from cited literature, consequently the idea that jellyfish are increasing has permeated through the literature to a greater degree than would be supported by the original studies. At the time of writing, two manuscripts have addressed the topic of global increases of jellyfish. Condon *et al.*[70] performed a meta-analysis using 37 primary data sets and found no evidence of a protracted increase, but did find an increasing trend from 1970. Due to the high variability of jellyfish populations, it was suggested this may be due to population oscillations present throughout the dataset. Brotz *et al.*[62] used 'fuzzy logic' (a system that allows information from multiple sources to be analysed by combining confidence scores and trend data) to analyse jellyfish trends using a wide range of sources. They found evidence of increasing jellyfish abundances in 62% of large marine ecosystems analysed. However, both studies carry caveats: the data sets used by Condon *et al.* were not designed to monitor jellyfish, and although this error should be systematic, sampling bias may be distorting the overall findings. For example, the Continuous Plankton Recorder was used to gather data for the north-Atlantic. However, the sampling aperture on this device is only 1.6 cm^2[71], while it has sampled fragments of jellyfish larger than the aperture such as *Pelagia noctiluca*, *Chrysaora hysoscella*, *Cyanea capillata*, and *Aurelia aurita*[72], it seems plausible it large jellyfish species, such as *Rhizostoma octopus* (which can be found in massive numbers in shallow waters[73]) go undetected and it's appropriateness for addressing jellyfish abundance trends is questionable. Brotz et al. have used a fundamentally different approach relying on a combination of scientific and anecdotal evidence in a 'fuzzy logic' system: however, this may have incorporated prevalent errors discovered in the literature by Sanz-Martin *et al.*[69]. Like many global studies, the paucity of data-sets in Africa, Asia, and South America is likely to have introduced geographic biases into both publications. As such, the global picture remains somewhat unclear: only with standardised jellyfish surveys can be we begin to build a more accurate picture. When considering blooms and increasing jellyfish populations, the best practice at present is to use local data: extrapolations to other systems, when data gaps are present, should be made cautiously, with particular attention paid to the processes and pressures affecting that ecosystem.

1.3.4 Impacts of jellyfish

Regardless of the cause of perceived jellyfish increases, blooms can have serious ecological and economic effects. Human enterprise can be adversely affected: jellyfish sting, and in some instances have caused fatalities[74]. Preventative measures such as beach closures, can negatively affect tourism revenues: following the death of two bathers in Australia, cancelled bookings amounted to USD $65 million in lost revenues[75]. Blooms also can block cooling pipes for power stations[76], or other industry. This can lead to power outages[34] and substantial remediation costs[77]. The fishing industry is also, unsurprisingly, affected by blooms. Jellyfish can overwhelm and burst nets. Additionally, since jellyfish compete with forage fish for the same prey items, they are thought to cap the fish population when zooplankton are limited[43]. In South Korea, jellyfish blooms have been linked with a 6.8-25.3% decrease in total landings valued at USD $68-204.6 million annually[78]. Aquaculture fares little better; blooms have eradicated entire fish stocks occasions in the Irish and United Kingdom waters[40].

Although mainly known for noxious effects, it should be noted that jellyfish are thought to provide positive outcomes too. Green fluorescent protein, a common bio-medical marker, was synthesised from jellyfish[79]. Jellyfish also provide cultural services: aquaria displaying jellyfish, and in some locations, recreational scuba diving can generate tourism revenues[80,81]. Jellyfish also have certain benefits for fisheries: juvenile fish have been observed sheltering among jellyfish for protection from larger predators[82], furthermore they are a food source for a variety of fish, bird, and reptile species[67,83] (see below for more detail). Jellies also have a direct use value: commercial fisheries now harvest jellyfish for medical collagen[84], and as a delicacy in Eastern Asian Markets[85].

1.3.5 Jellyfish as trophic dead-ends

Understanding the role jellyfish play in the ecosystem is an important first step towards predicting, and managing, blooms and their harmful effects and also providing important baseline information for an EAFM. Knowing who eats whom is one of the most basic building blocks of network ecology. Regrettably, this is another area for which we are currently data deficient. Historically, aside from leatherback turtles[86] (*Dermochelys coriacea*) and sunfish[87] (*Mola mola*), few predators of jellyfish were

documented. As such, they have been considered trophic dead ends[28,63]: *i.e* once nutrients enters jellyfish it is no-longer usable by predators occupying higher positions in the food web. This viewpoint may have originated from methodological limitations: viewing feeding events in the marine environment remains very challenging. Food webs have been reconstructed by morphological gut contents analysis, where stomachs were dissected out of a species of interest and hard parts such as bones and scales were used to identify predation. Although this technique has successfully identified jellyfish predation[88,89], it is likely underestimating the true extent of predation since the technique frequently fails to detect rapidly digesting, soft bodied organisms[90].

Recently, jellyfish predation has been identified using new techniques such as stable isotope analysis[83,91], static video loggers[92,93], animal-borne video loggers[94–96], acoustic sampling[97], and high throughput sequencing[98–100]. These studies have empirically demonstrated that jellyfish are not trophic dead ends and provided valuable methodologies to investigate diet. However, these feeding relationships have typically focussed on just one or two predators, and are scattered across many ecosystems. As such, many attempts to model and quantify the role jellyfish occupy in food webs still treat them like trophic dead-ends[101,102] and the true role jellyfish play in food webs remains poorly understood. There remains a need to systematically analyse predatory species for jellyfish consumption before a meaningful attempt to quantify their role in the food web can be quantified in any given ecosystem.

1.4 Common dietary analysis methodologies

1.4.1 Direct observation

Direct observation of feeding has been the mainstay for uncovering the diets of large terrestrial organisms such as birds[103] and mammals. It is conceptually simple and requires limited equipment. Additionally, it is non-invasive so is particularly well suited for endangered species. However, a number of caveats exist: the taxonomic resolution is dependent on the expertise of the observer, quantification of dietary items is nearly impossible, and the act of observation can change some species' natural feeding behaviour[90]. Furthermore, *in-situ* observation has been very difficult in some

environments, such as marine ecosystems[104]. As such, a range of other techniques have been employed to address these shortcomings.

Recently, direct observation has re-emerged as the miniaturisation and digitisation of cameras has enabled the study of previously inaccessible systems. For example: static[93,105] and animal-borne cameras[94–96] have enabled the study of jellyfish predation using direct observation. While some specialised equipment is now required, the need for expertise in the field is reduced as it is possible for a trained taxonomist to identify species from the obtained footage away from the field. Additionally, animal-borne cameras allow feeding behaviour to be detected that would be missed by other dietary elucidation techniques. For example, Sutton *et al*.[96] found Adélie penguins only feed on jellyfish if a hunt for more energy-rich food sources has failed.

1.4.2 Morphological gut content analysis

First used by Forbes in 1883[106] in his study on coleopteran predators, morphological gut contents analysis (GCA) continues to be used over 130 years later[89]. It has been employed where direct observation is very difficult and has been invaluable in understanding invertebrate and marine food webs. Relatively little specialist equipment is required: namely a dissecting kit and, in some instances, a microscope. An organism's gut contents are removed and prey are identified visually to the lowest possible taxonomic level using hard parts such as bones, otoliths and scales. Quantification of the diet can occur through the mass or relative abundance of removed prey items, alternatively a point system can be used (*e.g.* 0 for empty stomach, 1 for 0-25% full, 2 for 25-50% full etc.)[107].

There are a number of limitations to using morphological GCA. Firstly, a high-degree of taxonomic expertise is required[108]; the technique systematically underestimates soft-bodied organisms or life stages due to rapid digestion times and a lack of unique morphological characteristics[109]; secondly, the process is time intensive; furthermore the identification of prey species is impossible in species that liquid feed, such as spiders, or heavily macerate their prey such as many crustaceans[90]. Finally, lethal sampling is required for morphological GCA, although similar results can sometimes be obtained from morphological faecal analysis[110]. Other non-lethal

techniques, compatible with morphological GCA, have been investigated: stomach rinsing, otherwise known as gastric lavage, has now been used to source material for a number of species. Effectiveness seems to vary species to species: Barnett *et al.*[111] used gastric-lavage to conduct a dietary assessment on broadnose sevengill sharks (*Notorynchus cepedianus)*. A subset of sampled sharks were acoustically tagged and monitored for survival; 100% of these sharks were alive after 18 months indicating high survivorship. Conversely, Hartleb and Moring[112] experienced 60% mortality in golden shiners (*Notemigonus crysoleucas*) following stomach rinsing. This may be due to the small size of the fish, and the difficulty of inserting rinsing apparatus into the stomach. This suggests the unique physiology of the study species should be considered before non-lethal techniques are used.

1.4.3 Fatty Acid analysis

The move away from visual approaches began with the use of fatty acid trophic markers which were first suggested for dietary use in 1935 by Lovern[113]. However, it was nearly 30 years before fatty acid analysis was used in a marine diet context[114]. By removing the need to identify hard parts, soft bodied organisms were detectable by dietary studies. Furthermore, taxonomic expertise is not required once the initial quantitation of fatty acids takes place. Unique fatty acid compositions are present in primary producers, when consumed these markers are transferred to the feeding organism[115]. Although modification of fatty acids takes place during assimilation into the consumer it is possible to gain “calibration coefficients” through laboratory trials[116] and use these to get a rough quantitative breakdown of diet.

The benefits acquired from moving away from visual approaches come with some limitations. Fatty acid analysis necessitates lethal sampling and specialist facilities are required for the extract of lipids, and their analysis using gas chromatography[117]. The quantification of diet is entirely dependent on a calibration co-efficient, which may not exist for species of interest. Bayesian statistical approaches trained using a library of monoculture diets fed to a model organism offer an in-development work around[118]. However, these libraries are very limited at present[107]. Additionally, if fatty acid profiles are similar between prey items it is difficult to differentiate and some species will be missed from the analysis[119]: this may be fine if only a basic

understanding of the food web is required, but makes the technique unsuitable for work requiring finer taxonomic resolution.

1.4.4 Stable Isotope Analysis

Stable isotope analysis for food web reconstruction (SIA) first appeared in the late 1970s[120]. Stable isotopes, elements with nucleuses enriched with additional neutrons (referred to as 'heavy' isotopes due to the increased atomic mass) , occur naturally throughout the environment. Organisms preferentially retain molecules containing 'heavier' stable isotopes from the environment and diet, so the ratio of heavy isotopes to normal elements can be used to track a number of ecological metrics. Using a mass-spectrometer and elemental analyser, it is possible to calculate heavy to light isotopic ratios in tissue samples from an organism, as well as potential food items, to discern information about the food web[119]. ^{15}N accumulates at approximately 3.4‰ per trophic level[119] in a process known as trophic enrichment[107]. Although a number of other environmental factors are known to influence this process[120], diet is a sufficiently strong driver in the accumulation of heavy isotopes that it can be used to deduce trophic level: higher incidence of heavy nitrogen isotopes indicates a higher tropic level. ^{13}C:^{12}C values vary spatially, but do not undergo significant enrichment, as such it is possible to look at isotopic carbon values in an organism and discern the location of the primary producer underpinning that particular food chain[121]. Functional roles, and inter-specific feeding relationships can be discerned by using isotopic nitrogen and carbon values in tandem[83]. A numer of studies have also used Sulphur and oxygen isotopes: ^{34}S can be used to determine the location in the water column a marine organism resides in (benthic or pelagic)[107] . Isotopic hydrogen (^{2}H) can be used in a similar manner to differentiate between the input of freshwater or marine resources to an organism's diet[107], although the environment and the diet both influence this isotope strongly and accurate measures can become difficult to derive[122]. Stable isotope analysis has a number of unique advantages among the non-visually based techniques: because isotopic values are based on assimilated food items, it provides a longer term temporal view of diet and is good at determining which food items are actually important for the physiological maintenance and growth of an organism[107]. Furthermore, compound-specific SIA which looks at the isotopic values across multiple compounds (amino acids and fatty acids) within one sample has become more common[107]. The added compounds

offer a bigger range of isotopic values, and can be used to glean finer details on diet and spatial movements of an individual. Additionally, the factors that can affect isotopic values of a single compound are often more precisely quantified, granted higher confidence in the reconstructed food webs[120].

Limitations with SIA include the need for lethal sampling (or at the very least invasively obtaining a tissue sample from the organism), drivers other than diet affecting isotopic signatures[120], low taxonomic resolution due to overlapping isotopic values[123], and a heavy reliance on relatively few studies for fractionation correction values[120]. As such, while SIA certainly has some unique selling points: namely the ability to quantify biomass assimilated into an organism, the lack of precision can limit its utility if used in isolation.

1.4.5 Antibody based approaches

Antisera based approaches were an early molecular approach used to study predation without the need for visual identification of hard parts[124]. In its simplest approach, proteins from potential prey species are injected into a mammal (usually a rabbit), after some time the antibodies for the injected proteins are extracted. These are then introduced to gut contents either passively, or in an electrical field (to speed up the assay), the presence of the prey of interest is inferred from the production of white precipitate[90]. Like other non visual-based approaches, it is able at identify heavily macerated, or semi-digested prey items[125]. However, due to protein similarities between prey false positives have been known to occur[126]. Additionally, the approach is expensive and has poor reproducibility due to the idiosyncratic nature in which antibodies are produced: even using the same mammal to produce antibodies can yield different results[90].

With time improvements to address early limitations were made: the introduction of enzyme-linked immunosorbent assays[127], which uses an enzyme to facilitate the connection of the antibody and protein to greatly increase the sensitivity of the approach. Additionally, *in*-vitro monoclonal antibodies were developed which facilitated the detection of taxa-specific, or even life stage specific, protein sequences[128]. While these improvements made the approach excellent at detection

of specific prey the approach remained expensive and poorly suited to studying generalist predators[90]. It also faced ethical objections, because of the need to maintain populations of laboratory mammals.

1.4.6 DNA based approaches

An alternative molecular GCA approach was to use DNA to identify predation events. Predation is identified by matching fragments of DNA found in the stomach of a predator against a taxonomically-verified reference library. Genes from mitochondrial DNA (*e.g.* COI[129], 16s[130], 12s[131]), or chloroplast DNA (trnL[132]) are often used since multiple copies of these organelles exists within each cell, increasing the probability of successful detection.

The first published research exploring the viability of using mtDNA molecular GCA was conducted by Asahida *et al.*[133]. In summary, after feeding flounder to shrimp in laboratory conditions, shrimp were sacrificed and stomachs were removed. DNA was extracted using a TNES-urea buffer. Then, a polymerase chain reaction (PCR) using flounder-specific primers was conducted to amplify flounder DNA. Flounder predation was ascertained by the presence of an amplicon visualised using gel electrophoresis. This process using taxon-specific primers and electrophoresis to confirm predation is known as diagnostic PCR. It has been the mainstay of the field and has been used in variety of studies[134], it also has provided the base from which other molecular techniques were built on.

Real time PCR (rtPCR) assays are built on the same fundamental approach, but rely on a real a time PCR system and molecular probes. Molecular probes can amplify smaller quantities of DNA [135], and have greater taxonomic-specificity than standard PCR primers. In addition, while diagnostic PCR produces qualitative (detected / not detected) results, rtPCR quantifies the amount of DNA present in a sample. The development of multiplex rtPCR facilitated multiple fluorescently labelled (e.g TaqMan) primers for different taxonomic groups to be detected simultaneously in a PCR run [136,137]. Principally this reduces the time and cost of analysis, but also allows the presence of PCR inhibition and false-negative results to be evaluated[138]. Some studies have used the method to obtain relative abundance of food items in diet[135]. However, experiments formally testing this relationship suggest the technique should

not be used this way: Deagle and Tollit[130] formally tested if quantification was possible by feeding pinnipeds fixed quantities of fish, then using a rtPCR assay on faeces to see if the relative proportions obtained matched the diet. Although there was some evidence to suggest the amount of DNA quantified was not totally random, the output was significantly different from the dietary input. This could be a result of digestion biases, or different performance of primer sets. However, despite these limitations, rtPCR still remains the gold standard for investigating the predation of particular species due to its relative low-cost, sensitivity, taxon-specificity, and rapid production of results[109].

A modification of diagnostic PCR is introducing a sequencing step. Instead of solely relying on taxon-specific primers and gel electrophoresis to identify predation, sequencing of amplicons is used to match the amplicon against an online database such as BOLD[139] or Genbank[140], or a custom database. Taxon-specific primers are no longer required since the nucleotide sequences are used to identify a species, not the presence or absence of an amplicon during electrophoresis. Consequently, 'universal' primers covering a broad range of species are often employed to capture taxonomically diverse diets[131,141,142]. Early studies used vector cloning of PCR product to separate and reproduce different amplicons, prior to sanger sequencing, and identification with a sequence database[143,144]. While this approach allows generalist diets to be studied more easily, vector cloning and sanger sequencing is relatively slow and expensive.

First used for dietary purposes by Deagle *et al.*[145], high throughput sequencing (HTS) revolutionised this approach. HTS refers to a range of sequencing platforms that rely on sequencing many amplicons simultaneously, negating the need for vector cloning after an initial PCR. Vast quantities of data are produced facilitating the detection of rare trophic interactions[146]. An initial obstacle to overcome was predator DNA dominating the produced reads, thus reducing the ability to detect rare prey items. However, blocking primers have largely overcome this limitation. In a study carried out by Shehzad et al.[147] when blocking primers were not utilised 91.6 % of sequences obtained were that of the predator. Including blocking primers allowed the detection of seven addition prey species, a 63% increase compared to the original PCR run. In studies working with highly conserved genes, where the possibility of

blocking prey species' DNA existed, peptide nucleic acid clamps have been used to achieve similar results [100].

The ability of HTS to produce quantitative results (the reads produced corresponds to the initial biomass of different species in the sample) is a source of fierce academic debate. Initially it was hoped the number of sequences from a given taxa would be proportional to biomass consumed[148]: results have certainly been interpreted in this way by a variety of studies[149]. However, experimental evidence for this kind of usage is mixed. Initial results suggested there was no significant relationship between biomass and number of sequences produced[150,151]. However, recent studies suggest this may not be the case, and statistically significant relationships have been detected[152,153]. At present, there is no clear indication if particular organisms, genes, or sequencing platform are better or worse for producing quantitative results. Primer choice is very important as primers are known to preferentially bind to certain sequences over others, if a universal primer does this for one taxa over another, the sequencing results will reflect primer binding affinity rather than biomass[154]: Piñol *et al.*[155] in a mock-community experiment found the number of mismatches between a primer and template DNA explained 73% of the variation in sequence reads. As such, it seems possible phylogenetically similar taxa may have similar primer bias and be better suited for quantitative HTS studies than mixtures of more distantly related organisms, although this hypothesis has yet to be experimentally examined. Some improvement has already occurred: Thomas *et al.*[156] have used correction factors derived from laboratory trials to improve accuracy of quantifications, although it should be noted these correction factors were rather small. Recent opinion is that sequences from a HTS run probably contain some sort of quantitative signature[151]. Deagle *et al.* demonstrated, through the use of computer simulation, treating reads in a quantitative manner is often more accurate than detected/not-detected approaches[157]. While so many uncertainties remain, HTS is not ready for quantitative dietary studies en masse. However, sequences produced often loosely correspond with starting biomass, and with further research an accurate quantitative approach may soon be feasible.

To overcome primer biases the use of PCR-less shotgun sequencing is being explored in dietary studies[158,159]. Paula *et al.*[160] first used this approach to look at aphid

predation by harlequin ladybirds. Sample collection and DNA extraction is the same as other DNA-based techniques, however the extracted DNA is loaded directly into a high throughput sequencer. The deep sequencing depth enables the detection of fragmented mitochondrial and nuclear DNA. As primer biases are not present, quantification is possible. Furthermore, because the detection of prey is not dependant on the survival of a particular genetic marker (any DNA will do) greater sensitivity can be achieved. However, relatively few samples can be processed due to the deep sequencing depth required (most reads come from the predator and must be discarded) making this approach very expensive per sample. Furthermore, well annotated genomes are required in databases for reads to be identified. At present, databases have sparse genome availability so custom database construction is an, expensive, prerequisite step before this kind of study can take place.

Despite the fine taxonomic resolution achievable and lack of taxonomic expertise required, molecular GCA for dietary studies is far from a panacea: there are a host of issues worthy of consideration. Sequence databases are one source of uncertainty: although species coverage is constantly increasing as more sequences are submitted, large data gaps still exist[161]. This is particularly problematic in areas where it is difficult to obtain samples for sequencing, such as the deep sea[162]. Phylogenetic methods, (*e.g.* Bayesian-based[163] or Lowest common ancestor[98]), can be used to infer what an unidentifiable species is likely to be. In addition to database gaps, how representative of a species any given sequence is remains questionable: often only one sequence per species is available in a database. Whether this individual's sequence is typical of the species is unknown. This could be remedied by using a consensus sequence constructed out of many individuals [164]. Most concerningly, errors in sequence reads or taxonomic misidentifications are known to exist in sequence databases[108], removing these erroneous records is an important step towards increasing the reliability of molecular dietary studies.

Other issues are related to PCR and sequencing steps. The formation of chimeric sequences can lead to overestimates of dietary breath. Chimeras form when incomplete extension of a molecule occurs during PCR and the sequence binds onto a different DNA strand: creating a hybrid molecule. This can occur frequently in HTS studies with a PCR step: in extreme cases 70% of reads can be chimeric[165]. A suite

of programs exists for detecting such sequences[166], however in poorly sequenced taxa these programs may be of limited utility, and chimeric sequences have been identified on sequence databases.

Secondary predation is a very contentious issue: secondary predation occurs when the prey of a consumed item is erroneously attributed to the organism being studied[167]. This is particularly problematic for approaches using rtPCR or HTS due to the high sensitivity of the techniques[168]. Potential secondary predation can be identified by looking at the co-occurrence of prey items: if one apparent prey item is never present without another it is likely to be secondary predation[98]. Independently carrying out dietary studies of these prey items can further aid identification. eDNA (trace DNA found in the environment[169]) can also lead to false positives. Moving forward, as the relationship between number of sequence reads and biomass becomes better established it may be possible to identify certain reads as a product of background 'DNA' noise, until then using molecular GCA in tandem with one of the other approaches highlighted here can increase certainty in the results as eDNA contamination is less likely to be an issue with less sensitive techniques like SIA or fatty acid analysis.

Finally, attention must be paid to the technical aspects of the study's design. HTS is a very sensitive technique, prone to stochasticity in the results; creating robust repeatable methodologies is therefore difficult. Differences in primer choice, how technical replicates of the PCR are handled, sequencing depth, and OTU clustering (similar sequences grouped prior to taxonomic identification) can all affect the final outcomes of a dietary study. To highlight how much these choices can affect results Alberdi *et al.*[158] conducted a study using standardised source material, but different bioinformatic approaches: in the final results reconstructed dietary richness varied between 11 and 560 species. Variability from methodological choices is hardly limited to molecular GCA, nor does it 'invalidate' any given set of results, however it is worth being cognisant of these factors, and to design experiments and interpret results regarding this.

1.5 Modelling approaches to fisheries management

An EAFM requires combining disparate sources of data into an integrated, holistic approach. To this end, models are a widely-used approach, and have been instrumental in formally quantifying our knowledge of fisheries. Models have a number of uses including quantitative predictions, identification of knowledge gaps, 'gaming' to explore viable alternative ecosystem states (*i.e* 'what if' scenarios") and better understanding the network interactions underpinning food webs[170]. [170]. However, every model is a characterisation of the real world situation, so model choice must be carefully considered in tandem with data sources before any of the aforementioned uses can be implemented.

1.5.1 Single species models

Single species models for fisheries management were first implemented in the 1950s[171] and are still used in contemporary fisheries management[20,172]. Their wide use is facilitated by the relative ease of construction and statistical robustness compared to multi-species approaches[173]. Additionally, they offer superior short-term quantitative predictions than multi-species models[172,174]: in certain circumstances their use can still be advocated. It not a case of multi-species models being 'better' than single-species models; rather, it is choosing a model that matches needs and data availability.

However, ecosystems are complex and managing species individually is both conceptually flawed and, in practice, impossible. Since the models are trained on landings data rather than the physiological requirements of marine organisms, the models lack biological realism such as fully capturing the mechanisms behind the changes in recruitment (the number of juveniles entering the population) when populations are reduced[20]. Furthermore, theoretical studies have demonstrated that the actual harvestable yield of an ecosystem is less than the sum of its individual species' harvestable yields as predicted by models[175]. In practice, single species models have systematically underestimated declines in fisheries[20] and are sometimes listed as a contributing factor driving the collapse of many fisheries[176].

1.5.2 Extended Single Species Models & Minimum Realistic Models

Extended single species models possess much of the same statistical robustness, and conceptual simplicity as true-single species models, however a small step towards biological realism is taken by including important interspecific interactions for the species being modelled[177]. This reduces the chances of ecological surprises, for example: including predators in an extended species model of Alaska walleye pollock (*Gadus* chalcogrammus) enabled a more accurate representation of the inherent uncertainty in the fishery to be achieved compared to a traditional single species model[178]. However this approach still only models a small subset of the ecosystem and is a far cry from a true ecosystem approach to fisheries management.

Minimum realistic models, such as GADGET[179], act as a conceptual bridge between extended single species models and whole-ecosystem models. Rather than attempting to model all interactions within an ecosystem they focus on a small subset of important interactions within the ecosystem[174]. The most commonly used form of minimum realistic model is multi-species virtual population analysis and its derivatives. This approach explicitly models predation events between species in the model, as well as 'other predation' events. It benefits from a large user base, and has had many of its underlying assumptions well tested[177]. A 'forward mode' allows simulations to run through time[180]. However, the approach suffers from lacking an ability to estimate uncertainty in input variables, being very data intensive[177], treating predator growth and feeding rates as constant (which is biologically unrealistic)[180] and also ignoring portions of the ecosystem outside its partition. This last point can become particularly problematic in ecosystems experiencing significant bottom-up control as this possibility is totally unrepresented in a multi-species virtual population analysis approach[172].

1.5.3 Whole ecosystem models

Whole ecosystem models, such as Ecopath with ecosim (EwE)doy[181] and ATLANTIS[182], carry the most ecological realism: allowing species at all trophic levels, and the interactions between them, to be represented. As such, these models are well suited for use in EAFM. Many examples exist where whole ecosystem models have unveiled unlikely, multi-trophic, consequences within a perturbed ecosystem[180]. For example Bogstad *et al.* using MULTISPEC[183] were able to demonstrate increased

Minke whale (*Balaenoptera acutorostrata*) population sizes would lead to an increase in capelin (*Mallotus villosus*) population sizes despite being a predator of capelin. This was due to the interaction term of a third species: herring (*Clupea harengus*). It may be tempting to think of multi-species models as a panacea, especially when multi-species models seem to replicate the results generated by well-established single species models[174]. However, this increased biological realism comes at the cost of a lack of statistical robustness and greater uncertainty in the model outputs[177]. This is not through any fault in the models *per se*, but rather as a result of the complexity of the models: with more input variables, and interactions, within a model the likelihood of poor data entering the model, or abstract mathematical equations doing something biologically inappropriate increases. The likelihood of over-parameterising the model through the use of confounded variables increases for the same reason[184], although statistically testing data for co-variance before incorporation should decrease the likelihood of this occurring. It may not always be advisable to use multi-species models in place of single species models for generating specific quantitative predictions about a species. However, they are excellent for complimentary analysis and generating a holistic viewpoint.

1.6 Ecopath with Ecosim

1.6.1 An introduction to Ecopath with Ecosim

As the most commonly used whole-ecosystem model[185], the applications of Ecopath with Ecosim (EwE) vary greatly. EwE has been used to model a variety of freshwater, marine, and even terrestrial ecosystems[186]. The spatial scale of these models varies considerably: early models focussed on small spatial scales such as lakes and rivers due to the easily defined ecosystem boundaries. However, EcoOcean[187], a recent EwE modelling effort by Christensen *et al.* models the entirety of the world's marine ecosystems. The complexity of EwE models also shows great variation. EwE can, in its simplest form, can be used as a minimum realistic models[188]. However, most published EwE models adopt a true ecosystem approach incorporating taxa from a range of trophic levels. This range of previous applications and diversity of existing models showcases the flexibility of EwE, however this can make it bewildering to choose which particular EwE approach to use.

EcoBase, a database of published EwE models, has been created as a reference tool to facilitate easy access to pre-existing models, and help with the creation of new models using existing approaches[186].

Despite the great variety of EwE models, they are all based on a conceptually simple framework that can be broken into three main components. Ecopath, a static model developed by Polovina[189] to describe the interspecific interactions in a coral reef ecosystem, lies at the core of the approach. Ecosim, developed by Walters *et al.*[190] is a dynamic extension of Ecopath: the linear equations of Ecopath are replaced by differential equations to enable the model to simulate changes over time[23]. Ecospace, is the spatial extension: EwE is run in a grid system to allow simulation of an ecosystem both temporally and spatially[191].

1.6.2 A model description of Ecopath

Ecopath is parameterised under the assumption of mass balance over a specified period[23]. The model is constrained using two key equations: production and consumption. Production can be expressed as:

Production = catch + predation + biomass accumulation + net migration + other mortality

Or

$$P_i = Y_i + (B_i)(M2_i) + E_i + BA_i + (P_i)(1-EE_i)$$

Where P_i is the total production of species (i), Y_i is total fishery catch rate of species (i), $M2_i$ is the predation rate on species (i), E_i is the emigration rate of species (i), BA_i is the biomass accumulation of species, and $(P_i)(1-EE_i)$ represents all other mortality[192].

Three of the following four variables must be entered, (All four can be entered if data are available):

- Ecotrophic Efficiency (EE)
- Consumption / biomass ratio (Q/B)

- Production / biomass ratio (P/B)
- Biomass (B)

The following parameters must then be entered for each group (unless all four data parameters have been entered):

- Catch rate
- Net migration rate
- Biomass accumulation rate
- Assimilation rate
- Diet Composition

Relationships between different groups are then established, assuming energy balance as constrained by the consumption equation (see below). If mass balance is not achieved and incompatible values are found, input variables can be altered either manually or using an automated balancing procedure, taking into account user-defined uncertainty regarding input parameters.

Consumption = Production + respiration + unassimilated food

1.6.3 Analysis tools in EwE

EwE's core analysis tools can be broken down into sensitivity analysis tools and tools for the analysis of ecosystem indices.

Sensitivity analysis is of paramount importance in any model. This makes it possible to elucidate how dependent the model outputs are on the initial parameters. EwE offers a variety of sensitivity analysis tools. The simplest sensitivity analysis routine varies the input variables in steps of 10% to +50 % and -50% to test the effect of input variables on the calculated 'missing' variables' generated by the model[192]. Ecoranger offers a more sophisticated approach, and uses a Bayesian resampling routine to allow the probability distributions for each of the basic inputs, in addition to catch rate and diet compositions[193], to be calculated. A Monte-Carlo approach is then utilised to vary the input parameters within user-defined uncertainty estimates. This allows the

probability of any given input variables and transformed parameters occurring within the EwE model to be calculated. Mixed trophic impact (MTI) quantifies the change in biomass of one modelled group on all other groups within the modelled ecosystem[194]. As such it can be used to assess the 'keystone-ness' of a modelled group. It can also be used a sensitivity analysis tool: if a modelled group makes no quantitative difference on any of the other modelled groups it is not functionally important, and may warrant removal, or merging with another group during model simplification[195].

A number of specialist modules have been developed within EwE. The move to Microsoft's .net framework has improved the ease with which EwE can communicate with other programs and modules can be developed[185]. The most frequently used modules are Ecotracer and Ecotroph. Ecotracer is used to track the bioaccumulation and flow of contaminants, such as heavy metals and persistent organic pollutants, through the functional groups of an ecosystem[196]. Ecotroph uses biomass, and kinetics statistics, to calculate the impact of fishing on an entire ecosystem[197]. The network analyses plug-in adds to EwE's built in ecosystem indices, and allows the utilisation of concepts central to information theory such as ascendancy, flow from detritus, primary production required, trophic level decomposition, and keystoneness[186,198].

1.6.4 EwE Weaknesses

Issues that exist with EwE can be broken into two categories: issues inherent with the model, and common user errors. Weaknesses inherent with the model are the 'steady state' assumptions when using the Ecosim module, inadequate handling of uncertainty, uncertain biological underpinnings of the foraging theory, no account for energetic differences in prey, poor scaling from the microscale to the macroscale, and some mathematical inconsistencies[188]. While some degree of user error occurs with any modelling approach, the particular ease of model construction in EwE, due in part to its user-friendly interface, makes poor-quality models a particular issue. This is usually facilitated by uncritical use of default settings in EwE and poor data quality being used in model parameterisation[193]. When interpreting a model, a reader should be very wary of user error. Christensen and Walters[193] provide an excellent overview of common pitfalls and strategies to avoid them. Additionally, the inclusion of

the pedigree index, which describes data quality and origin, should serve to help identify weak models attributable to user error[198].

That ecosystems are assumed to be in a 'steady state' (no major fluxes in biomass occur in the period Ecopath is modelling) can present some serious problems. Ecosim and Ecospace, work by using a static Ecopath model and projecting the model stepwise through time and space respectively. For some ecosystems, like coral reefs, a long average may be appropriate (although recent coral bleaching events highlight the dynamic nature of even apparently 'stable systems'[199]). For other more dynamic systems, a much shorter time scale will be better suited[188]. To its credit, EwE features some ability to handle inherent variability: a seasonal forcing function can be applied to account for intra-annual variability[193]. Furthermore, if the system is known to be flux for the time period in which the Ecopath model was created, the change in biomass can be entered into the model[193]. However, catastrophic shifts in ecosystems are known to occur in marine ecosystems[200], and even with mediating functions applied, the inherent dependence on the static nature of the Ecopath model will make EwE ill-suited for highly dynamic ecosystems. Consequently, the use of EwE to make predictions about the ecosystem when conditions are no longer analogous to the time the Ecopath base model was created, or is actively in flux, is not advocated[190,193]. Should a highly variable ecosystem need to be modelled – constructing multiple Ecopath models, over different time periods, has been advocated as an appropriate approach[186,201] .

EwE's handling of uncertainty in both the input parameters, and the model itself, has been widely criticised[177,180,188]. A lot of effort has been directing into correcting these shortfalls: EwE now features a true Monte-Carlo approach that can be conducted on EcoSim runs to help interpret model output[198]. In addition, the inclusion of data pedigree and a Bayesian approach, Ecoranger, for incorporating parameter uncertainty into the model are both major additions.

EwE's approximation of predation using a 'foraging arena' is another source of criticism. The foraging arena theory states that predators must spend time foraging, and competing, for prey groups defined as being vulnerable to them. Conceptually, this has some appeal: predators are rarely found with full stomachs suggesting the

foraging theory has some degree of biological realism[202]. However the biological underpinnings of this are very "controversial and uncertain"[177]. A plethora of evidence exists, suggesting that many species do not behave in this manner[188]. Furthermore, predators do not act in isolation: if one predatory species decreases, prey populations rarely explode as other species apply compensatory predation. The foraging arena does not include these compensatory mechanisms, and therefore can systematically underestimate predation[172]. Moreover, it is based on a micro-scale with interactions being simulated on the individual scale[202], how these processes scale to ecosystem, the scale which the model operates, has not been properly elucidated[188]. In addition to questionable biological grounding, the mathematics implemented suggest it is not modelling the foraging arena mechanisms well: Plaganyi and Butterworth[188] fully describe the mathematical assumption in the appendix and conclude that *de facto* "about half the prey population numbers N_i are vulnerable to predator j, effectively irrespective of the value of v_{ij} (vulnerability)."

The handling of energetics is another shortcoming of the EwE approach. The master equations in Ecopath deal solely with biomass: the energetic content of modelled groups is assumed to be the same[188]. This is a gross oversimplification, the energetic content of species varies largely and predators alter feeding behaviours accordingly. For illustration, gelatinous zooplankton contain less than 8-27% caloric value of fish per unit of biomass (depending on this fish and jellyfish species)[203]. In its current form, EwE would grossly overestimate the energetic potential of gelatinous zooplankton, and could conceivably make erroneous assertions about gelatinous zooplanktons' role in the ecosystem. It is conceivable that using relative biomass flows as an analogue for energy flows (i.e, however extreme caution must be applied as outputs would be easy to misinterpret).

Another shortcoming is the functional response curves embedded in the model were developed to simulate fish species. The scope of EwE has expanded significantly since this initial development, but the response curves remain the same. Consequently, EwE still handles population dynamics of certain organisms, such as birds and mammals, particularly poorly[172].

The final criticism levied at EwE is its handling of life history characteristics[172,177,188]. Different life stages were not incorporated into the original version of the model: some marine organisms can grow 1,000 fold during their life, so treating them identically was clearly less than ideal. However, EwE now allows organisms to be broken into multiple stanzas to allow different ontogenetic stages to be modelled in a biologically realistic manner[198].

1.6.5 EwE strengths

Despite some limitations, there are reasons to be positive about EwE's use. Many limitations apply to any whole ecosystem model: more complex models inherently have more parameters, and more opportunities to introduce errors. EwE strikes an excellent balance between biological realism and complexity, and the addition of a user interface makes creating and using models very accessible[188,198]. As a true ecosystem model it is allows the investigation of topics not possible using MSVPA or single species models such as network function, ecosystem health, trophic cascades and ecosystem wide impacts of fishing[188] . EwE is also particularly good at comparing ecosystems. Comparison requires that the same analytical framework has been used: EwE's low data requirements, good documentation, database of published models[186], and conceptual simplicity facilitate this[204]. EwE currently dominates ecosystem-modelling approaches, and has a large and active scientific community: as of October 2008 there were 5,649 registered users and over 300 publications featuring EwE (ecopath.org). It is also constantly being improved and updated, many of the criticisms expressed in the early literature have now been addressed: EwE now features predator satiation and prey switching, in addition to making material steps to quantify uncertainty in the model[198]. The incorporation of Microsoft's .net platform had increased the modularity of the model – and now allows users to readily develop their own modules. For example Steenbeek et al.[185] used the .net framework to incorporate true GIS capability into the EwE framework – allowing environmental forcing on the ecosystem to be explicitly captured.

1.7 Aims of this thesis

Implementing an EAFM requires a working knowledge of the major components of the ecosystem, and the interspecific interactions between them. Our knowledge of jellyfish trophic ecology, when compared to other marine taxa of comparable biomass, in ecosystems around the UK and Ireland is limited. This is concerning as jellyfish populations in the Irish Sea have been increasing[66], and elsewhere in the region adverse effects, such as the loss of fish stock in aquaculture installations has occurred[40]. In order to start to fill these data gaps the following aims are investigated in this thesis:

1. What is the trophic ecology of jellyfish? Traditional morphological gut contents analysis can systematically underestimate jellyfish predation, so diagnostic screening was used to screen 50 taxa taken from multiple years of sampling for jellyfish predation. The results are presented in chapter two.
2. Does predation mortality vary across jellyfishes' life cycle? Jellyfish undergo a dramatic transformation over the course of their life cycle. Time is spent as a sessile polyp, a planktonic juvenile, before developing into adult medusa. Predation during different life stages has important consequences for jellyfish populations, as well as the nutrients derived for predators. Jellyfish predation is investigated at different times of year, using the diagnostic PCR developed in chapter two, and is presented in chapter three.
3. To what extent can HTS be used to quantify trophic interactions? Having established jellyfish predation in different locales, and in different time of years there is a need to gain more context. HTS can provide complete diets, however it is unclear if the reads generated correspond to the amount of biomass consumed. In chapter four we conduct a meta-analysis on HTS studies to ascertain how the results of a HTS should be interpreted.
4. What is the context of jellyfish predation? Are predators preferentially selecting jellyfish as a prey, or are they only utilised when other prey are unavailable? What is the relative consumption of jellyfish: are they rarely ingested or important pathway for energy in the food webs. These questions are

investigated using HTS and universal primers, the results are presented in chapter five.

5. How are jellyfish handled in whole ecosystem models? Is there any evidence of particular studies influencing the entire field? In chapter six, existing EwE models are examined, drawing upon both literature and the findings of this thesis. Recommendations are made on best-practice for including jellyfish in whole ecosystem models.

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Chapter 2: Jellyfish on the menu: mtDNA assay reveals scyphozoan predation in the Irish Sea

2.1 Abstract

Localised outbreaks of jellyfish, known as blooms, cause a variety of adverse ecological and economic effects. However, fundamental aspects of their ecology remain unknown. Notably, there is scant information on the role jellyfish occupy in food webs: in many ecosystems few or no predators are known. To identify jellyfish consumers in the Irish Sea, we conducted a molecular gut content assessment of 50 potential predators using cnidarian-specific mtDNA primers and sequencing. We show that jellyfish predation may be more common than previously acknowledged: uncovering many previously unknown jellyfish predators. A substantial proportion of Herring and Whiting were found to have consumed jellyfish. Rare ingestion was also detected in a variety of other species. Given the phenology of jellyfish in the region, we suggest that the predation was likely targeting juvenile stages of the jellyfish lifecycle.

2.2 Introduction

Cnidarian jellyfish (hereafter referred to as “jellyfish”) are a common feature of many marine ecosystems. Localised outbreaks, known as blooms, can cause negative economic and ecological effects such as fish death, interference with marine infrastructure, and tourism losses [1]. Understanding the ecology of jellyfish is essential if the blooms are to be predicted and adverse effects avoided.

One area of jellyfish ecology that is poorly characterised is their role in food webs. Jellyfish have historically been viewed as trophic dead-ends, *i.e.* once nutrients enter jellyfish they are lost to organisms occupying higher trophic levels [2,3]. This viewpoint may originate from difficulties observing marine interactions *in situ* and the inability of

morphological gut contents analysis (GCA) to identify rapidly digested, soft bodied organisms such as jellyfish [4]. New analytical techniques have revealed that some animals do feed on jellyfish [5–8]. However, many of these jellyfish predators are scarce and not thought to play a significant role in controlling jellyfish populations [9]. Furthermore, most of these studies have focused on single species and therefore, the extent of jellyfish predation in food webs remains unknown.

The Irish Sea makes for an excellent case study: it has experienced adverse effects from an increasing jellyfish population [10] yet aside from small populations of leatherback turtles and sunfish [11] no predators are known. Systematically identifying predators of jellyfish is a prerequisite step before the broader role jellyfish play in ecosystems can begin to be adequately addressed. As such, the aim of this study was to identify Irish Sea jellyfish predators, using a newly developed cnidarian specific polymerase chain reaction (PCR) assay. This approach identifies gut contents by matching amplified DNA fragments against a DNA database, circumventing issues associated with morphological GCA. Therefore, even highly digested jellyfish can still be detected.

2.3 Methods

2.3.1 Sample collection & Extraction of DNA

Sample collection, processing, and molecular work is detailed in full in [12]. In summary: gut samples were collected aboard the RV Cefas Endeavour in the eastern Irish Sea between 25/02/08 - 02/03/08, and 19/02/09 - 28/02/09. Trawling gears were deployed to capture predators from throughout the water column to maximise active predator-prey interactions. For each haul, vinyl gloves were sealed, then inserted inside an additional vinyl glove which itself was then sealed. The innermost gloves were processed as stomachs, with the outer gloves simulating a fish during dissection: this negative control was used to detect any potential contamination introduced during initial processing. Seven hundred and fifty-one and 1762 samples were collected in 2008 and 2009 respectively. The length of each sampled organism was recorded before the removal of the stomach on-board. Gloves were changed between the dissection of different species, and instruments were decontaminated between hauls

with Microsol detergent to minimise the possibility of contamination. Removed stomachs were stored at -20°C.

DNA was extracted from the stomach contents in a molecular laboratory. Whole sprat (*Sprattus sprattus)* and shrimp *(Crangon sp.*) stomachs had DNA extracted and purified using a salt protocol, since their stomachs were small enough to avoid issues with PCR-inhibitory substances. Samples were homogenised in 300 µl of extraction buffer (30 mM Tris-HCL pH 8.0, 10mM EDTA pH 8.0, 1% SDS), before 5 µl Proteinase K (Qiagen) was added. The samples were then incubated at 55°C overnight. Purification of DNA took place using a salting out protocol [13].

All other species' stomachs had DNA extracted using a CTAB (hexadecyltrimethylammonium bromide) method [12]. Contents were scraped out of the dissected stomachs and homogenised in autoclaved 1.5 ml Eppendorf tubes with 350 µl of 2% CTAB buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20mM EDTA pH 8.0, 2% CTAB, 0.2% 2-mercaptoethanol), then mixed with 5 µl Proteinase K (Qiagen) and incubated at 55°C overnight for the sample to digest. DNA purification was performed using two choloroform-isoamyl washes followed by a sodium acetate precipitation (3M, pH 4.8). Both CTAB and salt extracted samples were dissolved in 100 µl water and stored at -20°C.

2.3.2 Jellyfish group-specific primer design

Available 16S sequences of jellyfish present in UK coastal waters (Supplementary table 2.1) were obtained from GenBank [14] (Supplementary table 2.2) and aligned using MUSCLE [15] with default settings. Positions in the 16s alignment where nucleotides were conserved among jellyfish, but different in non-gelatinous species (Supplementary table 2.3) were identified. Jellyfish specific primers: SCY_16S_F4 (TTAAATGGCCGCGGTAACT) and SCY_16S_R4 (GCTCAATAGGGTCTTTTCGTCT) were designed using Primer3 [16] to amplify a 135 bp fragment that included the unique jellyfish sequences. The primers were tested *in-silico,* on non-gelatinous species (Supplementary table 2. 3), using Amplify4 [17] prior to PCR validation across a panel of jellyfish and non-gelatinous marine species (Supplementary table 2.4) to ensure specificity to jellyfish.

2.3.3 PCR amplification and sequencing

PCRs were conducted in 10 µl reactions containing 1 µl DNA, 1 µl 10x ReddyMix PCR Buffer IV (ABgene), 1 µl dNTPs (2 mM), 0.05 µL Thermoprime plus Taq DNA polymerase (5 U μl^{-1}) (Thermo Scientific), 1 µl of Scy_16s_F1 & Scy_162_F2 (10 µM), 1.2 µl BSA (20 mg ml^{-1}) (New England Biolabs), 0.6 µl $MgCl_2$ (25 mM) (Thermo Scientific), and 3.15 µl H_2O. Cycling conditions were: 95°C for 4 minutes, followed by 35 cycles of 95°C for 0:30s, 65°C for 0:30s, 72°C for 0:30s, with a 10 minute incubation at 72°C. Negative and positive controls were included on each plate. The presence of jellyfish DNA was determined based on the presence of a band at 177 bp on 1.5% ethidium-bromide stained agarose gels.

A subsample of positive amplifications were purified with Exo1 (Thermo Scientific) and FastAP (Thermo Scientific) prior to Sanger sequencing (Eurofins UK). Sequences were trimmed of primers and low read-quality bases, and chromatograms visually inspected for quality. Sequences were identified using nucleotide megablast [18] against the GenBank nucleotide database, and reported as % BLAST identity values.

2.4 Results

2.4.1 2008 Survey

Jellyfish mtDNA was detected in 18 out of 751 samples from 9 of the 34 surveyed taxa (Table 1). All positive samples were identified as moon jellyfish (*Aurelia aurita*) (supplementary table 2.5). Five sequences from dab (*Limanda limanda*), whiting (*Merlangius merlangus*), herring (*Clupea harengus*), and squid (*Loligo* sp.) had a 100% identity match with moon jellyfish across the 135bp amplicon. The remaining sequences also matched with Moon jellyfish, but with BLAST identity values varying from 85% - 96%.

2.4.2 2009 Survey

Cnidarian mtDNA was detected in 141 samples out of 1762 samples from 7 of the 38 surveyed taxa (Table 1). Predation was much more frequent in herring and whiting than in 2008: Jellyfish were detected in 27.6% and 11.6% of herring and whiting stomachs respectively, compared to just 1.4% and 2.6% observed in stomachs from 2008.

Samples from dover sole (*Solea solea)*, sprat, and a subsample of herring (n=15) and whiting (n=21) were successfully sequenced. A sequence could not be obtained from the flounder (*Platichthys flesus*) amplicon, consequently flounder was not included in further analysis. Twelve sequences from herring stomach samples were identified as moon jellyfish with 3 unidentified sequences. Whiting had mainly consumed mauve-stinger jellyfish (*Pelagia noctiluca*) (n=16), although 3 mtDNA sequences derived from whiting stomachs were identified as oaten-pipe hydroids (*Tubularia indivisa*) (100% match), one sample as soft coral (*Alcyonium sp.*) (99% match), and one unidentified sequence. In contrast to 2008, most samples had 98%+ identity match (supplementary table 2.5).

Table 2.1. Taxa tested for jellyfish feeding events.

	2008			**2009**		
Taxa	**Stomachs screened**	**Stomachs with jellyfish consumption detected**	**Frequency of Occurrence (%)**	**Stomachs screened**	**Stomachs with jellyfish consumption detected**	**Frequency of Occurrence (%)**
Agonus cataphractus	1	0	0	13	0	0
Ammodytes marinus	0	0	0	4	0	0
Arnoglossus sp.	0	0	NA	28	0	0
Aspitrigla cuculus	4	0	0	9	0	0
Blennius ocellaris	0	0	NA	4	0	0
Buglossidium luteum	0	0	NA	14	0	0
Callionymidae sp.	12	4	33.3	30	0	0
Cancer pagurus	0	0	NA	3	0	0
Ciliata mustela	2	0	NA	0	0	0
Clupea harengus	143	2	1.4	369	102	27.6
Corystes cassivelaunus	0	0	NA	21	0	0
Crangon crangon	9	0	0	60	0	0
Cyclopterus lumpus	0	0	NA	1	0	0
Echiichthys vipera	13	0	0	22	0	0
Engraulis encrasicolus	3	0	0	0	0	NA
Eutrigla gurnardus	31	1	3.2	31	0	0
Gadus morhua	3	0	0	2	0	0
Hippoglossoides platessoides	2	0	0	0	0	NA
Limanda limanda	70	1	1.4	171	1	0.6
Liocarcinus depurator	0	0	NA	25	0	0

Liparis liparis	0	0	NA	2	0	0
Loligo sp.	36	1	2.8	1	0	0
Majidae sp.	0	0	NA	20	0	0
Melanogrammus aeglefinus	13	0	0	0	0	NA
Merlangius merlangus	76	2	2.6	294	34	11.6
Microchirus variegatus	0	0	NA	18	0	0
Microstomus kitt	0	0	NA	7	0	0
Necora puber	1	0	0	0	0	NA
Nephrops norvegicus	12	0	0	0	0	NA
Octopodidae sp.	0	0	NA	1	0	0
Pagurus cuanensis	0	0	NA	45	0	0
Palaemon serratus	0	0	NA	2	0	0
Pandalus sp.	1	0	0	0	0	0
Platichthys flesus	22	0	0	39	1	2.6
Pleuronectes platessa	8	0	0	0	0	NA
Polybius holsatus	15	0	0	0	0	NA
Pomatoschistus sp.	1	0	0	10	0	0
Raja clavata	7	0	0	0	0	NA
Raja montagui	0	0	NA	12	0	0
Scomber scombrus	2	0	0	17	0	0
Scyliorhinus canicula	16	2	12.5	11	0	0
Sepia officinalis	3	0	0	0	0	NA
Sepiola atlantica	1	0	0	21	0	0
Solea solea	0	0	0	25	1	4
Sprattus sprattus	192	4	2.1	412	1	0.2
Trachurus trachurus	4	0	0	0	0	NA
Trigla lucerna	7	0	0	8	0	0
Trisopterus esmarkii	10	0	0	0	0	NA
Trisopterus luscus	1	0	0	0	0	NA
Trisopterus minutus	30	1	3.3	10	0	0

2.5 Discussion

2.5.1 Jellyfish consumption amongst common species

Dragonet (Callionymidae sp.), grey gurnard (*Eutrigla gurnardus*), poor cod (*Trisopterus minutus*), lesser-spotted dogfish (*Scyliorhinus canicula*), squid, herring, whiting, dover sole, and sprat were identified as taxa that consume jellyfish (Figure 2.1). High year-to-year variability was seen, particularly for whiting and herring. It is unclear what drove this variation, particularly without data for jellyfish abundance or alternative food sources for the species. However, this does highlight the importance of repeated sampling: had we formed our data from a single year some predation events would have been missed, while other estimates would have been more inaccurate.

Jellyfish predation in the Irish Sea is not novel: sunfish and leatherback turtles are known predators [11]. However, the relative biomass of these species is tiny relative to the taxa described here (supplementary table 2.6). The discovery that common species prey on jellyfish is unexpected since jellyfish predators were thought to be scarce [9]. This could be important since even apparently low levels of jellyfish consumption amongst common species could potentially exert comparable, or greater, levels of influence on jellyfish populations than rare predators.

2.5.2 Which species and life stages are being targeted?

Moon and mauve-stinger jellyfish were both found to be consumed in this study. Non-exact matches (85 - 96% Blast identity) could be a result of intraspecific variation, un-sequenced cryptic species, or other jellyfish absent from GenBank. The amplification of oaten-pipes hydroid and soft coral demonstrates that the primers also amplify non-scyphozoan cnidarians (some of which are jellyfish [19]), highlighting the importance of a post-PCR sequencing step to identify and remove any false positives.

Moon jellyfish possess a meta-genetic life cycle, characterised by functionally different life stages [20]. In the autumn, adult jellyfish (medusae) reproduce sexually: fertilised planktonic planulae are released, and spend several days in the water column [21] before settling on hard substrata to form sessile polyps. Medusae then begin to die

off, while polyps overwinter [20]. Polyps are sessile until strobilation (the asexual production of free-swimming ephyrae) is stimulated by the onset of cooler temperatures [22] in February, March, and April [23]. Ephyrae continue to develop in size, becoming medusae in around 4 weeks [24]. Mauve-stinger jellyfish have a similar life history, although notably lack a polyp life-stage [25]. Predation on different life stages could have varying effects on jellyfish populations, and the nutrients available to predators. At the time of sample collection (February and March) the majority of moon and mauve-stinger jellyfish would not have yet matured into medusae [20]: it therefore seems probable the detected predation was on juvenile ephyrae or perhaps moon jellyfish polyps.

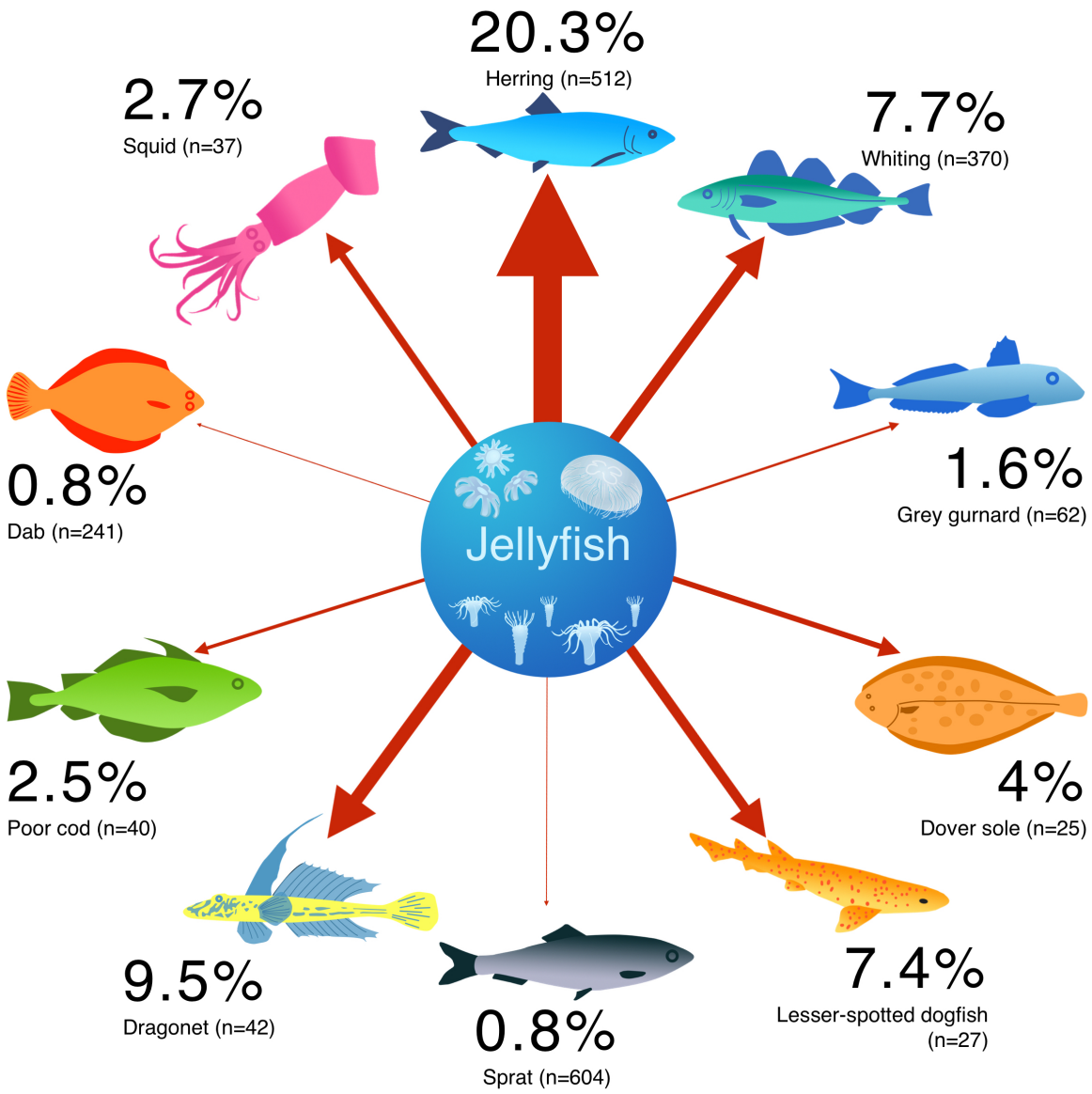

Figure 2.1. Species that feed on jellyfish in the Irish Sea validated using sequencing. Thickness of arrow is representative of the percentage of stomachs jellyfish were detected in (also displayed as a percentage) across the years 2008–2009. Reported sample sizes (*n*) refer to the number of stomachs sampled from each species. Species that jellyfish were not detected in are detailed in Table 2.1

2.5.3 A molecular approach: advantages and limitations

A variety of approaches have been used to detect jellyfish predation. Multiple studies have identified jellyfish predation using morphological GCA approaches [5]. Shortcomings of this technique, such as systematically underestimating soft-bodied prey and taxonomic uncertainty, are well documented [4]. Recently, video loggers recorded benthic scavenging of jellyfish carcasses [26]. However, the static nature of video cameras means capturing mid-water interactions, where jellyfish spend most of their life cycle, is logistically extremely challenging. Stable isotope analysis (SIA) [6] is free of the limitations of both morphological GCA and visual observation, and also has the advantage that it provides an estimate of biomass consumed. However, SIA cannot elucidate interspecific relationships to a fine taxonomic resolution due to overlap in isotopic values between different species [27], nor is it effective at detecting rare prey species in the diet.

Molecular GCA also overcomes the limitations of morphological GCA and observational approaches, and has been used with high throughput sequencing (HTS) to identify jellyfish predation in herring in coastal waters of New Brunswick [8]. Additionally, unlike SIA, it inexpensively provides species-specific identifications. Consequently, large sample sizes can be investigated which, in this instance, proved essential to detect jellyfish consumption.

Molecular GCA does have limitations: unlike SIA, molecular GCA cannot provide biomass consumption estimates. In addition, although not widely discussed, the possibility of contamination from eDNA (trace DNA found in the environment [28]) could exist, though it is typically found at very low concentrations. In this instance, it is extremely unlikely to be problematic: in an eDNA study of Japanese Sea nettle jellyfish (*Chrysaora pacifica*) the highest concentration of eDNA, detected on the sea floor, had a concentration of 2.49 $x10^{-10}$ ng μl^{-1} [29]. The primers used here detected moon jellyfish DNA diluted to a concentration of 0.03 ng μl^{-1}, but no further. A related issue using molecular approaches is that secondary predation (when a consumed prey species has consumed jellyfish) cannot be distinguished from direct consumption of jellyfish. This is particularly problematic if using HTS: the high sensitivity makes the

probability of detecting small amounts of DNA from secondary predation more likely than using the gel-imaging approach used here [30]. Secondary predation can be diagnosed by identifying predatory species in the gut alongside the jellyfish, then independently testing those species for jellyfish predation. However, by using cnidarian-specific primers, the co-occurrence of other non-cnidarian species in the guts cannot be examined; so the possibility of secondary predation should not be disregarded. Balancing the requirements of precision, cost, and time needs to be carefully considered when choosing between dietary assessment methodologies: the technique employed here is fast, easy to conduct, and inexpensive, but lacks the precision and sensitivity of HTS, or biomass estimates of SIA. Therefore, it is best used as a low-cost diagnostic tool for initial screening of samples to aid in the design of HTS studies, or as a complementary analysis to provide finer taxonomic resolution to SIA.

2.6 Conclusion

The evidence presented here refutes the notion that jellyfish predation is rare: sequencing suggests that Herring and Whiting frequently feed on jellyfish. Dragonet, sprat, dover sole, dab, squid, lesser-spotted dogfish, and poor cod were also seen to infrequently ingest jellyfish. When considering phenology of jellyfish in this region [20], it seems probable this predation is targeting juvenile jellyfish, although ingestion of Moon jellyfish polyps also remains a possibility. Quantifying such feeding relationships, and testing for adult jellyfish predation later in the year are therefore important future foci towards understanding the trophic role jellyfish play in ecosystems and predicting jellyfish blooms.

2.7 References

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2.8 Supplementary information

Supplementary Table 2.1. Jellyfish species present in the British Isles[1–5].

Genus	Species
Aegina	citrea
Aeginura	grimaldii
Aequorea	forskalea
Aequorea	vitrina
Aequorea	pensilis
Aglantha	digitale
Amphinema	dinema
Amphinema	rugosum
Annatiara	affinis
Atolla	wyvillei
Atolla	vanhoeffeni
Atolla	parva
Aurelia	aurita
Botrynema	brucei
Bougainvillia	britannica
Bougainvillia	macloviana
Bougainvillia	principis
Bougainvillia	ramosa
Bougainvillia	pyramidata
Bougainvillia	superciliaris
Bythotiara	murrayi
Chromatonema	rebrum
Chrysaora	hysocella
Cladonema	radiatum
Clytia	hemisphaerica
Clytia	islandica
Codonium	proliferum
Colobonema	sericeum
Corymorpha	nutans
Coryne	eximia
Cosmetira	pilosella
Craspedacusta	sowerbii
Crossota	rufobrunnea
Cyanea	capillata
Cyanea	lamarckii
Dipleurosoma	typicum
Ectopleura	dumortierii
Eirene	viridula
Eleutheria	dichotoma
Eucheilota	maculata
Eucodonium	brownei
Euphysa	aurata
Eutima	gegenbauri
Eutima	gracilis
Eutonina	indicans
Gonionemus	vertens
Halicreas	minimum
Haliscera	bigelowi
Halopsis	ocellata
Helgicirrha	schulzei
Hybocodon	prolifer
Laodicea	undulata
Leuckartiara	nobilis
Leuckartiara	octona
Liriope	tetraphylla
Lizzia	blondina
Lovenella	clausa

Margelopsis	haeckelii
Melicertum	octocostatum
Mitrocomella	brownei
Mitrocomella	polydiademata
Modeeria	rotunda
Muggiaea	atlantica
Nanomia	cara
Nausithoe	atlantica
Nausithoe	globifera
Neoturris	breviconis
Neoturris	pileata
Obelia	geniculata
Obelia	dichotoma
Obelia	longissima
Obelia	lucifera
Obelia	nigra
Octophialucium	funerarium
Orthropyxis	integra
Pandea	conica
Pandea	rubra
Pantachogon	haeckeli
Paraphyllina	ransoni
Pelagia	noctiluca
Periphylla	periphylla
Phialella	quadrata
Phialopsis	diegensis
Physalia	physalis
Podocoryna	borealis
Podocoryna	carnea
Podocoryna	areolata
Podocoryna	minima
Proboscidactyla	stellata
Rathkea	octopunctata
Rhizostoma	pulmo
Rhopalonema	velatum
Rhopalonema	funerarium
Sarsia	tubulosa
Slabberia	halterata
Solmaris	corona
Solmissus	incisa
Stauridiosarsia	gemmifera
Stauridiosarsia	producta
Stauridiosarsia	ophiogaster
Staurostoma	mertensii
Tetraplatia	volitans
Thamnostominae	sp.
Tiaropsis	multicirrata
Tima	bairdii
Trichydra	pudica
Turritopsis	nutricula
Velella	velella
Zanclea	costata

Supplementary Table 2.2. Jellyfish 16S sequences, from GenBank, used for designing group-specific jellyfish primers

Species	Accession Number
Aurelia aurita	U19373.1
Atolla vanhoeffeni	JX393250.1
Atolla wyvillei	JX393251.1
Cyanea capillata	KM114287.1
Pelagia noctiluca	JX393260.1

Supplementary Table 2.3. Non-gelatinous species 16S sequences, from GenBank, used for designing group-specific jellyfish primers

Species	Accession Number
Actinia equina	KP090930.1
Aequipecten opercularis	AJ245397.1
Alcyonium digitatum	AF530482.1
Antedon bifida	KC626604.1
Bispira porifera	HM800968.1
Buccinum pemphigus	FJ875946.1
Buglossidium luteum	KJ128718.1
Crangon crangon	EU868649.1
Inachus dorsettensis	KC866331.1
Labrus bergylta	KJ128797.1
Limanda limanda	AY368897.1
Liocarcinus holsatus	GQ268540.1
Lophius piscatorius	KJ128815.1
Luidia sarsi	AY652495.1
Microchirus variegatus	FN688074.1
Mullus surmuletus	KJ128836.1
Munida sp.	AY351197.1
Nemertesia antennina	FJ550458.1
Ophiura ophiura	AY652508.1
Pandalus montagui	EU868698.1
Pasiphaea telacantha	KP725635.1
Processa guyanae	EU868708.1
Psammechinus miliaris	AY652516.1
Trisopterus minutus	KJ128939.1
Trisopterus luscus	KJ128937.1
Tritonia plebeia	AJ223393.1

Supplementary Table 2.4. Jellyfish and non-gelatinous species used to test and optimise group-specific primers on

Taxa	Grouping
Aequorea sp.	Jellyfish
Aurelia aurita	Jellyfish
Chrysaora hysoscella	Jellyfish
Cyanea lamarckii	Jellyfish
Pelagia noctiluca	Jellyfish
Rhizostoma pulmo	Jellyfish
Actiniaria sp.	Non-gelatinous
Aequipecten opercularis	Non-gelatinous
Alcyonium digitatum	Non-gelatinous
Antedon bifida	Non-gelatinous
Arnoglossus laterna	Non-gelatinous
Ascidiella scabra	Non-gelatinous
Buccinum sp.	Non-gelatinous
Buglossidium luteum	Non-gelatinous
Cellaria sp.	Non-gelatinous
Cirolana sp.	Non-gelatinous
Crangon allmani	Non-gelatinous
Hyalonema sp.	Non-gelatinous
Hyperoplus immaculatus	Non-gelatinous
Inachus dorsettensis	Non-gelatinous
Labrus bergylta	Non-gelatinous
Limanda limanda	Non-gelatinous
Liocarcinus holsatus	Non-gelatinous
Loligo forbesii	Non-gelatinous
Lophius piscatorius	Non-gelatinous
Luidia sarsii	Non-gelatinous
Microchirus variegatus	Non-gelatinous
Mullus surmuletus	Non-gelatinous
Munida rugosa	Non-gelatinous
Nemertesia sp.	Non-gelatinous
Ophiura ophiura	Non-gelatinous
Pagurus prideaux	Non-gelatinous
Pandalus sp.	Non-gelatinous
Pasiphaea sp.	Non-gelatinous
Porifera sp.	Non-gelatinous
Processa sp.	Non-gelatinous
Psammechinus miliaris	Non-gelatinous
Pycnogonum sp.	Non-gelatinous
Scalpellum sp.	Non-gelatinous
Symphodus melops	Non-gelatinous
Thyone sp.	Non-gelatinous
Trisopterus luscus	Non-gelatinous
Trisopterus minutus	Non-gelatinous
Tritonia hombergii	Non-gelatinous
Zeugopterus regius	Non-gelatinous

Supplementary Table 2.5. Sequencing a subsample of positive results identifies, via a megablast query on GenBank, which gelatinous species was present in the samples.

Species	Jellyfish identity	Blast Identity (%)	Year
Callionymidae sp.	Aurelia aurita	90	2008
Callionymidae sp.	Aurelia aurita	92	2008
Callionymidae sp.	Aurelia aurita	93	2008
Callionymidae sp.	Aurelia aurita	85	2008
Clupea harengus	Aurelia aurita	87	2008
Clupea harengus	Aurelia aurita	100	2008
Eutrigla gurnardus	Aurelia aurita	88	2008
Limanda limanda	Aurelia aurita	100	2008
Limanda limanda	Aurelia aurita	85	2008
Limanda limanda	Aurelia aurita	89	2008
Loligo sp.	Aurelia aurita	100	2008
Merlangius merlangus	Aurelia aurita	87	2008
Merlangius merlangus	Aurelia aurita	96	2008
Merlangius merlangus	Aurelia aurita	100	2008
Scyliorhinus canicula	Aurelia aurita	92	2008
Scyliorhinus canicula	NA	NA	2008
Sprattus sprattus	Aurelia aurita	89	2008
Sprattus sprattus	NA	NA	2008
Sprattus sprattus	Aurelia aurita	94	2008
Trisopterus minutus	Aurelia aurita	100	2008
Clupea harengus	NA	NA	2009
Clupea harengus	Aurelia aurita	95	2009
Clupea harengus	NA	NA	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	NA	NA	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	95	2009
Clupea harengus	Aurelia aurita	98	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	99	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	100	2009
Clupea harengus	Aurelia aurita	100	2009
Merlangius merlangus	Alcyonium sp.	99	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	92	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	99	2009
Merlangius merlangus	Pelagia noctiluca	97	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Pelagia noctiluca	94	2009
Merlangius merlangus	Pelagia noctiluca	100	2009
Merlangius merlangus	Tubularia indivisa	100	2009
Merlangius merlangus	Tubularia indivisa	100	2009
Merlangius merlangus	NA	NA	2009
Merlangius merlangus	Tubularia indivisa	100	2009
Solea solea	Pelagia noctiluca	100	2009
Sprattus sprattus	Pelagia noctiluca	100	2009

Supplementary Table 2.6. Biomass of jellyfish predators in the Irish Sea. This assumes the Irish Sea area is 58000 km2. Sources for the data used in these calculations are referenced in the relevant data cell.

	M. mola	D. coriacea	C. harengus	Callionymidae sp	M. merlangus	Eutrigla sp.	S. canicula	Trisopterus sp.	S. sprattus	S. solea
Density (individuals km^{-2})	0.043 [6]	0.000775862 *[7]								
Body mass (kg)	55 **[8]	455 [9]								
Predator biomass (tonnes km^{-2})	0.0024	0.0004	4.0415 [10]	0.1710 [11]	0.5070 [11]	0.4440 [11]	0.2880 [11]	0.9740 [11]	0.7378 [10]	0.1100 [11]
Total Biomass (tonnes)	137.17	20.4750	234409.8	9918.0	29406.0	25752.0	16704.0	56492.0	42790.5	6380

*Based on 45 Leatherback sightings, in the Irish sea in 2012

**Body mass is based on mean values obtained from Figure 3A

2.9 Supplementary references

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Chapter 3: Cryptic diets of forage fish: jellyfish consumption observed in the Celtic Sea and Western English Channel

3.1 Abstract

Multiple, independent, sources of evidence suggest jellyfish are not trophic dead-ends, but instead play a role in supporting fish stocks. However, dietary data have been frequently collected in an ad hoc manner and whether fishes' feeding relationships with jellyfish vary with time, if at all, is unknown. To establish if fishes' consumption of jellyfish changes through the year, we conducted a molecular gut content assessment on opportunistically sampled species from the Celtic Sea in October and compared these to samples previously collected in February and March from the Irish Sea. Mackerel were found to feed on hydrozoan jellyfish relatively frequently in autumn, with rare consumption also detected in sardine and sprat. This is in contrast to sampling in February and March where moon jellyfish ephyrae were heavily predated. By October, moon jellyfish appeared to have escaped predation, potentially through somatic growth and the development of stinging tentacles. No significant change in predation rate was observed in sprat. However, jellyfish predation by mackerel feeding in autumn was significantly higher than that seen during winter: this increase in consumption appears to be driven by the consumption of different, smaller jellyfish species than were targeted during the winter.

3.2 Introduction

Fisheries in the Irish Sea are important for the regional economy: in 2016 the UK-based fleet landed 36, 600 tonnes worth £57.8 million[1], while the Irish fleet caught a further 11, 253 tonnes[2]. However, Irish Sea fisheries are facing challenges from an increasing scyphomedusae jellyfish (hereafter referred to as 'jellyfish', unless stated otherewise) population[3]. Jellyfish blooms (instances when jellyfish become super abundant in a localised area) in other regions have caused economic losses to fisheries by bursting fishing nets, contaminating catches, reducing the abundance of fish by competing for the same resources, and killing fish[4]. Recently, an aquaculture installation in the region experienced total loss of stock when overrun by a jellyfish bloom[5]. Preventing jellyfish blooms from affecting human enterprise has been difficult, and many direct interventions have been ineffective[4].

Until recently, it was thought that predators of jellyfish were rare or non-existent in many ecosystems. However, this view is no longer widely-held: using new techniques such as stable isotope analysis[6], stationary underwater cameras[7], remote operated vehicles[8], and acoustic surveys[9] a variety of taxa are now known to feed upon jellyfish. Previously, using molecular gut content analysis it was shown commercially-important fish species such as Herring (*Clupea harengus*), Whiting (*Merlangius merlangus*), and Dragonet (Callionymidae sp.) consumed jellyfish[10]. This suggests, that jellyfish populations may offer benefits to fisheries by supporting fish stocks. However, the observed scyphomedusae consumption occurred when jellyfish in the Irish Sea were juvenile and lacked the size or defensive structures to deter predation; It remains unknown if they are consumed throughout the year or used as a seasonal resource.

Complex and dynamic interspecific relationships are common in marine ecosystems: assuming unchanging predation through the year is likely to drastically mischaracterise a species' trophic role. For example, herring and sprat (*Sprattus sprattus*) are known to limit cod recruitment by feeding on ichtyoplankton stages of cod (*Gadus morhua*)[11]. However, upon maturation, cod feed on small herring[12], reversing the interspecific relationship. A dynamic relationship like this may be present in jellyfish as

they have a complex life cycle featuring multiple, functionally different life stages[13]. During a previous investigation, jellyfish early in the year (February and March) were producing mobile juvenile ephyrae from their sessile life stage in a process known as strobilation. Upon strobilation, ephyrae are just a few millimetres in diameter[14] and typically lack defence mechanisms: the first moon jellyfish (*Aurelia aurita*) ephyrae retain the tentacles from the polyp, however all subsequent ephyrae take several weeks to develop these stinging structures[14]. The observed predation of this vulnerable state is therefore unsurprising.

Although all Irish and Celtic Sea jellyfish ephyrae measure a few millimetres in diameter, there is considerable variation in size and stinging ability by maturation[14]. Mauve stinger jellyfish (*Pelagia noctiluca*) remain small, with a mean size of 4.5 (±1.2) cm in diameter, although large individuals can reach 12 cm[15]. Other common species are known to grow larger: moon jellyfish diameters can reach 25 cm[16], while Barrel jellyfish bells (*Rhizostoma pulmo*) are known to approach 1 m in diameter[17]. While large predators like turtles are known to feed on whole medusae[18,19], it remains to be seen if the pelagic fish species identified as jellyfish consumers in the Irish Sea previously maintain this trophic relationship throughout the year. It is plausible that large size of jellyfish relative to the predatory fish and the development of stinging tentacles may limit predation, however other predatory fish species have been observed biting, and consuming, parts of jellyfish despite these structures[20] so jellyfish may yet be viable prey.

Here, drawing on samples collected from the Celtic Sea in October, adult jellyfish predation is characterised with the aim of testing whether jellyfish support predatory fish populations through the year, or if predation is reduced or stopped altogether as jellyfish develop defensive structures and grow.

3.3 Methods

3.3.1 Sampling

Samples were collected aboard the RV Cefas Endeavour as part of the PELTIC 15 research survey. Collection permits were not required, with all samples being caught

and processed following the guidelines detailed in 'Animals in scientific studies at Cefas' [21]. Full details on the PELTIC 15 survey can be found in Appendix 5 of ICES WGIPS report[22]. In summary: between 05/10/2015 and the 20/10/2015 acoustic data acquisition and plankton sampling were undertaken along transects as shown in figure 3.1. A 20 x 40 m v d K Herring Trawl using KT nets was deployed opportunistically at 18 locations when fish schools were observed in the echograms (shown in figure 3.1). Upon retrieval of fish, they were identified to species level (shown in table 3.1), measured, weighed, and had their stomachs removed and frozen on-board. Scalpels and gloves were changed, and cutting boards cleaned using fresh water between species dissection. If jellyfish were found in the haul, they were identified to species level and a small sample of bell tissue was preserved in 100% ethanol.

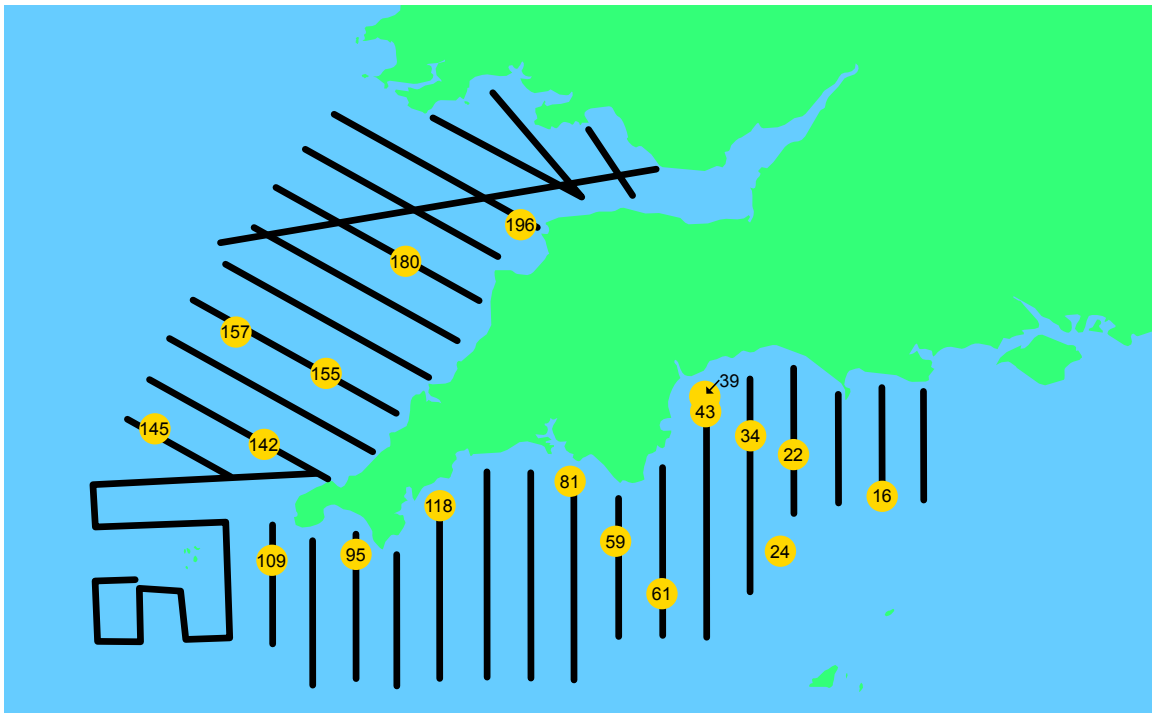

Figure 3.1. Map of survey area in the Western Channel and Celtic Sea. Lines denote location of acoustic transects. Yellow circles show sampling locations, numbers denote to sampling station ID. Diagram adapted with permission from ICES (2016).

Additional jellyfish samples were obtained from plankton sampling. Plankton sampling occurred at night when the ship was stationary at designated sampling points (shown in [22]) . Ring-nets, equipped with a General Oceanics mechanical flowmeter (model 2030RC, which includes a mechanism to prevent the rotor from turning backwards) with either an 80 µm or 270 µm mesh were used, and took a vertical sample of the entire water column (more details in [23]). Caught jellyfish, had bell tissue preserved in

100% ethanol. Note this sampling was not jellyfish population sampling, rather a method for identifying jellyfish species present in water during the survey.

Table 3.1. Species collected from PELTIC 2015 cruise.

Common name	Binomial nomenclature	Sample size
European Anchovy	*Engraulis encrasicolus*	20
Horse Mackerel	*Trachurus trachurus*	77
John Dory	*Zeus faber*	5
Lesser Spotted Dogfish	*Scyliorhinus canicula*	1
Mackerel	*Scomber scombrus*	95
Sardine	*Sardina pilchardus*	70
Red Gurnard	*Chelidonichthys cuculus*	5
Saury Pike	*Scomberesox saurus*	5
Sea Bass	*Dicentrarchus labrax*	4
Sprat	*Sprattus sprattus*	90
Whiting	*Merlangius merlangus*	3

3.3.2 DNA extraction

Stomachs were thawed and contents dissected on a separate disposable paper towel and using flamed scissors, scalpel and forceps to prevent contamination. DNA was extracted using a salt extraction technique[24]: Stomach contents were macerated and a small volume (approximately 1-8 mm^3) was placed into 300 µl digestion buffer (30 mM Tris-HCl ph 8.0, 10 mM EDTA, 1% SDS, with 10 µl Proteinase-K (Qiagen)) in a 1.5 ml Eppendorf tube, incubated overnight at 55°C. One hundred µl of 5 M NaCl was added to each sample and centrifuged for 5 minutes at 13,000 rpm. 250 µl supernatant was transferred to a new Eppendorf tube, taking care to avoid the precipitate. 500 µl ice-cold 100% ethanol was added, before being cooled at -20°C overnight. The Eppendorfs were centrifuged at 13,000 rpm for 30 minutes, and the ethanol was tipped off. The DNA pellet was washed once with 1 ml 70% ethanol, before an additional 5 minutes in the centrifuge at 13,000 rpm. The DNA pellet was then dried at 50°C (approximately 20 minutes), 200 µl molecular grade water added and the samples incubated at 37°C for 30 minutes. In addition to the stomach samples, negative controls, where nothing was dissected, but the tweezers were

dipped in the digestion buffer at the beginning of the process, were included as contamination controls.

3.3.3 PCR & Sequencing

The protocol developed previously by Lamb et al.[10] was used here. In brief, the cnidarian-specific 16s mtDNA primers SCY_16S_F4 and SCY_16S_R4 were used to amplify a 135 bp amplicon in a PCR. The presence of a band at 177 bp on an ethidium-bromide stained 1.5% agarose gel indicated cnidarians had been eaten. Positive PCR product was cleaned, using Exo1(Thermo Scientific) and FastAP (Thermo Scientific), then sanger-sequenced (Eurofins UK). Identification of the consumed cnidarians based on BLAST identity was performed using the nucleotide megablast algorithm[25] on the GenBank nucleotide database[26].

3.3.4 Statistical analysis

All statistical analyses were performed using R[27]. Samples from a previous study in February and March from the Irish Sea were also included[10]. For species that were detected eating cnidarians, where sample size permitted (for both early- and late-season sample collection), a Fisher's exact test was performed to determine if differences in predation could be observed between seasons. Since multiple hypotheses (different species) were tested, a one-stage false detection rate correction[28] was applied (reported a q-values) to avoid the chance of a type-2 error.

3.4 Results

3.4.1 Jellyfish predation

Cnidarian DNA was detected in three species: sardine, mackerel, and sprat. Predation was rare in sardines, and sprat with only 3.3% and 1.4% samples containing jellyfish DNA in their stomachs respectively. Predation was common in mackerel, with 23.2% stomachs containing jellyfish DNA. The consumed cnidarians were identified as the scyphozoan mauve-stinger jellyfish, as well as the hydrozoans *Geryonia proboscidalis*, *Scolionema suvaense*, and *Liriope tetraphylla* (no common names). Six mackerel samples and one sprat sample could not be sequenced, these samples were excluded from the positive sample list. The successfully sequenced samples had Blast identity values between 86% and 100%. Results are shown in table 3.2.

Table 3.2. Species of jellyfish predators, the sampling station (as denoted in figure 3.1), and the jellyfish preyed upon that were detected using a 16s mtDNA assay. The blast ID, which shows the percentage of shared nucleotides with the sequence in the database, and the length of the sequence used to identify the species are also shown.

Species	Sampling station	Blast ID	Identity %
Pilchard	95	*Pelagia noctiluca*	100
Mackerel	59	*Liriope tetraphylla*	94
Mackerel	59	*Pelagia noctiluca*	100
Mackerel	59	*Geryonia proboscidalis*	86
Mackerel	59	*Liriope tetraphylla*	99
Mackerel	180	*Liriope tetraphylla*	97
Mackerel	180	*Liriope tetraphylla*	97
Mackerel	180	*Liriope tetraphylla*	97
Mackerel	180	*Liriope tetraphylla*	93
Mackerel	180	*Liriope tetraphylla*	95
Mackerel	180	*Liriope tetraphylla*	100
Mackerel	180	*Liriope tetraphylla*	100
Mackerel	180	*Liriope tetraphylla*	96
Mackerel	180	*Liriope tetraphylla*	99
Mackerel	180	*Liriope tetraphylla*	100
Mackerel	180	*Liriope tetraphylla*	99
Mackerel	180	*Liriope tetraphylla*	100
Mackerel	180	*Liriope tetraphylla*	100
Mackerel	196	*Liriope tetraphylla*	90
Mackerel	196	*Scolionema suvaense*	95
Mackerel	196	*Liriope tetraphylla*	93
Mackerel	196	*Liriope tetraphylla*	100
Mackerel	196	*Liriope tetraphylla*	99
Sprat	118	*Pelagia noctiluca*	91
Sprat	118	*Liriope tetraphylla*	92
Sprat	118	*Liriope tetraphylla*	95

3.4.2 Seasonal variation

Figure 3.2 demonstrates that predation of jellyfish by mackerel was common in October (n=22, 23.2% stomachs contained cnidarian DNA) (late season), but was not detected in February or March (aggregation of 2008 and 2009 data); a Fisher's exact test suggested this was a significant difference (q=0.02, p=0.01). Jellyfish appeared to be a rare prey item in both seasons (2015 data: n=3, 1.4% stomachs contained jellyfish DNA; 2008/2009 data: n=5, 0.8% stomachs contained jellyfish DNA) for sprat and no significant difference was detected (q=0.07, p=0.07).

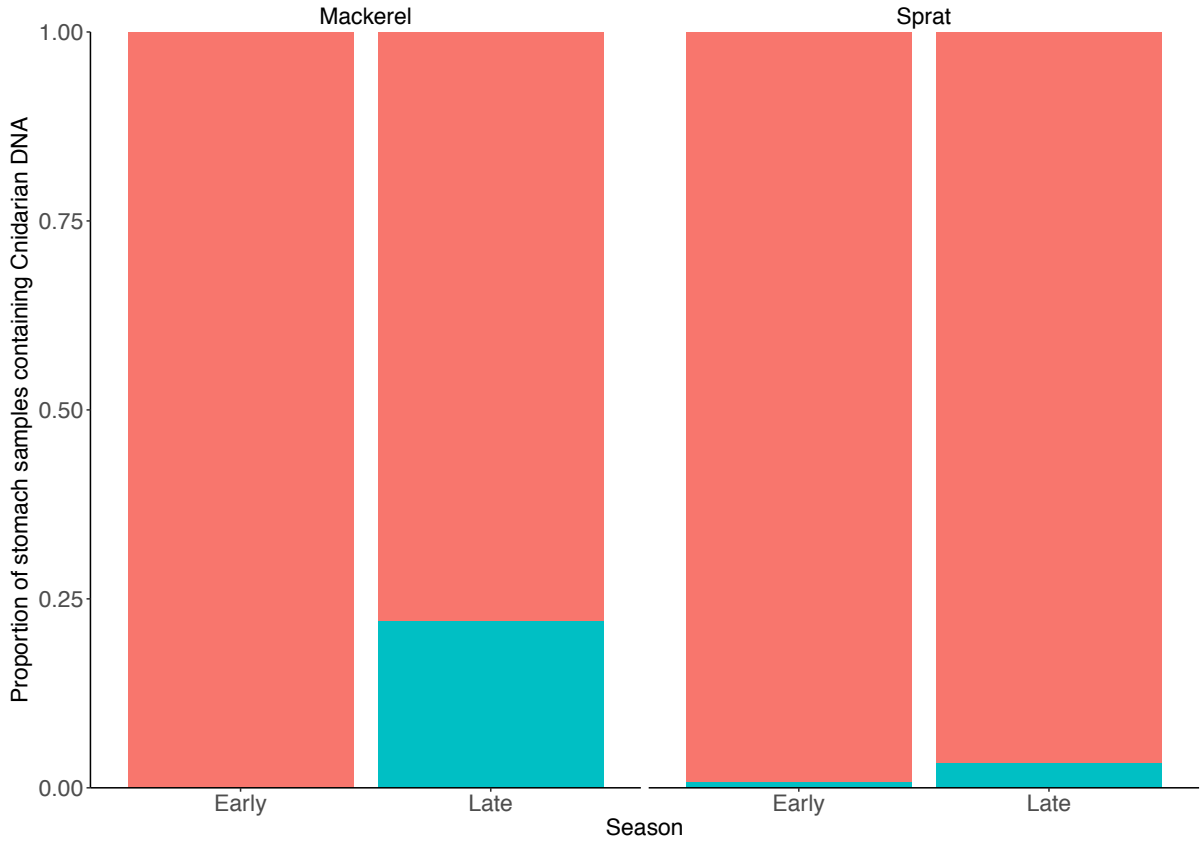

Figure 3.2. Proportion of stomachs that jellyfish were detected in for mackerel, and sprat. Red indicates no cnidarians were detected, while blue shows consumption of cnidarians had occurred.

3.5 Discussion

3.5.1 Observed predation

Three fish species: sprat, mackerel, and pilchard were observed eating jellyfish. A single instance of mauve stinger jellyfish consumption was observed in all 3 species. The ingestion of *G. proboscidalis* and *S. suvaense* was observed only once in mackerel, although it should be noted the low BLAST ID (86% and 95% respectively) suggests a high degree of uncertainty in the taxonomic assignment at the species or

genus level: particularly as these species are not associated with the region. *L. tetraphylla* accounted for all remaining predation in mackerel and sprat.

3.5.2 The pelagic food-web

The largest consumer of jellyfish in the early season (February & March) was herring[10], however despite being a pelagic fish, none were captured in this study and we were unable to draw comparison between early and late season. The absence of Herring in the autumn may be linked to their migratory behaviour[29] or relatively low population size in the area surveyed[30]. Seasonal comparisons in the other detected predators also presented challenges: in the early season a large sample size and a variety of trawling techniques were employed to capture both benthic and pelagic communities of fish[31], facilitating the detection of rare predation. The late-season samples were collected opportunistically through mid-water trawling. As a consequence, we were unable to capture the late-season benthic component of the food web. Furthermore, unlike the late-season survey, the early-season survey was devoted to surveying an entire predator community, and single-individual processing of opportunistic samples further limited sample sizes.

Although these factors limit our ability to assess temporal variation for most species, these data still refine our understanding of jellyfish predation in the food-web. Figure 3.2 shows that small jellyfish were a food source for mackerel. Jellyfish predation by *S. scombrus* has been observed previously: mackerel switched from filter feeding to a biting feeding behaviour to consume the small hydrozoan *Aglantha digitale*[32] (10-40 mm bell height). However, in contrast to the widespread predation by fish during February and March[10] very little predation on jellyfish was observed across the pelagic community during October. A possible explanation of diet shifts may be related to the relative abundance of other prey items. For example, sprat switch to preying on fish eggs in the winter when zooplankton levels are depressed[33]. It is possible that widespread predation of scyphomeduase jellyfish ephyrae in the February and March is in response to poor availability of other zooplankton; greater zooplankton availability in October may result in a switch away from jellyfish and result in the observed predation rates.

3.5.3 Escaping predation?

Although no difference in seasonal predation was detected in sprat, statistical analysis demonstrated mackerel fed on jellyfish more frequently in the samples collected in October than those in February and March. This was unexpected, as we anticipated the consumption of larger jellyfish to be more difficult and that rates of predation would therefore decline later in the year. Upon closer inspection however, the results do not contradict this hypothesis: *L. tetraphylla* has a bell diameter of 1- 3 cm[34]: 69 times smaller in area than a large moon jellyfish (bell diameter of 25 cm[16]). Larger jellyfish species such as mauve-stinger, moon, barrel, compass (*Chrysaora hysoscella*), and blue jellyfish (*Cyanea lamarckii*) were caught incidentally during the research cruise but were not detected with the dietary assay. While quantified population estimates are not available, this suggests that the complete absence of prey is unlikely to be responsible for jellyfishes' absence in the dietary data. Prey switching could occur due to decreased medusae populations which typically decrease, and experience mortality, later in the year[13] (although overwintering populations have been recently been recorded in other ecosystems[35,36]). Another explanation is that larger species of jellyfish, particularly moon jellyfish, which were frequently predated upon early in the season, may have escaped predation through somatic growth, leaving only small species like *L. tetraphylla* vulnerable to predation (see figure 3.3). Finally, it possible that an unknown sea-specific phenomena may be driving the observed differences.

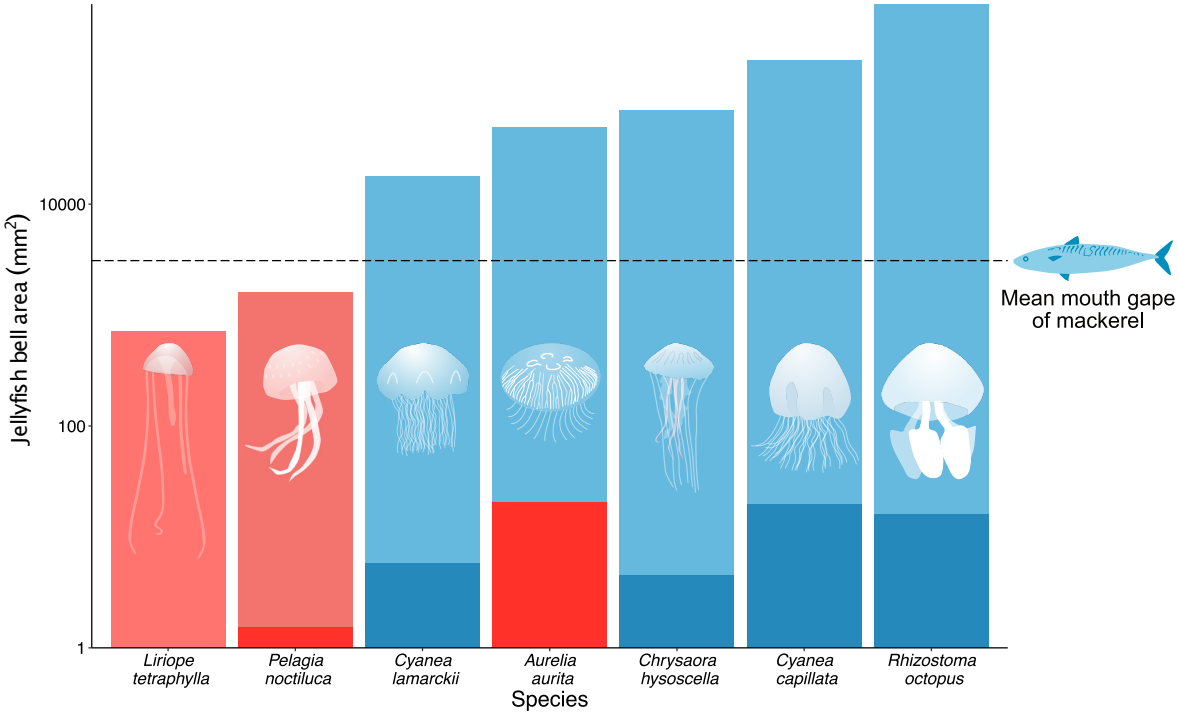

Figure 3.3. Jellyfish predation as a function of size. Bar graphs are overlaid, not stacked: the top of the graph details the size of the medusae. Detection of predation (red) and non-detection (blue) are shown for medusae (light blue and red) and ephyrae (dark blue and red). Mean *Lirope tetrayphylla* size and moon jellyfish were taken from literature: [34] and [15] respectively. Mauve-stinger jellyfish ephyrae size is taken from [37]. All other mean medusae, and ephyrae, bell areas are reported in [14]. The mouth gape of mackerel was calculated using mean mackerel size in this study and a mackerel-specific allometric scaling function[38].

3.6 Conclusion

The data presented here show that, in contrast to early-season sampling, late-season predation is limited: mackerel were the only species to frequently feed on jellyfish, although rare predation was also detected in sardine and sprat. The type of jellyfish consumed also changed: the small hydrozoan species *L. tetraphylla* was the preferred prey item in October, accounting for 80.7% predation across all species. The shift from widespread predation of juvenile jellyfish to rare predation of adults suggests energy flows from jellyfish to fish stocks are dynamic throughout the year. Although jellyfish are not an energy-rich food item when compared to other components of the plankton[39], the high abundance in which they can occur suggests they could play a role in supporting a range of forage-fish populations during the winter. In late-season sampling, consumption of jellyfish is less frequently seen. Possible explanations for this shift are changes in jellyfish availability, escape of predation through somatic growth, or sea-specific phenomena. Collecting data on jellyfish populations throughout the

year in one location, in tandem with diet-sampling could elucidate which of these hypotheses, if any, are responsible for the observed predation patterns.

The jellyfish-specific assay used here reveals the presence of a trophic link, but is not well suited to quantifying energy flows. Consequently, it is difficult to say exactly how important jellyfish are in the diet mackerel. Techniques such as stable isotope analysis[6] could be used to quantify the energy flows between jellyfish and fish stocks. Additionally, high throughput sequencing with universal primers could reveal the broader context of diet: are jellyfish the only consumed prey or are they part of a generalist diet? Future research could use combination of both techniques to quantify jellyfish–fish trophic links.

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Chapter 4: How quantitative is metabarcoding: a meta-analytical approach.

4.1 Abstract

Metabarcoding has been used in a range of ecological applications such as taxonomic assignment, dietary analysis, and the analysis of environmental DNA. However, after a decade of use in these applications there is little consensus on the extent to which proportions of reads generated corresponds to the original proportions of species in a community. To quantify our current understanding we conducted a structured review and meta-analysis. The analysis suggests that a weak quantitative relationship may exist between the biomass and sequences produced (slope = 0.52 ±0.34, $p<0.01$), albeit with a large degree of uncertainty. None of the tested moderators: sequencing platform type, the number of species used in a trial, or the source of DNA were able to explain the variance. Our current understanding of the factors affecting the quantitative performance of metabarcoding is still limited: additional research is required before metabarcoding can be confidently utilised for quantitative applications. Until then, we advocate the inclusion of mock communities when metabarcoding as this facilitates direct assessment of the quantitative ability of any given study.

4.2 Introduction

Metabarcoding, the use of a polymerase chain reaction (PCR) and high throughput sequencing (HTS) to characterise organisms present in a sample, has been used to address an array of ecological questions [1] (PCR-free sequencing is an emerging technology [2,3] but is not the focus of this analysis). For example, metabarcoding has allowed the taxonomic identification of many specimens simultaneously using a standardised DNA region [4] without the need for on-the-ground taxonomic expertise.

Similarly, environmental DNA (eDNA) studies, which sequence DNA in soil and water [5] without first isolating any organisms, facilitate rapid biodiversity monitoring with only small sediment or water samples. Metabarcoding has also played an important role in uncovering diets and resolving food webs [6], as well as reconstructing community dynamics temporally using ancient DNA preserved in sedimentary layers [7].

Early adopters of metabarcoding were hopeful that outputs would be quantitative, *i.e* that reads obtained from a sequencing run would correlate with biomass in the original sample [8] in a similar manner to other applications such as RNA sequence analysis [9] and the characterisation of microbial communities (where it is referred to as meta-genomics). However, several factors, detailed in figure 4.1, can introduce bias into the results and yield inaccurate biomass estimates. Yet, despite these factors being well documented, after more than a decade of use there is no clear consensus as to what extent metabarcoding is quantitative. Many studies report their findings in a quantitative manner where the relative read abundance (RRA) [10] is interpreted as the relative abundance of biomass[11–14]. Others use a frequency of occurrence (FOO) approach, also referred to as weighted occurrence [10], where the proportion of samples in which a given sequence was detected is used to infer a different sort of quantitative measure[15,16]. It is also common to incorporate a qualitative approach (detected / not detected), sometimes simply referred to as occurrence [10] or a 'species list', alongside these quantitative approaches [17].

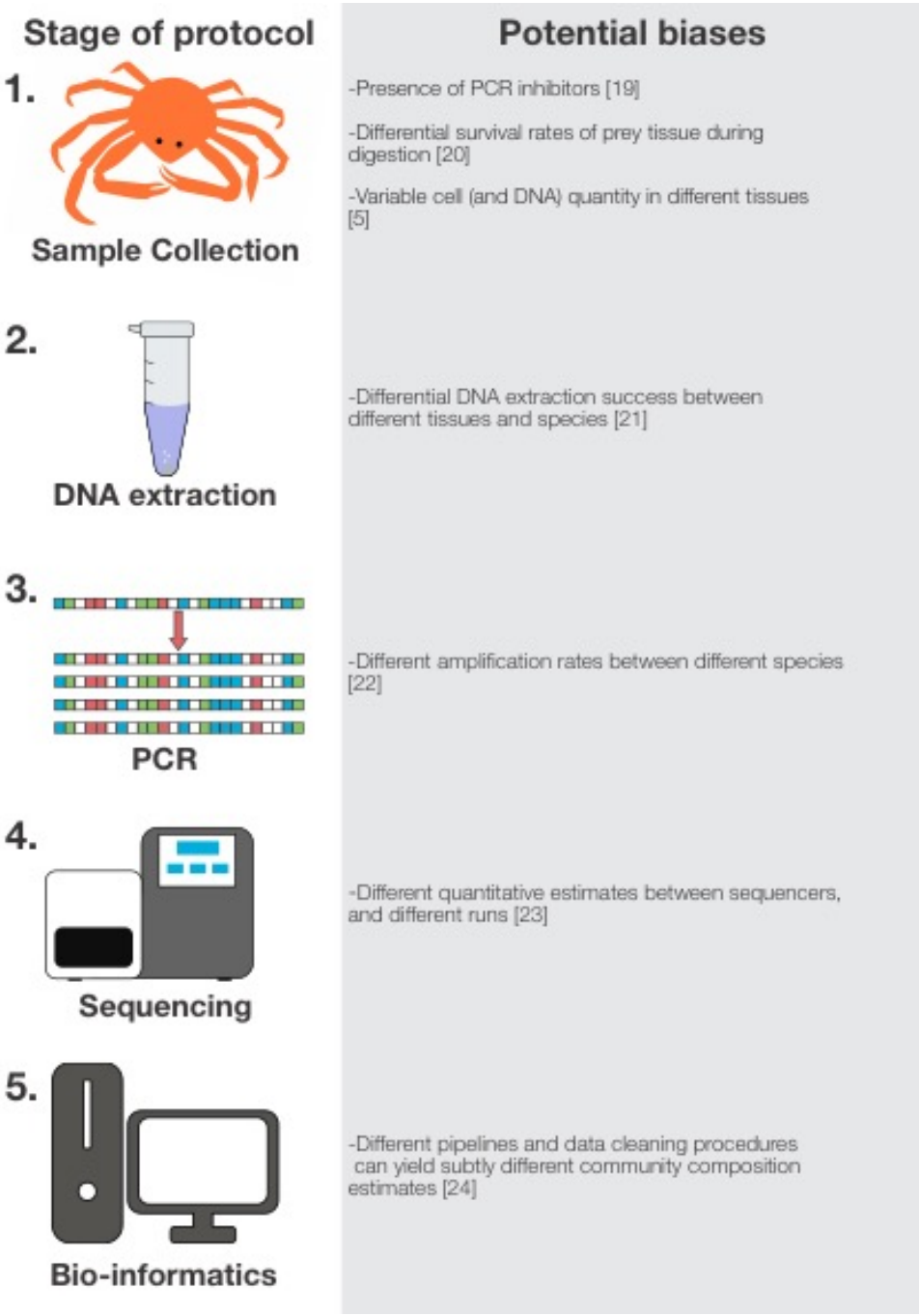

Figure 4.1) Overview of HTS procedure, and factors that can influence the quantitative output[6,18–23].

Empirically determining the extent to which metabarcoding is quantitative should be relatively simple: take a mixture of organisms with known biomass, PCR and sequence, then compare the results of the HTS run to the original biomass of each community member. Indeed, many studies have used this approach [24,25]. However, often only one primer set is used and the output may be a result of primer bias (the differential amplification of target DNA due to different numbers of nucleotide mismatches between the primer and target DNA between samples) rather than a reflection of the ability of metabarcoding techniques. Even if multiple primers are used,

they are normally used on the same sequencing run, in which case results cannot be considered independent. An experiment featuring enough sequencing runs to gather sufficient statistical power to disentangle the various factors that may affect quantitative performance would be prohibitively expensive for most research groups. Consequently, there is an *ad hoc* collection of methodologies that provide different levels of quantitative performance, but little certainty as to whether the variance is due to unique parameters in the experimental set up or a result of more general drivers.

In this study, we aim to address this knowledge gap. A structured review was conducted to collate our knowledge about the extent to which metabarcoding for taxonomic assignment is quantitative. Subsequently a meta-analysis was conducted to investigate the degree to which metabarcoding is quantitative across multiple independent studies. Factors affecting the quantitative performance such as platform choice, the experimental set up (does using biomass, individuals, or DNA as the input unit affect quantitative estimates?) and the number of species incorporated in a study were also investigated. Factors that could not be addressed are also discussed to direct future research.

4.3 Methods

4.3.1 Search strategy

Articles that used quantified multi-species assemblages, PCR, and HTS platforms for taxonomic assignment with metabarcoding were targeted using specific search terms. Identifying optimised search terms was important since metabarcoding is now widely used across evolutionary, ecological, and medical research. After assessing a variety of search terms an appropriate combination was finalised: the Web of Science was searched on 31/10/2017 for English language articles for all available years using the following search terms: ((quant* OR diet OR biomass) AND (barcod* OR metabarcod*)). In total, 1262 articles were retrieved.

4.3.2 Article screening

Initial filtering of the articles was based on their titles: any articles that obviously had no relevance to quantification of biomass using metabarcoding were discarded. After

initial filtering 262 articles remained. These articles were manually inspected and any that included a quantified community of biomass, individuals, or DNA as starting material and reported the proportion of reads obtained from a HTS platform were used for data extraction. Since the slope of a fitted linear model was to be used as an effect-size (see below), variation in the amount of input material was also required (equal amount of starting biomass could not be used). In total, 22 articles (table 4/1) were used in the meta-analysis.

Table 4.1. Articles that were included in the meta-analysis.

Author	Species per trial	Sequencer	Starting material	Organisms	Marker
Albaina, Aguirre, Abad, Santos, & Estonba (2016)	6	454	Biomass	Marine invertebrates (Crustaceans, Annelids)	18s
Blanckenhorn, Rohner, Bernasconi, Haugstetter, & Buser (2016)	4 to 9	Illumina	Biomass	Macroinvertebrates (Coleoptera, Diptera, Hymenoptera)	COI
(Bokulich & Mills, 2013)	12	Illumina	DNA/RNA	Yeast	ITS
(Deagle, Chiaradia, McInnes, & Jarman, 2010)	3	454	Biomass & Faecal	Fish	16s
(Diaz-Real, Serrano, Piriz, & Jovani, 2015)	3	454	Individuals	Feather mites	COI
(Egge et al., 2013)	11	454	DNA/RNA	Haptophytes	18s
(Elbrecht & Leese, 2015)	52	Illumina	Biomass	Macroinvertebrates (freshwater)	COI
(Elbrecht et al., 2016)	52	Illumina	Individuals	Macroinvertebrates (freshwater)	COI
(Elbrecht et al., 2017)	52	Illumina	Biomass	Macroinvertebrates (freshwater)	16s
(Geisen, Laros, Vizcaíno, Bonkowski, & De Groot, 2015)	8	454	Individuals	Protist culture	18s
(Hatzenbuhler, Kelly, Martinson, Okum, & Pilgrim, 2017)	5	454	Biomass	Fish	COI
(Hirai et al., 2015)	33	454	Biomass	Copepods	LSU
(Iwanowicz et al., 2016)	12	Illumina	DNA/RNA	Plants	ITS
(Klymus, Marshall, & Stepien, 2017)	11	Illumina	DNA/RNA	Bivalves, Gastropods	16s
(Kraaijeveld et al., 2015)	6 to 11	Ion Torrent	Individuals	Plants (pollen)	TrnL
(Pochon, Bott, Smith, & Wood, 2013)	9	454	DNA/RNA	Marine invertebrates (Echinoderms, crustacenas, ascidians , molluscs, annelids)	18s
(Porazinska et al., 2010)	38	454	Individuals	Nematodes	18s
(Rocchi, Valot, Reboux, & Millon, 2017)	9	Illumina	DNA/RNA	Fungus	ITS2
(Saitoh et al., 2016)	9	454	Biomass	Macroinvertebrates (springtails)	16s, COI
(Smith, Kohli, Murray, & Rhodes, 2017)	10	Illumina	Individuals	Dinoflagellates	Cyt b, LSU, 18s
(Thielecke et al., 2017)	5	Illumina	DNA/RNA	Plasmid contructs	n/a
(Thomas, Deagle, Eveson, Harsch, & Trites, 2016)	3	Illumina	Biomass	Fish	16s

4.3.3 Data extraction

The composition of the community assessed (either biomass, number of individuals, or concentration of DNA) and the proportions of reads corresponding to the relevant species in the test community obtained from the sequencing platform were recorded for each trial within an experiment. The sequencing platform, number of species used, and the source of input material for each trial within any given study was recorded. The main manuscript and supplementary information were inspected: if possible data were taken from a table, if tables were unavailable the data were manually extracted from figures using *Web Plot Digitizer* [26]. If data were not presented in the main article, the corresponding author was emailed to obtain the data.

The composition of the mock community, and corresponding sequence data were converted in percentage values (see figure 4.2 (a)). For the Elbrecht et al. (2017) study using individuals of varying sizes [27], the composition of individuals in the mock community, and the output of reads, was presented grouped by size (large, medium, and small individuals) and unsorted. In this instance, we calculated input and output percentages by the sorted size groupings as this was most similar to the approaches used in other included studies.

Slope is a commonly used effect size when the relationship between two continuous variables is being investigated [28]. In this instance, it was chosen as it is easy to interpret and meets the statistical assumptions of the meta-analysis model without transformation (in this instance because slopes did not approach vertical asymptotes and little skewness was present in the data).

4.3.4 Meta-analysis model fitting

Slope (the effect size) was calculated by fitting a linear model for each trial detected in the review using *R* [29], such that the proportion of reads produced from the sequencing run would be a function of the proportion of starting material in the experiment. The variance of the slope was calculated in R, and used as the sampling variance as described by Rosenberg, Rothstein, & Gurevitch (2013). Figure 4.2 illustrates how the results of a mock community experiment are incorporated into this analysis.

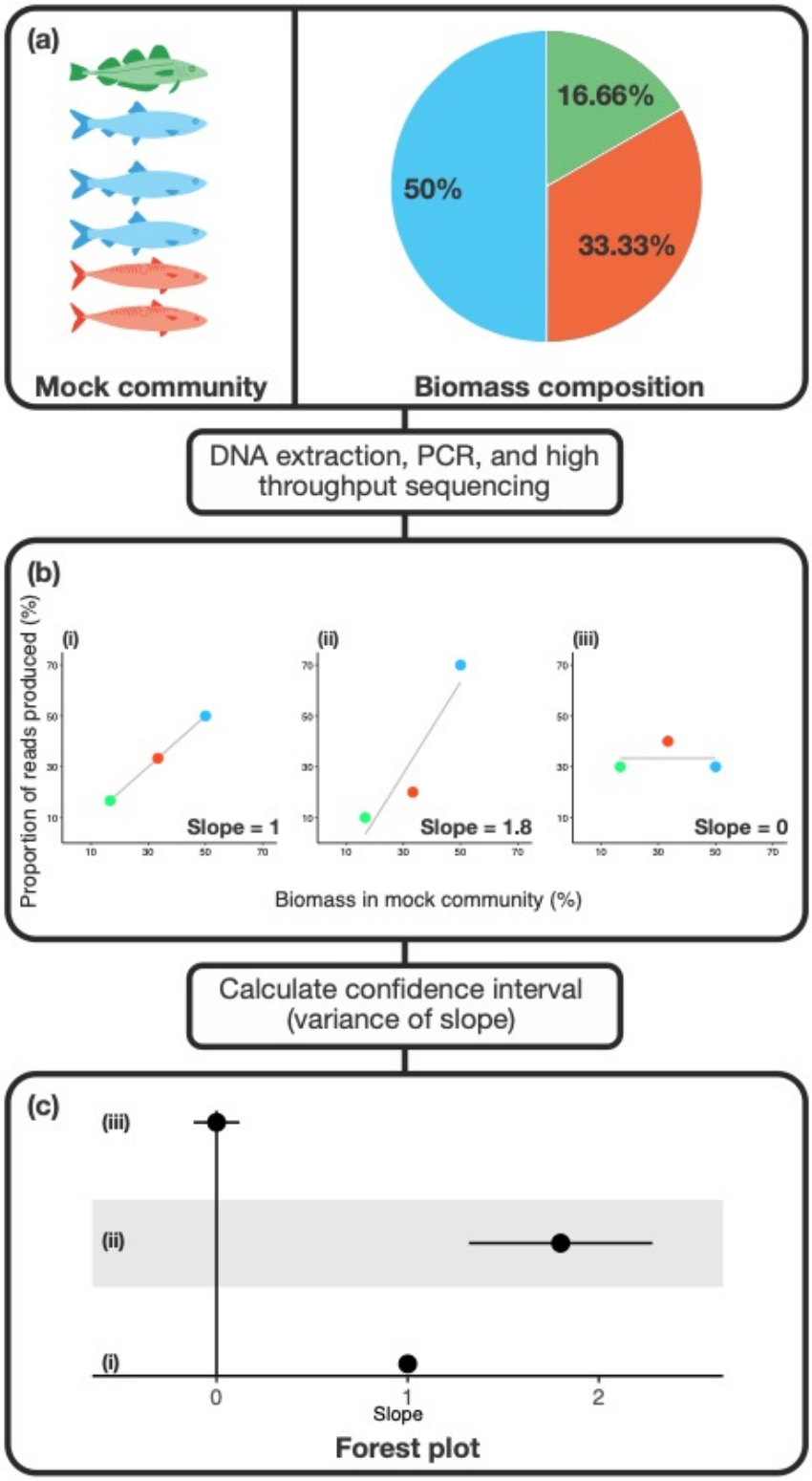

Figure 4.2. A schematic illustrating how data are utilised in the meta-analysis. (a) The mock community with quantified biomass. (b) three hypothetical outcomes of the metabarcoding step: (i) a perfect quantitative relationship between biomass and sequencing yield i.e. a 1% increase in biomass yields a 1% increase in reads, generating a slope = 1 (ii) a quantitative signal in which rank abundance is same in the mock community, but with over-representation of common sequences and under-representation of rare sequences resulting in a slope greater than 1. A slope of between 0 and 1 would be produced when common sequences are under-represented and rare sequences over-represented (not shown). (iii) no quantitative information, with a slope close to 0. Negative slopes would also be indicative of non-quantitative signals. (c) shows how (i),(ii), and (iii) would be visualised in a forest plot with corresponding variance of slope denoted by error bars.

All meta-analysis was conducted in the customisable, open-source, meta-analysis package 'metafor' [30] in R. Many studies used multiple trials within a single study, however, these trials cannot be treated as statistically independent from one another. To account for this non-independence, a cross-study slope-estimate was determined using a two-level nested random effects model using a restricted likelihood function. Trials within an experiment were nested at the study level. The influence of sequencing platform, and DNA source material, were tested by including them as moderating factors in the model. Terms were iteratively omitted from the model, and AIC was used to select the final model.

Weighting of each study in the meta-analysis model was determined solely by the number of sequencing runs used in each study (e.g. 1 for 1 run, 2 for 2 runs etc). However, when multiple trials were conducted within a single study, the weight of each trial was calculated by dividing the number of reads produced for the *trial* by the total number of sequences produced by the sequencing run within the study. This allows different sequencing depths within a single study to be accounted for (using a nested model) whilst maintaining sequencing runs as independent data points. For example in Saitoh *et al.* (2016) a single sequencing run was used and a meta-analysis model study weight of one was assigned. Within this study, there were two trials: the 16s *trial* produced 45% of the reads, therefore it accounted for 45% of model weight within the nested model (at the study level).

4.3.5 Sensitivity testing

Assessing publication biases (the increased probability of positive results being accepted for publication) in meta-analytical models is challenging for nested-models: Funnel plots are difficult to interpret: studies cluster together due to statistical dependencies rather than genuine biases [31]. Egger's regression test [32], another commonly used metric, is not supported for nested-models in the current version of metafor. Consequently, it was not possible to assess if publication bias may be present in the data set. However, influential trials in the meta-analysis were visually identified using hat values, which show the importance of any given trial in relation to the model as a whole [33], plotted against the standardised residuals of the meta-analysis model.

4.4 Results

Across all studies a significant ($p<0.01$) relationship existed between the proportion of input material for each species present and the proportions of sequences obtained from metabarcoding. A large amount of observed variation was due to actual differences in the inter-study slope estimate ($I^2 = 88.5\%$). Across all studies, an effect size estimate (slope) of 0.52 (±0.34 variance of slope) was identified.

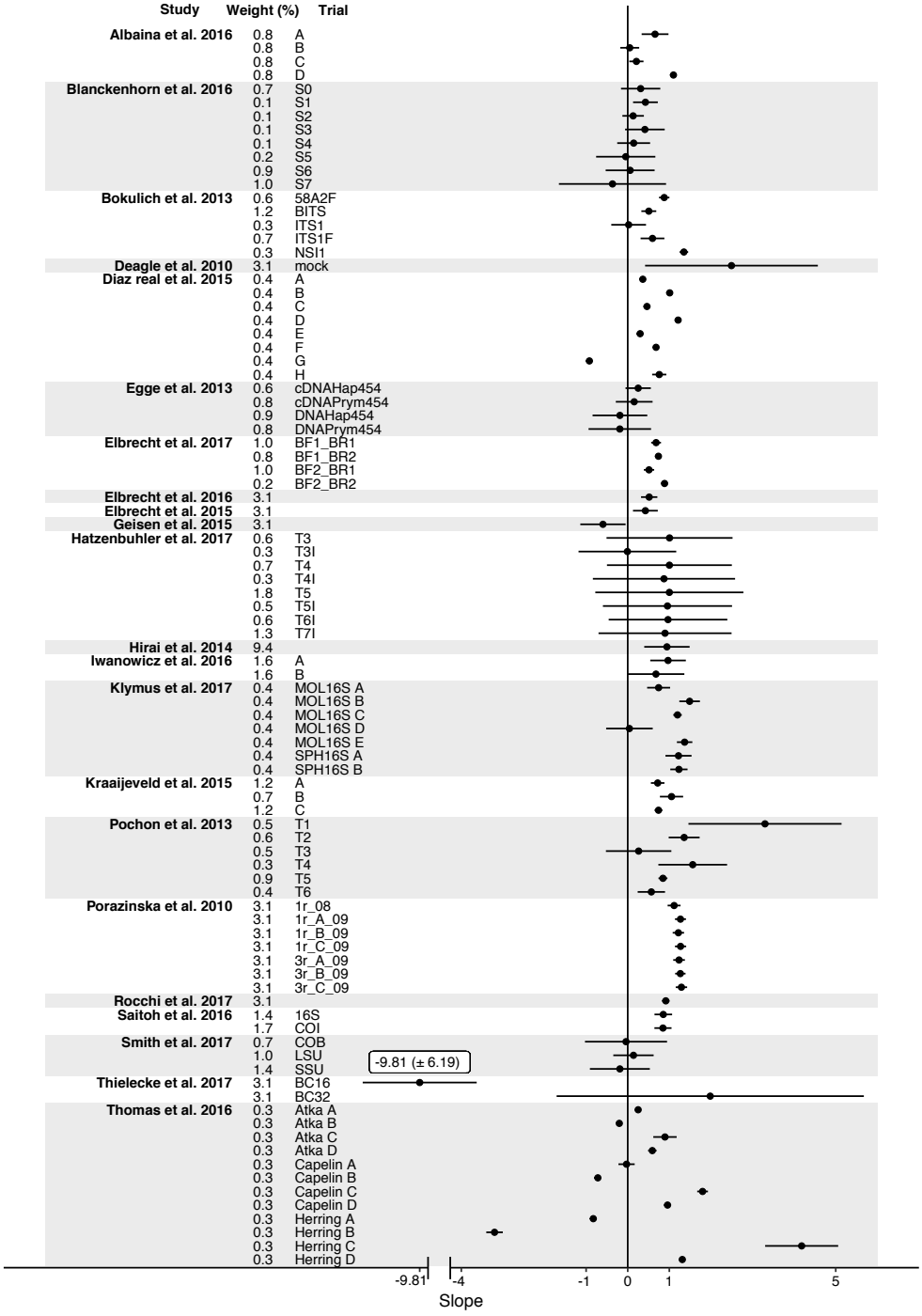

Figure 4.3. Forest plot showing the slope estimate for all trials in the meta-analysis (± 95 % confidence intervals). Trials are clustered at the paper level denoted by the grey and white shading.

None of the tested moderators: type of sequencer, number of species used in a trial, or type of starting material had a significant effect ($p>0.05$ in all instances) on the estimate provided by the meta-analysis model. Figure 4.4 illustrates the lack of difference in quantitative ability (a) between the materials used for meta-barcoding, (b) among the sequencers, and (c) the number of species used in a trial.

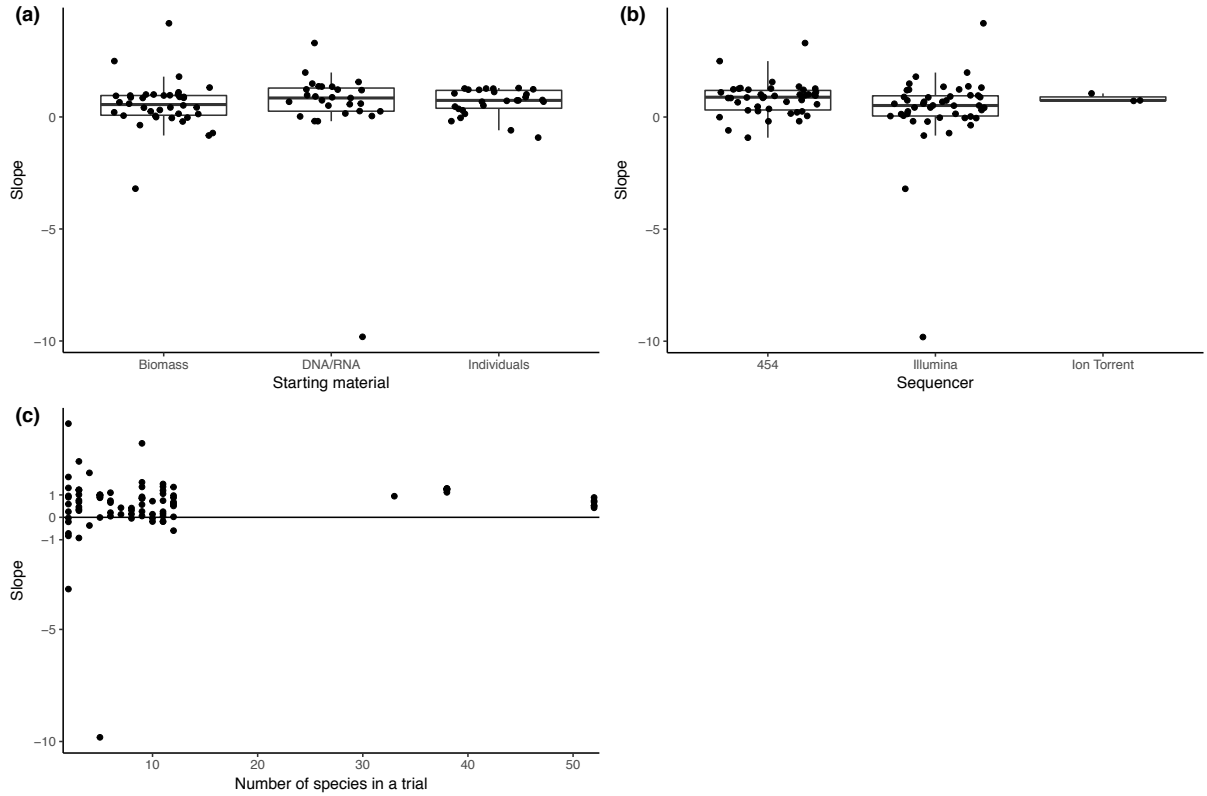

Figure 4.4. The quantitative ability of metabarcoding using (a) various starting materials, (b) different sequencing platforms, and (c) different number of species within in a trial. Note that each point represents a trial, which may not be fully independent from one another. However, this non-independence is accounted for in the meta-analysis model.

Sensitivity testing, using hat values and residuals (figure 4.5) appear to show a single trial [34] was having a large influence on the final output of the model. However three sequencing runs were used for a single trial in this study, and as such it has a relatively greater weight in the meta-analysis compared to most other trials that only used a single sequencing run.

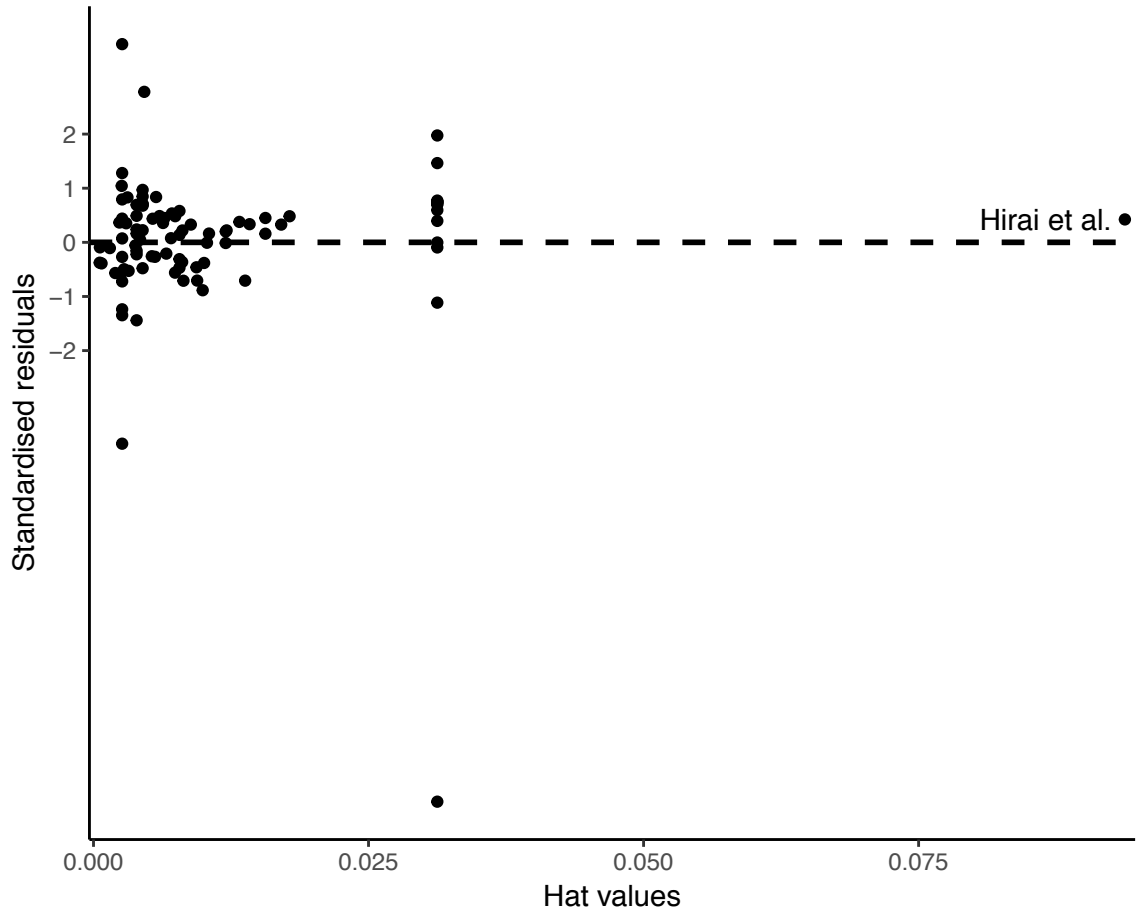

Figure 4.5. Hat values of trials included within the meta-analysis (a measure of influence on the meta-analysis model plotted against standardised residuals. Outlying trials are labelled. Note the points correspond to trial-level influence, not study level influence.

4.5 Discussion

4.5.1 How quantitative is DNA metabarcoding?

Across all studies, a slope estimate of 0.52 was identified as the relationship between biomass and sequence read number. This shows that the RRA produced from a metabarcoding loosely corresponds to the relative occurrence of species in the starting material. If no data about composition of a sampled community exists, metabarcoding data interpreted quantitatively could therefore be more informative than treating it in a strictly detected / not-detected manner even if the accuracy is low. This supports evidence from simulations presented in Deagle *et al.* (2018), which suggest that a more accurate interpretation of communities can be achieved by treating metabarcoding data quantitatively rather than relying solely on qualitative measures. However, this estimate has a large degree of uncertainty: ±0.34 variance of slope suggests that in real world applications metabarcoding can be either somewhat quantitative, or

produce a very weak signal. This uncertainty is reflected within some of the experiments themselves: figure 4.3 shows that while many of the included trials appeared to produce quantitative results, their variance of slopes were sufficiently large, overlapping with 0, that a non-trivial probability exists that non-quantitative data will be produced on any given sequencing run. Furthermore, there are several trials included in this meta-analysis in which metabarcoding produced extremely poor quantitative performance. With such variation between studies, and no easy way to diagnose whether any given metabarcoding study has produced quantitative results, it is easy to see how different opinions on the quantitative ability of metabarcoding has arisen. Focusing on the factors influencing the quantitative performance is essential to further clarify this situation.

The influence of sequencing technology, and initial experimental design were included as moderating factors in the initial model. The sequencing platforms did not significantly differ in quantitative ability. This was unexpected, as the different platforms have different technical approaches towards sequencing [35], and different levels of bias were expected. Additionally, Illumina platforms produce many more reads than other platforms, so a greater level of precision might have been expected. This is not to say platform choice is not important when undertaking a metabarcoding study: read length, sequencing accuracy, and cost will all play a role in determining the best choice for a given study. However, these results suggest that in terms of attaining quantitative data, any difference between sequencing technologies is too subtle to be detected in this meta-analysis, and the factors driving quantitative performance perhaps lie elsewhere in the experimental set up.

It has been hypothesised that including a higher number of species in a metabarcoding study will improve the quantitative performance as different amplification efficiencies will have diminishing effects on the overall quantitative performance as the number of species used increases [10,36]. However, this relationship was not detected here. This may be due to most of the included studies using relatively few species: only three studies had more than 30 species. Thus, the lack of relationship may be driven by lack of variation in the data. Additionally, it is expected that different primer sets, or other factors tested here, would explain much of the variation. Our ability to detect subtle trends in a noisy dataset is limited with relatively few studies. This relationship could

be better characterised with empirical studies, or if the amount of data available for meta-analysis were to increase substantially.

Different input materials had no explanatory ability in the final model: sequences were able to replicate the original biomass, quantity of DNA, or individuals in a study equally well. We believe this may be because counts of individuals were frequently used for species of similar size: if there is little variation in size of individuals between different species, count data can be regarded as a proxy for biomass. A notable exception, in using counts of individuals from species of similar sizes was the Elbrecht *et al.* 2017 study: here species were sorted by size prior to sequencing. The authors demonstrated that sorting individuals by size affected the quantitative ability of metabarcoding by comparing a mock community sorted by size, and a mock community where individual size was not considered. We used the sorted data treatment as this was most similar to other studies in the meta-analysis. However, given that counts of individuals and biomass were proxies in many studies, and empirical evidence suggests that the RRA does not correspond with the number of individuals if significant size differences are present [27] we would advocate caution when inferring count data from metabarcoding data without *a priori* knowledge of minimal size variation between individuals.

No difference in quantitative performance existed between studies using quantified DNA as a starting material, and those that used biomass. Given DNA extraction is the only step (figure 4.1) separating these points in the protocol, this suggests it is not a source of significant bias in the studies included in the meta-analysis. However, it must be noted that this is not always the case: Pornon *et al.* (2016) reported a 300-fold difference in DNA concentrations after extraction. It is possible that structural differences in the exine (the tough protective coating of pollen) may have driven the variable DNA yield. Although not a significant factor in this study, best practice would dictate that quantifying the relationship between biomass and DNA yield in the target organisms is advised prior to metabarcoding.

4.5.2 Future directions

This analysis has shed light onto some, but not all, of the factors that influence the quantitative performance of metabarcoding. Although not considered here, primer bias

is likely a large source of variation in the quantitative performance of metabarcoding studies: Piñol et al. (2015) empirically tested the relationship between primer mismatch and amplification efficiency and found mismatches accounted for 75% of variation. We had hoped to explore the effect of primer bias on the quantitative performance of metabarcoding by using the nucleotide pairwise diversity at the primer binding site of the mock community as a moderating factor in the final model. Unfortunately, this was not possible: the sequence in the target DNA at the primer site could not be inferred from the studies included in this meta-analysis as, at most, only the primer sequence can be obtained. For a number of studies, sequences covering the primer binding-region were not present in DNA databases. Additionally, even for those species which had relevant sequences, inter-individual variation was a concern: amplification-efficiency is very sensitive to both the type of nucleotide mismatch between the target DNA and the primer, and the location of the mismatches in the primer sequence [38]. Without knowing the actual sequence present in the individuals used in the studies, we opted to omit primer site mismatches from this analysis. However, the effect of nucleotide mismatches in primer sequences on quantitative performance of metabarcoding is explored in detail through the use of simulations in this issue [36]. This topic will be an ongoing research area, and until we accurately determine the quantitative performance of any given primer set we would advocate reporting all *in-silico* testing to assess the quantitative ability of primers, and the inclusion of a mock community control on each sequencing run to gauge how accurately RRA corresponds with the starting material.

4.5.3 Reflection on meta-analysis

It is important to remember what is entailed in a meta-analysis: a consensus of studies included in the analysis, weighted by sample size. Studies were included based on their detection in a structured review; although this presents a transparent, repeatable, way of including literature, our approach may have missed some relevant studies. Indeed, not all of the high-quality literature detected in the structured review was included [24,25], due to their experimental-design being incompatible with our analytical framework, rather than any shortcomings of the work or relevance to contribute further understanding on the topic.

It should be noted, that incorporating results into meta-analysis necessitates some loss of nuance in the results. Most notably, in this study, we used the slope derived from a linear model as an effect size to facilitate synthesis. However, the quantitative nature of the relationships reported in this analysis may well be more complex than reported by a linear model. As such, we would encourage readers to use this manuscript only as reference material, and assess the cited literature themselves, as a perfect distillation of included literature is inherently not possible.

Furthermore, publication bias remains an unknown factor. Using a nested-model to account for non-independence makes using most common tests for publication bias problematic as they detect the structure implemented in the model, not genuine publication bias. Not accounting for the non-independence of trials run on the same sequencing run was, we felt, a more immediate flaw than accounting for publication bias. That unfortunately leaves us in a position where the extent of any publication bias is unknown, and we are unable to say how important, or trivial, the issue may be: as such we reiterate that any synthesis drawn from this model may have been influenced by the omission of unpublished data, as much as the studies included.

Another issue worth considering is the relative weighting given to each study. Meta-analyses differ from a simple vote-count by assigning increased weighting to studies with a larger sample size. Here, weighting was assigned based on the number of sequencing runs used in a study. We feel this weighting is more appropriate than a simple vote count but it is worth highlighting the results presented here are influenced more heavily by some studies than others, e.g. Porazinska et al. (2010) had the greatest influence on the model (21.7%) due to the study's use of seven sequencing runs.

Finally, it should be noted this analysis quantifies the understanding of the field at a point in time rather than attempting to be a final point of authority. As highlighted above, much more research is still to be done in this area, and we hope the shortcomings and gaps highlighted will be filled as exciting new research reveals a more mechanistic understanding of this topic

4.6 Conclusion

Our meta-analysis suggests that metabarcoding possesses some quantitative ability: a cross-study slope-estimate of 0.52 was found, suggesting a weak quantitative signal is present, albeit with a large degree of uncertainty (±0.34 variance of slope). Quantitative ability did not appear to differ among sequencing platforms, the amount of species included in a trial, or with different starting materials: biomass, individuals, or DNA. We remain sceptical that individual count data can be reliably inferred from metabarcoding if there are large size differences between the individuals being assessed and would advise against count-based inferences without *a priori* knowledge of the community being assessed. All presented results have probably been influenced by the relatively small sample sizes: additional research is warranted to reveal the mechanistic factors driving quantitative performance. While metabarcoding may eventually become a quantitative tool, many uncertainties remain. Moving forward, we suggest explicitly testing the relationship between read abundance and input biomass using mock communities included as quantitative controls during metabarcoding. Not only will this allow researchers to assess their own study, but it will also assist future meta-analyses. We also recommend presenting all trials and simulations used in primer selection to make the rationale behind primer choice transparent. Finally, we would encourage additional empirical research into the mechanistic factors behind primer bias in metabarcoding since this is difficult to study using meta-analytical techniques, yet potentially holds the key to truly quantitative metabarcoding.

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Chapter 5: Putting jellyfish predation in perspective: pelagic fish diets revealed by DNA metabarcoding

5.1 Abstract

Consumption of hydrozoan and scyphozoan jellyfish, in different seasons, has been detected using molecular techniques in the Irish and Celtic Seas. However, very little context for this predation exists: it is unclear if fish are actively targeting jellyfish, or if they are part of their broader diet. To establish the role jellyfish play in the diet of fish we tested nine fish species opportunistically sampled from around the British Isles from August to September using two sets of universal primers and high throughput sequencing. Cnidarian, ctenophore, and chordate-jellyfish were detected in the stomach contents of fish. Hydrozoans appeared to be a frequently consumed jellyfish. Mackerel appeared to be the most frequent jellyfish predator, with approximately 40% of individuals possessing jellyfish-DNA in their stomachs. However, all tested species had jellyfish detected as part of a broad diet with no clear evidence of specialisation. By contrast with samples collected during winter, active scyphozoan predation by fishes during summer was rare, suggesting that as scyphozoans grow and mature, they play an increasingly minor role in fish diets.

5.2 Introduction

Jellyfish (defined here as cnidarians, ctenophores, and pelagic tunicates), are understudied relative to other marine ecosystems, yet are responsible for a variety of both positive[1] and negative effects [2] on human enterprise in marine ecosystems. Historically, few predators of jellyfish were known, and jellyfish were thought to divert nutrient flows away from the rest of the food web. However, recent evidence suggests that not only are they an integral part of marine food webs[3–5], but they may also act as a bridge between marine and terrestrial ecosystems[6,7]. In the Irish Sea, the study

conducted in chapter two established that predation was not limited to leatherback turtles (*Dermochelys coriacea*)[8] and sunfish (*Mola mola*)[4], but was a feature of many commercially-exploited species. A follow-up study in the Celtic Sea (chapter three) established that predation occurs throughout the year. However, the pattern of predation appeared dynamic: consumption was no longer widespread among predators but was concentrated in mackerel (*Scomber scombrus)*, and was directed toward smaller jellyfish species.

These recent findings suggest that, far from being purely a nuisance species, jellyfish may play some role in supporting Irish Sea fisheries. However, at present, only the relative frequency of jellyfish consumption per species is known. The quantity consumed, or the context in which jellyfish are consumed (generalist feeding or actively predated) remains unknown. Gaining insight in these areas is key step towards further understanding the trophic ecology of jellyfish and the functional role they play within the marine ecosystems.

Metabarcoding, the use of universal primers in a polymerase chain reaction (PCR) followed by high throughput sequencing (HTS), is an approach technology well-suited for in-depth dietary studies. Like all molecular-based dietary assays, this approach removes the need for visual identification of prey, and is therefore suited to working with soft-bodied, rapidly digested organisms such as jellyfish[9]. Additionally, online sequence databases such as GenBank[10] and BOLD[11] facilitate the study of a system without *a priori* knowledge of the diet[12]. However, unlike other techniques, HTS offers the ability to amplify, sequence, and identify many species in parallel[13], enabling the study of the whole diet of organism. Furthermore, HTS offers much greater sensitivity than Sanger sequencing-based approaches and is therefore better suited to detect rare feeding events. Jellyfish predation has been successfully studied using HTS before: Bowser *et al.* detected moon jellyfish predation by herring (*Clupea harengus*)[14], while McInnes *et al.* (2017) detected jellyfish in the diets of albatrosses.

In this study, two universal primers and HTS are used to investigate the context of adult jellyfish predation across nine common fish species with the aim of identifying which jellyfish are being consumed, and at what rates. Are jellyfish actively targeted, or are they part of a generalist diet? Obtaining these data is a vital step towards

understanding how jellyfish fit into the marine food web, supporting the ecosystem approach to fisheries management.

5.3 Methods

5.3.1 Field methods

Samples from 9 species were collated from four cruises (see table 5.1 and figure 5.1). The North Sea International Beam Trawl Survey (IBTS) (8 August – 6th September 2016), Eastern channel beam trawl (17th– 30th July 2016), and the Poseidon (3rd -21st October 2015) research cruises were collected aboard the RV Cefas Endeavour. Samples from the Celtic sea herring acoustic survey (CSHAS) (7th-27th October 2016) were collected aboard the RV Celtic explorer. Full details of biological sampling are detailed in the cited cruise reports. In brief, the Poseidon cruise[15] and CSHAS[16] used pelagic trawls to obtain fish for sampling, while a 4 m beam trawl and hybrid GOV trawl were used for the Eastern Channel[17] and North Sea IBTS[18] research cruises respectively. On the Poseidon cruise stomachs from samples were removed on board, and frozen at -20°C. Gloves and scalpels were changed between species, and cutting boards cleaned using fresh water between species to limit contamination. On the other cruises, samples were frozen whole at -20°C and had DNA extracted at a later date in the molecular labs based at the University of East Anglia.

Table 5.1. Species, sample size, and cruises samples from which samples were collected.

Species	Sample size	Cruise
Grey gurnard	15	Eastern Channel Beam Trawl, North Sea IBTS
Haddock	15	North Sea IBTS
Herring	36	CSHAS, North Sea IBTS
Horse mackerel	15	Eastern Channel Beam Trawl, North Sea IBTS
Lemon sole	16	Eastern Channel Beam Trawl
Mackerel	38	Poseidon
Plaice	15	Eastern Channel Beam Trawl
Saithe	10	North Sea IBTS
Whiting	28	Eastern Channel Beam Trawl, North Sea IBTS

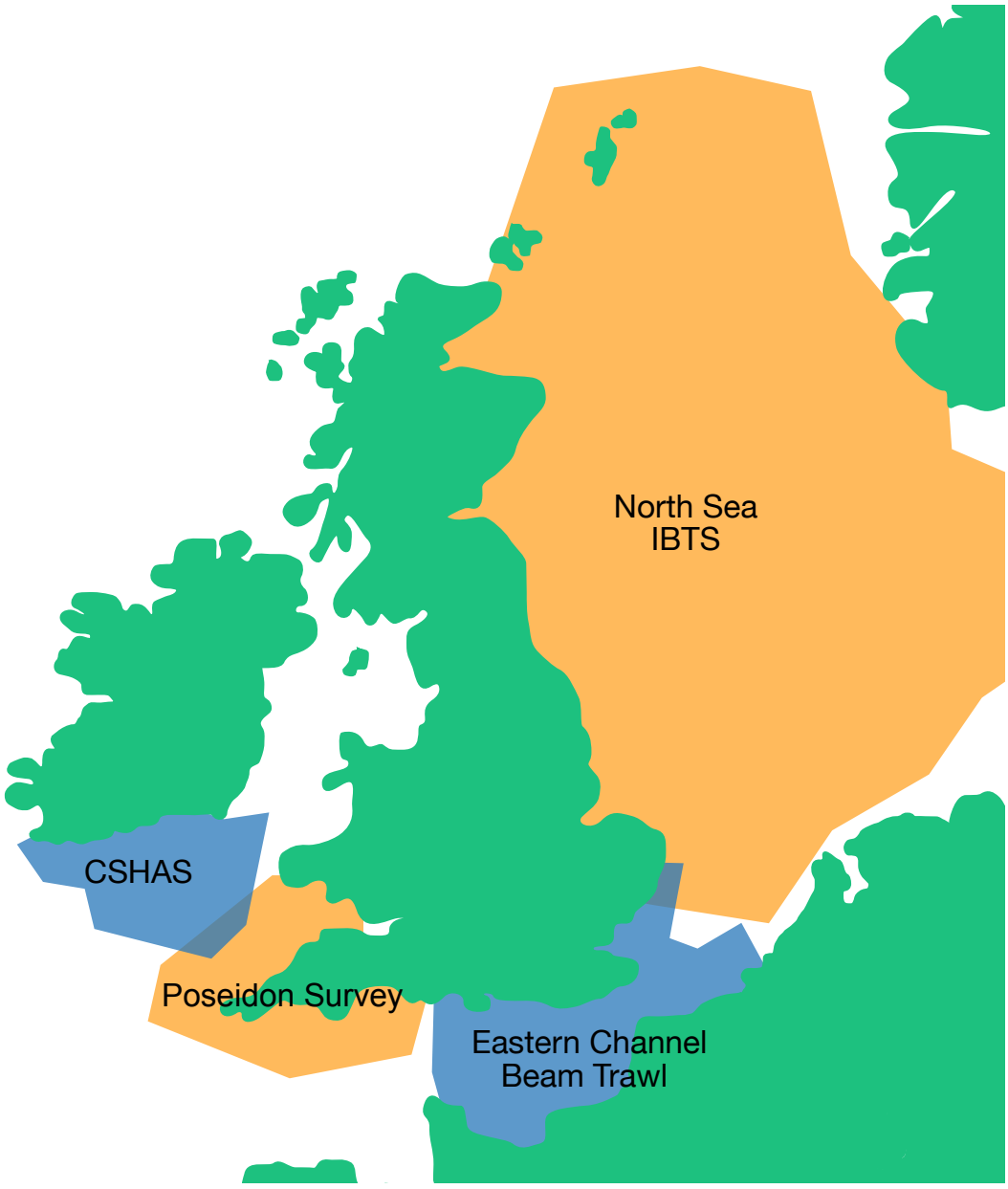

Figure 5.1. Location of surveys from which samples were obtained.

5.3.2 Salt DNA extraction

Samples from the Poseidon research cruise had DNA extracted using a salting-out technique[19]. Stomachs were thawed, and contents removed. Each stomach was processed on a separate paper towel that was discarded after dissection. Dissection tools were sterilised between samples by heating them by brief immersion in 70% ethanol and then flaming in a Bunsen burner. Stomach contents were macerated and a small quantity (1- 8 mm^3) added to 300 µl digestion buffer (30 mM Tris-HCl ph 8.0, 10mM EDTA, 1% SDS, with 10 µl Proteinase-K (Qiagen)) in a 1.5 ml Eppendorf tube then incubated overnight at 55°C. One hundred µl of NaCl was pipetted into each sample, before being centrifuged for 5 minutes at 13000 rpm. Two hundred and fifty µl supernatant was transferred to a separate Eppendorf tube. Five hundred µl ice-cold 100% ethanol was added to the supernatant, before being placed in a -20°C freezer overnight. The samples were spun at 13000 rpm for 30 minutes in a centrifuge and ethanol was discarded. The DNA pellet was cleaned with 1 ml 70% ethanol, prior to centrifugation at 13000 rpm for 5 minutes. The pellet was then dried for 20 minutes in an incubator set at 50°C, before resuspension in 200 µl molecular grade water added and incubated at 37°C for an additional 30 minutes to facilitate DNA entering solution.

5.3.3 CTAB DNA extraction

Samples from the North Sea IBTS survey, Eastern Beam trawl survey, and CSHAS had DNA extracted using a CTAB methodology[20]. CTAB was preferred to salt extraction, as it is better able to extract DNA from stomachs with PCR inhibitory substances. Samples were partially-thawed such that the sample could be dissected, but the stomach content was still frozen: minimising DNA degradation time. The cutting boards the samples were dissected on were disinfected with 0.5% Sodium hypochlorite and thoroughly cleaned with running distilled water between samples. Dissection tools were sterilised by flaming with 70% ethanol. Stomach contents were extracted, macerated, and a small amount (1-8 mm^3) placed into 1.5 ml Eppendorf tubes containing 400 µl 2X CTAB buffer (100 mM Tris-HCl (pH 8.0); 1.4 M NaCl; 20 mM EDTA; 2% CTAB; 2% PVP-40; 0.2% mercaptoethanol) and 20 µl Proteinase-K (Qiagen). Negative controls were generated by dipping the dissection equipment in the CTAB buffer prior to DNA extraction to identify any lab contamination. The eppendorfs were inverted for 1 minute in a tube rotator, prior to incubation at 59°C overnight.

Samples were cooled outside the incubator for 20 minutes. In a fume hood, 400 µl Phenol : Chloroform : Isoamyl alcohol (24:24:1) was pipetted into each Eppendorf, followed by 4 minutes of inversion in a tube rotator. 300 µl of supernatant was transferred to a new Eppendorf, along with 300 µl of Phenol : Chloroform : Isoamyl alcohol (24:24:1) before being inverted for a further 4 minutes in a tube rotator. The Chloroform: supernatant boundary was visually inspected for proteins (a layer of white viscous liquid). If no white liquid was observed the supernatant was transferred to a new tube, if proteins were still visible an additional cleaning step took place: 250 µl of supernatant was again transferred to a new Eppendorf, along with 250 µl of Phenol : Chloroform : Isoamyl alcohol (24:24:1) before another 4 minutes inversion. 200 µl of supernatant was transferred to a fresh Eppendorf tube.

2x volume of ice-cold 100% Ethanol (stored at -20°C) was added to the supernatant, and inverted in a tube rotator for 3 minutes. The mixture was then cooled at -20°C overnight. The eppendorfs were centrifuged for 30 minutes at 13,000rpm before the ethanol was discarded. The remaining DNA was cleaned with 1 ml 70% ethanol, prior to 5 minutes in a desktop centrifuge for 5 minutes. The pellet was then dried for 20 minutes in an incubator set at 50°C, then 200 µl molecular grade water was added and the sample incubated at 37°C for an additional 30 minutes.

5.3.4 1st Round PCR

PCR was performed in two separate batches for both an 18s and COI set of universal primers (table 5.2) that had been adapted to work with metabarcoding indexes developed by Bista *et al*[21].

Table 5.2. First round 18s and COI primers, including metabarcoding sequences

Primer	Sequence	Reference
ILF_ProSSU3'F_1 (18s forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNCACCGCCCGTCGCWMCTACCG	[22]
ILR_SSU3'R (18s reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCACCTACGGAAACCTTGTTACG	
Uni-MinibarF1 (COI forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTCCACTAATCACAARGATATTGGTAC	[23]
Uni-MinibarR1 (COI reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAAATCATAATGAAGGCATGAGC	

PCRs were conducted in 20 µl reactions containing 10 µl 2x Q5 Hot Start High-Fidelity Master Mix (New England Biolabs), 0.4 µl forward primer (10 µM ; IDT Ultramer DNA Oligos), 0.4 µl reverse primer (IDT Ultramer DNA oligos), 7.4 µl molecular grade water, 1 µl BSA (20 mg ml−1; New England Biolabs), and 1 µl DNA.

Cycling conditions for the 18s primers were as follows: 98°C for 5 minutes, followed by 20 cycles of 98°C for 5 seconds, 67°C for 20 seconds, 72°C for 20 seconds, finishing with 1 minute incubation at 72°C. Cycling conditions for COI primers were as follows: 95°C for 2 minutes, followed by 5 cycles of 95°C for 1 minute, 46°C for 1 minute, 72°C for 30 seconds, then a further 20 cycles of 95°C for 1 minute, 53°C for 1 minute, 72°C for 30 seconds, with a final incubation of 72°C for 5 minutes.

Cleaning took place by mixing 20 µl of Agencourt AMPure XP cleaning beads (Beckman Coulter) with 20 µl PCR product and pipette mixing 20 times. The mixture was incubated at room temperature for 5 minutes to maximise DNA yield. The mixture was then spun down in a plate centrifuge, and placed on a magnet plate for 5 minutes to separate the cleaning beads from solution. Approximately 32 µl solution was aspirated and discarded. The remaining beads had 3 rounds of ethanol washing: 200 µl of 80% ethanol added, incubated at room temperature for 30 seconds, then aspirated and discarded. The beads were incubated at room temperature until ethanol evaporated, then 25 µl molecular grade water added. The solution was then incubated at 37°C for 30 minutes for the DNA to enter solution. Finally, the plate was placed on a magnet plate, and 15 µl of solution from each sample was transferred to a new plate.

5.3.5 2nd Round PCR & Sequencing

Final library prep and sequencing took place at Sheffield diagnostic genetics service. A 2nd PCR was performed to attach a unique nucleotide index (table 5.3) for each sample.

Table 5.3. Second round primers used to attach a unique nucleotide sequence (index) onto each samples sequences

Primer	Sequence	Reference
Round 2 forward primer	AATGATACGGCGACCACCGAGATCTACAC - i5 Index - ACACTCTTTCCCTACACGACGCTC	[21]
Round 2 reverse primer	CAAGCAGAAGACGGCATACGAGAT - i7 Index – GTGACTGGAGTTCAGACGTGTGCTC	

PCRs were conducted in 25 µl reactions containing 12.5 µl 2x Q5 Hot Start High-Fidelity Master Mix (New England Biolabs), 0.5 µl forward primer (10 µM ; IDT Ultramer DNA Oligos), 0.5 µl reverse primer (IDT Ultramer DNA oligos), 6.4 µl molecular grade water, and 1 µl cleaned round 1 PCR product. Cycling conditions were as follows: 98°C for 3 minutes, followed by 15 cycles of 98°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, finishing with a 5 minute incubation at 72°C. The duplicate PCR products were pooled and cleaned with Agencourt AMPure XP cleaning beads (Beckman Coulter) as described in PCR round 1. Using a Qubit dsDNA BR assay kit, the amplicon library was diluted to 3 ng μl^{-1} using molecular grade water as needed. If samples had undetectable quantities of DNA, they were pooled with the library. The amplicon library was then sequenced using an Illumina MiSeq (2x 150bp).

5.3.6 Bioinformatics

Sequences were automatically demultiplexed using MiSeq Reporter software (v2.5.1). The COI and 18s amplicons were then split into separate files using a custom UNIX script by identifying the round one primers. Although the COI and 18s amplicons used the same pipeline, from this point forward they were processed independently. Round one primers were trimmed using Cutadapt (v1.15)[24], and imported into DADA2 [25]. Sequences were processed to keep Q-scores above 35: 18s sequences were truncated at 121 bp and 76 bp, COI sequences were truncated at 117 bp and 78 bp, on the forward and reverse sequences respectively. The DADA2 parametric error model was trained on trimmed data before reads were dereplicated into unique sequences, paired reads merged, operational taxonomic units (OTUs) clustered, and chimera detection and removal performed. An OTU table was constructed to import the data into the QIIME2[26,27].

Separate 18s and COI databases for taxonomic assignment of OTUs were constructed. COI sequences were downloaded from BOLD[11] with PrimerMiner[28]. Taxonomy files were downloaded directly from BOLD. Eukaryotic 18s sequences, and associated taxonomic information excluding environmental samples, were downloaded from GenBank[10]. The downloaded sequences were dereplicated using the ObiUniq feature in Obitools (v1.01)[29] leaving only unique sequences in the dataset. Sequences were cross referenced against the taxonomy files using a custom

python script, and only those with taxonomic information retained. The taxonomy files were then trimmed using another custom python script to only contain information on the sequences remaining in the associated fasta files. The sequence and taxonomy files were combined into a QIIME2 database artefact inside the QIIME2 environment.

Taxonomy was assigned inside the QIIME2 environment using consensus-blast[30] and the local databases, retaining only matches with over 90% match identity. Rarefaction curves and a result table, with sequences reported to taxonomic order were also generated in QIIME2. Predator sequences, those not belonging to marine organisms (Supplementary table 5.1), those belonging to Siluriformes (as these are used as a model species in the same lab as where DNA extraction took place), and rare sequences (less than 10 sequences) were removed. Sequences which occurred in negative controls were removed from predatory species' sequences to minimise the chances of type II errors. To normalise sampling effort between individuals (individuals with high sequencing depth have a greater chance of a richer reported diversity), taxonomic groups that constituted less than 1% of an individual's total reads were removed[31]. This normalisation between samples also prevents the over-representation of very rare sequences, and sequences present due to secondary predation, in the final dataset. Data were transformed from number of sequences to detected / not detected.

5.3.7 Statistics

All statistics were carried out in R (v 3.5.0)[32]. Diet, as described by both 18s and COI amplicons, was plotted using metacodeR[33] to show the relative frequency of prey items, and visualise the overall taxonomic diversity of the predator's diet . Non-metric multidimensional scaling (NDMS) was carried out in vegan[34]. A Morisita-Horn index[35] was used to analyse diet similarity treating each predatory species as a group using count data (sum of detected / non-detected sequences).

5.4 Results

5.4.1 PCR and sequencing success

The 18s amplicon sequencing was largely successful: 187 samples, and 3 negative controls produced 5,398,140 reads. Despite the primers being optimised for invertebrate amplification, vertebrate species also amplified: predator sequences ranged from 47.5% (±5.7% SE) of total reads in whiting, to 80% (±8.4% SE) in horse mackerel (figure 5.2). By contrast the COI amplicons yielded 1,329,123 reads from 111 samples and 1 negative control. The number of predator reads in the COI dataset was much more variable, although this must be interpreted considering the smaller sample sizes achieved with COI sequencing: a low of 23.0% (±12.1% SE) was seen in haddock, while 93.6% (± 3.6 % SE) stomach DNA belonged to the host in herring (figure 5.2).

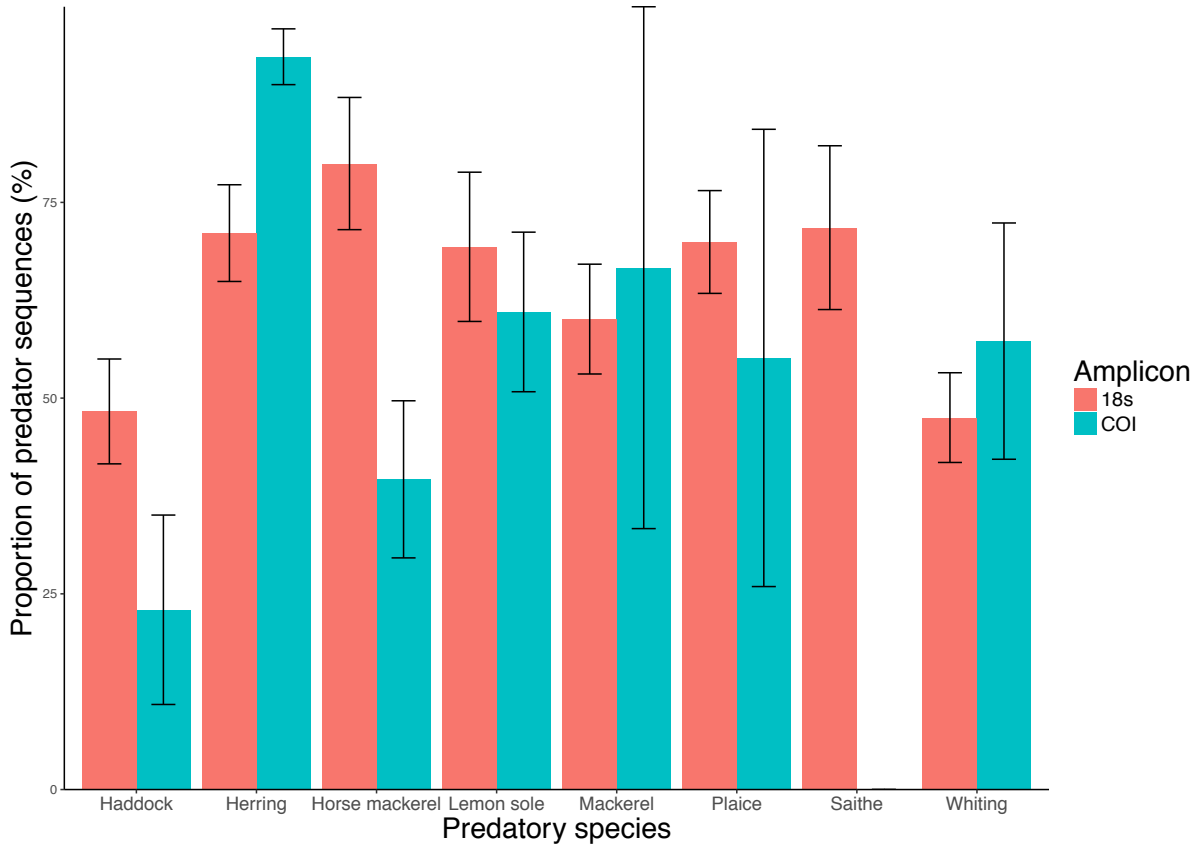

Figure 5.2. Amount of predator DNA detected (%) in stomach samples using COI and 18s amplicons.

Rarefaction plots (figure 5.3) for both amplicons show that sequencing depth was adequate to capture the diet of assessed species.

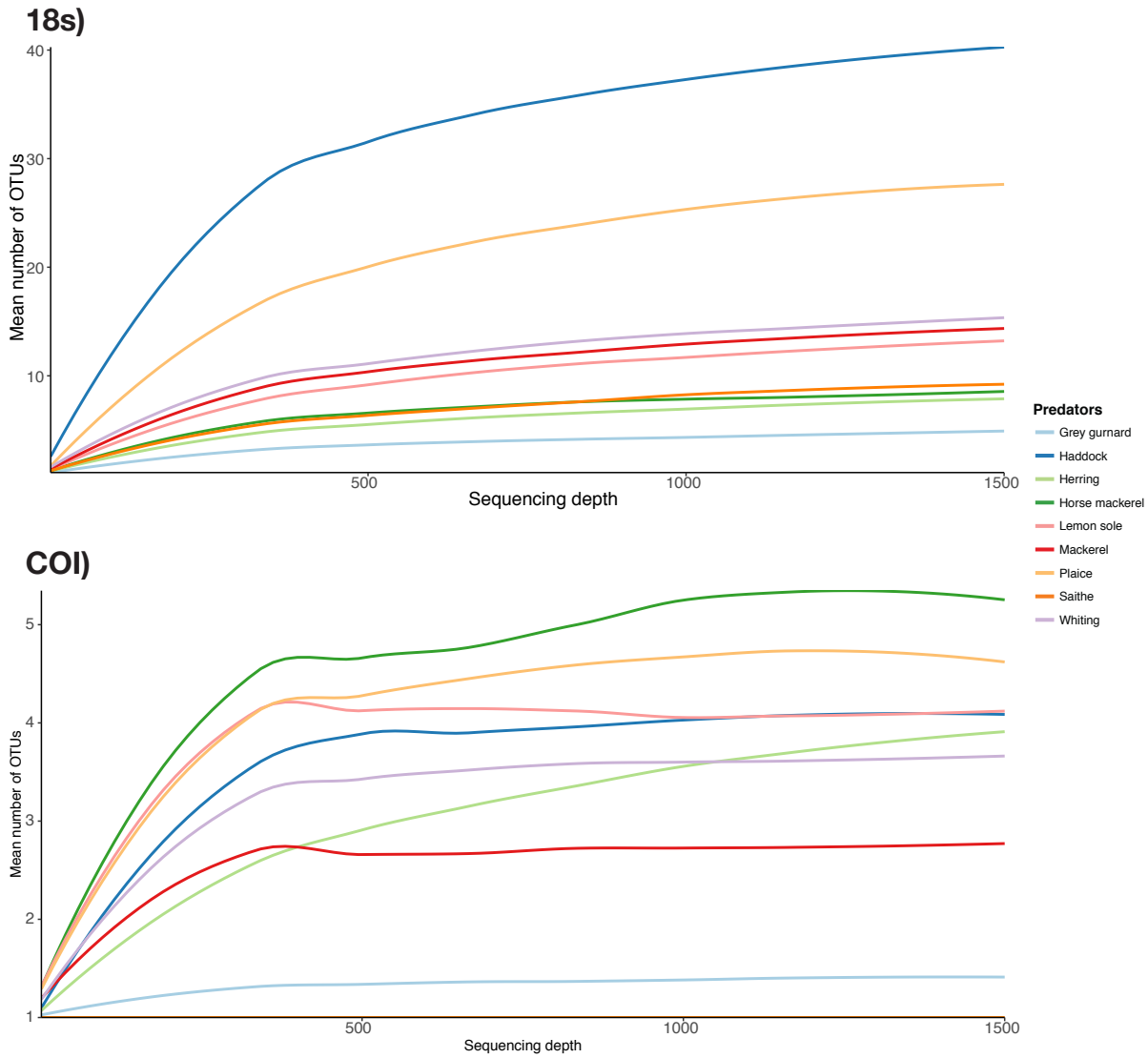

Figure 5.3. Rarefaction curves showing detection of OTUs as a function of sequencing depth in COI and 18s amplicons.

5.4.2 Diet

In all surveyed species 18s amplicons revealed a much wider variety of consumed species than the COI. Across all samples, 18s revealed 76 prey items while COI identified 16 in total. Seven prey items were identified only by COI, while 67 prey items were found uniquely using the 18s assay.

Figures 5.4-5.12 show the frequency that prey sequences were detected within the predators, a grey node indicates that predation was detected in few individuals, while a red node shows that predation was more common. The figures also show the taxonomic relatedness of prey items, and the overall richness of the predator diet.

Jellyfish sequences, belonging to cnidarians and ctenophores were detected using the 18s amplicon in the stomachs of haddock, herring, lemon sole, mackerel, plaice, and whiting using the 18s amplicon. Cnidarian predation was detected in mackerel, and chordate-jellyfish (salpida) detected in saithe, using the COI marker. No jellyfish predation was detected in either haddock or horse mackerel with either dietary assay. Figures 5.34-5.12 show that predation on any given jellyfish order by predators was relatively rare. The diet of surveyed species was rich, suggesting that none of these species are obligate jellyfish feeders and instead occupy a role in a more general diet. Predation at higher taxonomic levels appeared uncommon, with the notable exception of mackerel which frequently consumed hydrozoans. Scyphozoan DNA ('true-jellyfish') was rare or absent in all species except mackerel and haddock (mackerel DNA extracted in chapter 3 was utilised here, so mackerel data presented here should not be interpreted as an independent piece of evidence).

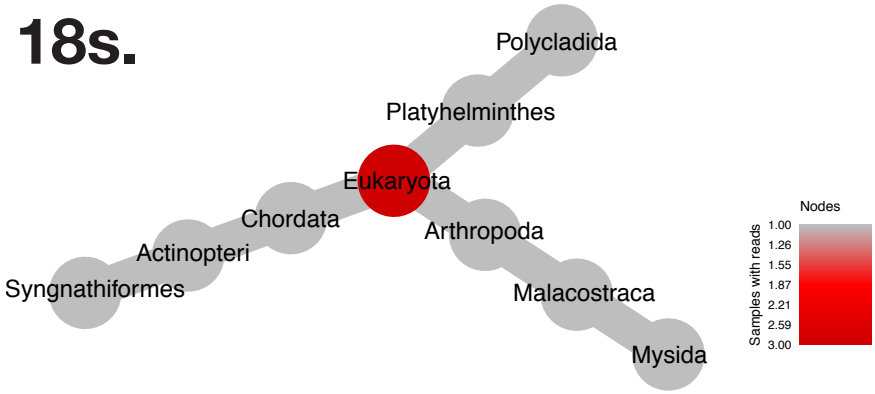

Figure 5.4. Taxa detected in grey gurnard stomachs using 18s metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

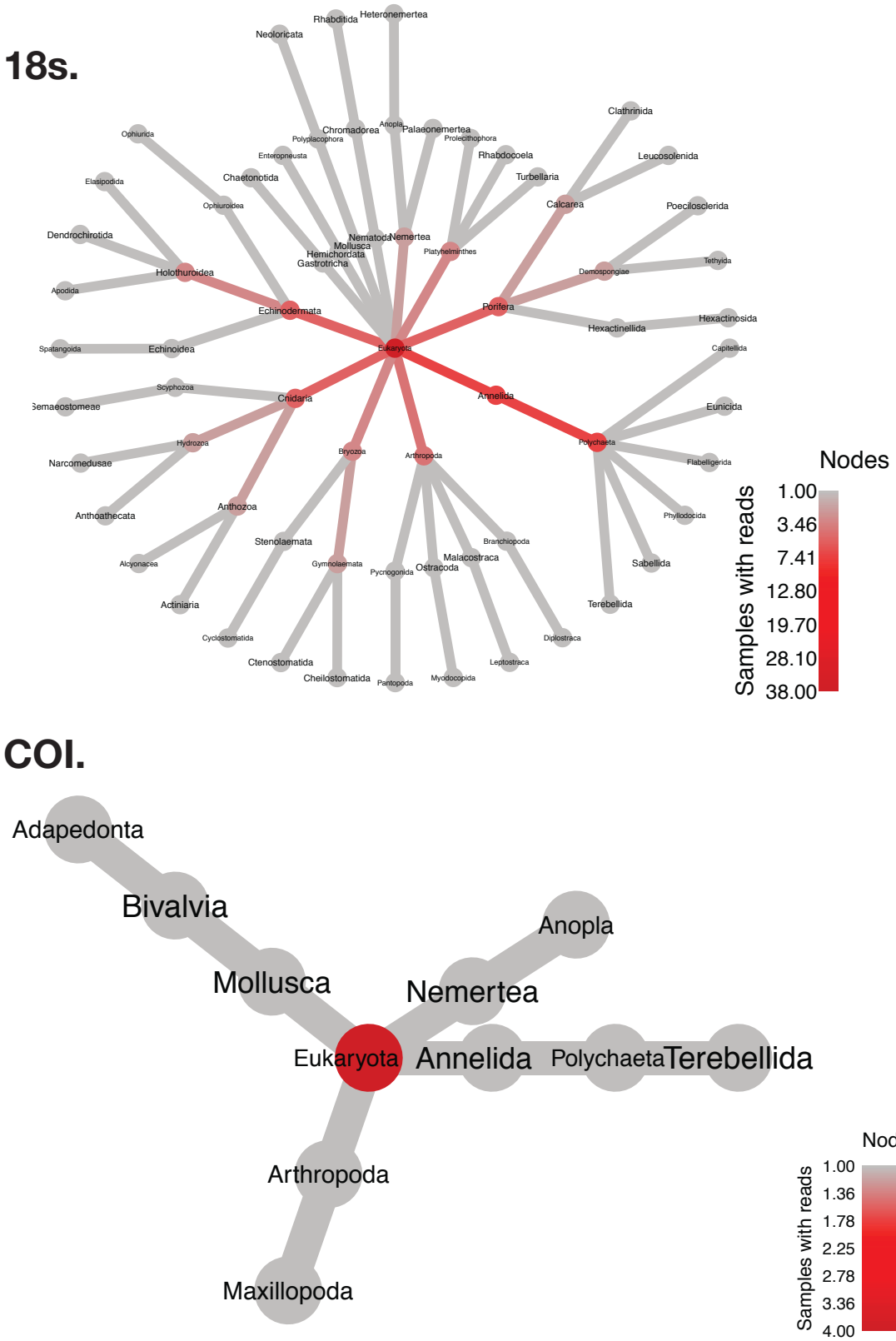

Figure 5.5. Taxa detected in haddock stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

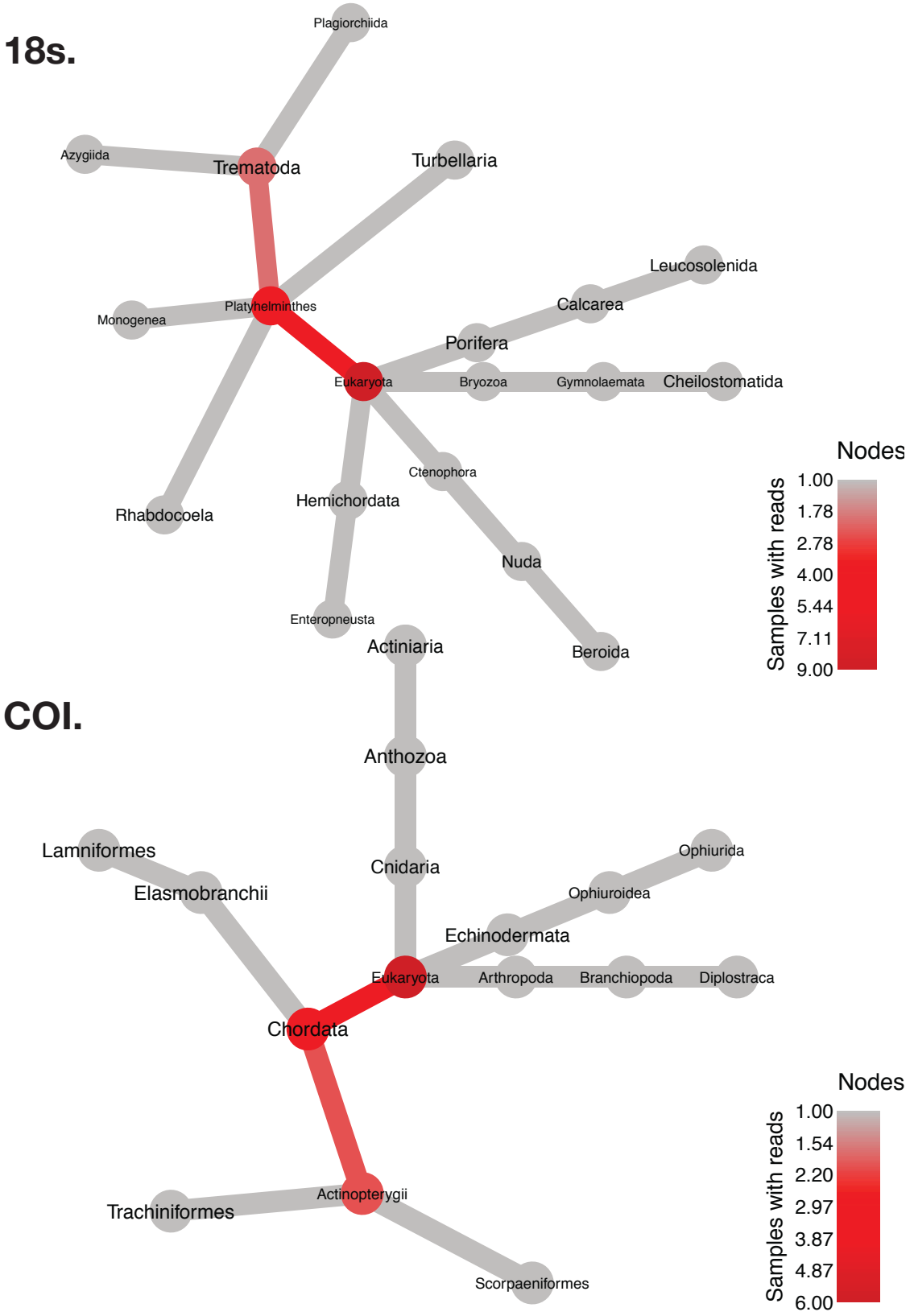

Figure 5.6. Taxa detected in herring stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

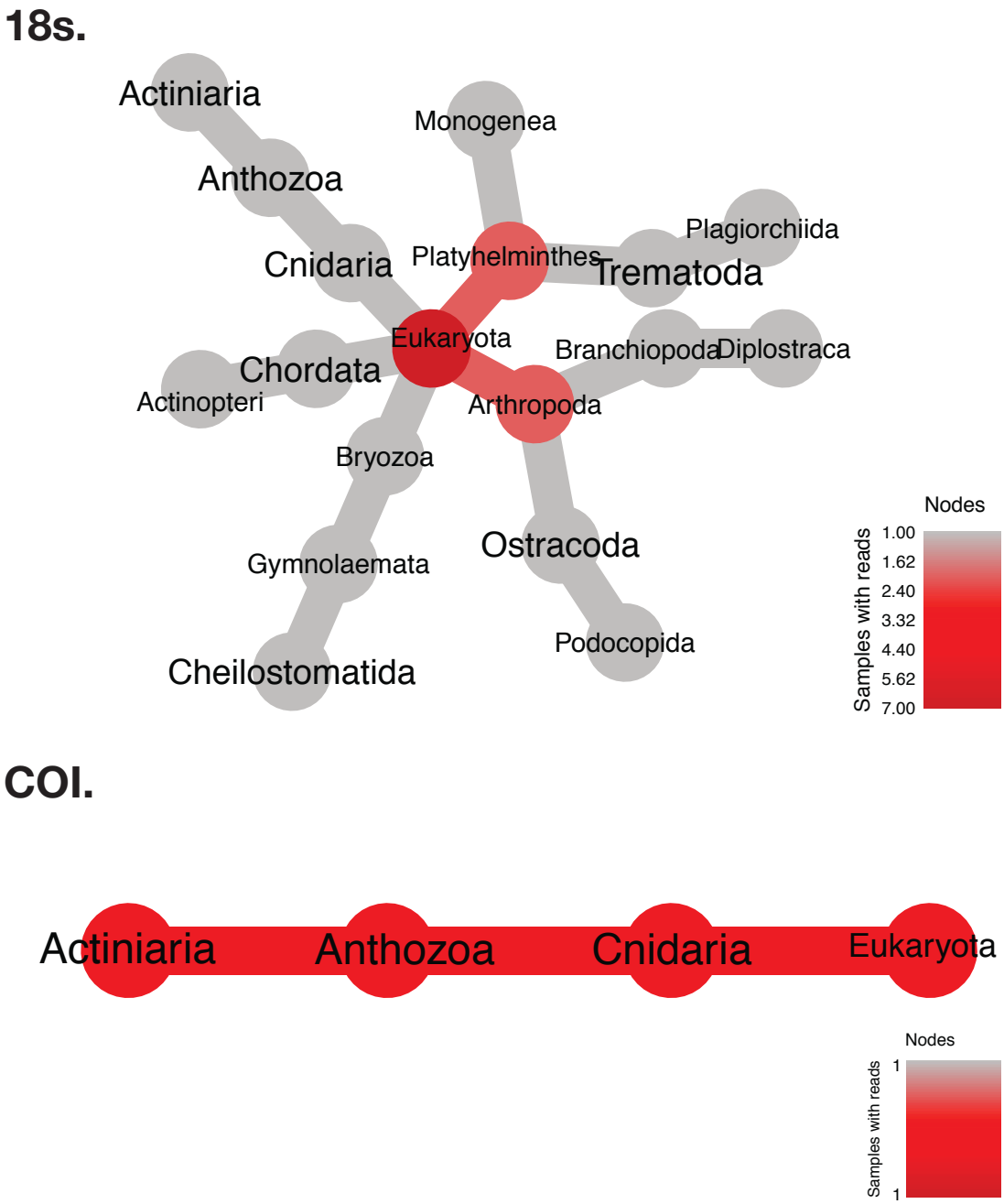

Figure 5.7. Taxa detected in horse mackerel stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

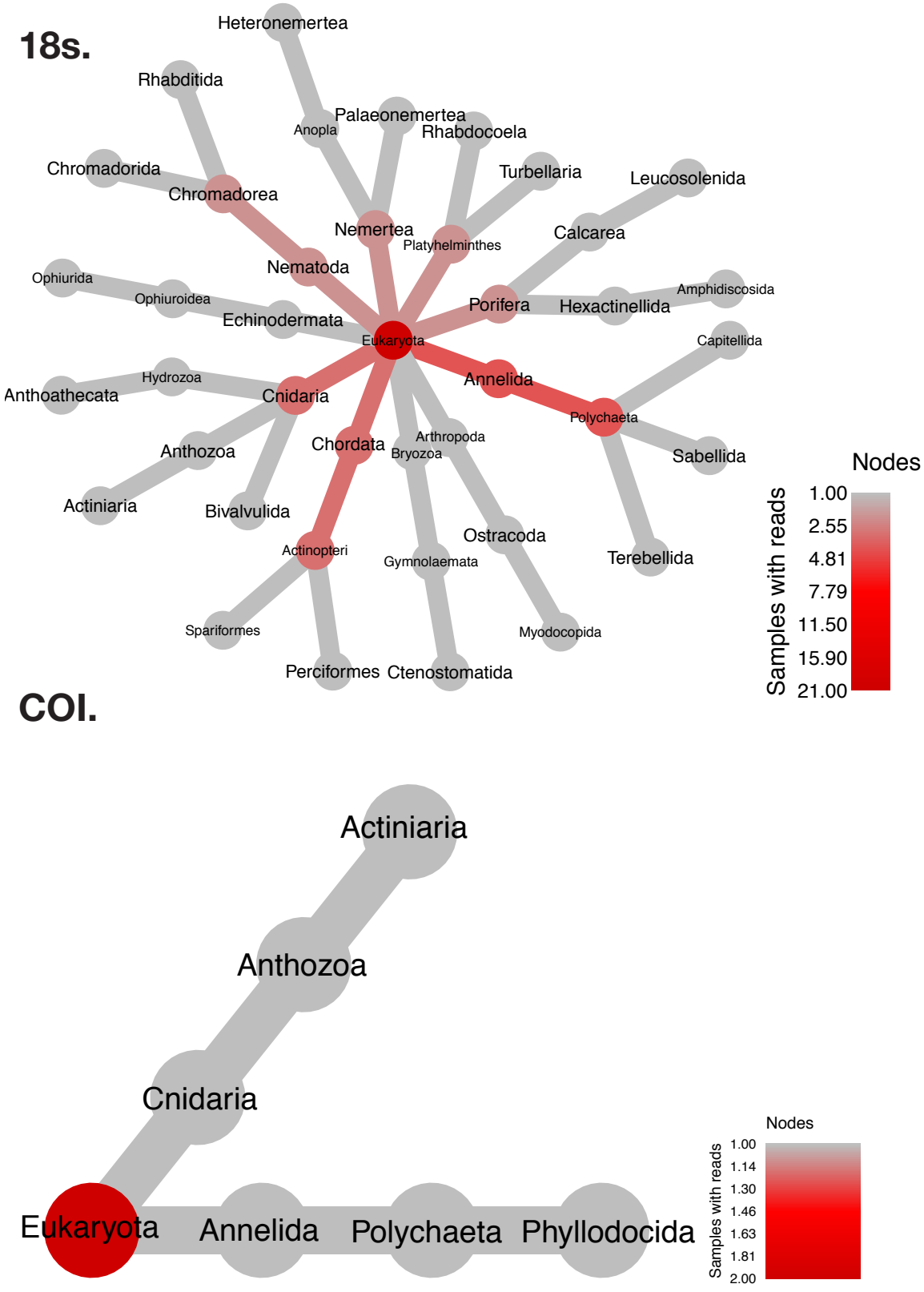

Figure 5.8. Taxa detected in lemon sole stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

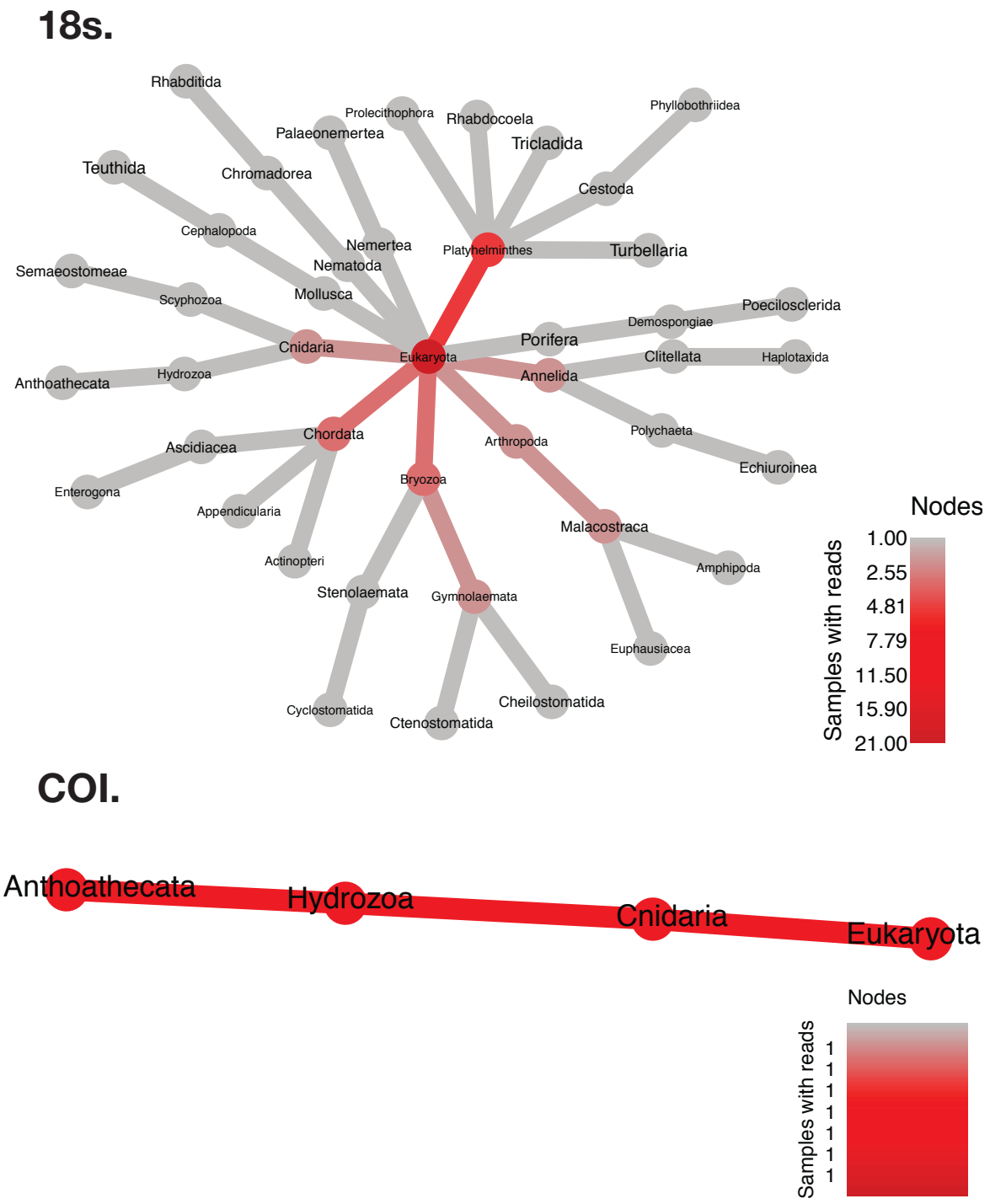

Figure 5.9. Taxa detected in mackerel stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

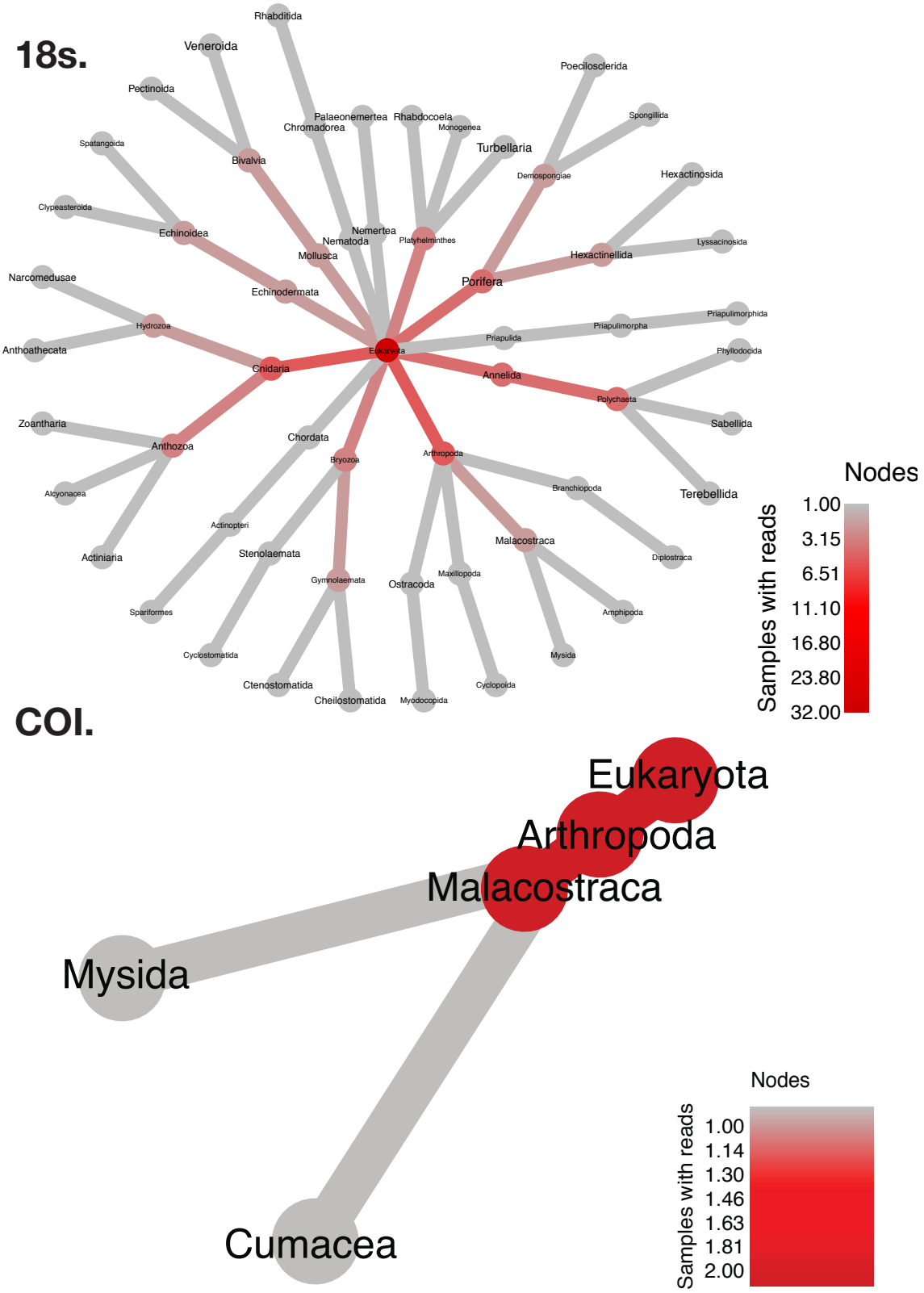

Figure 5.10. Taxa detected in plaice stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

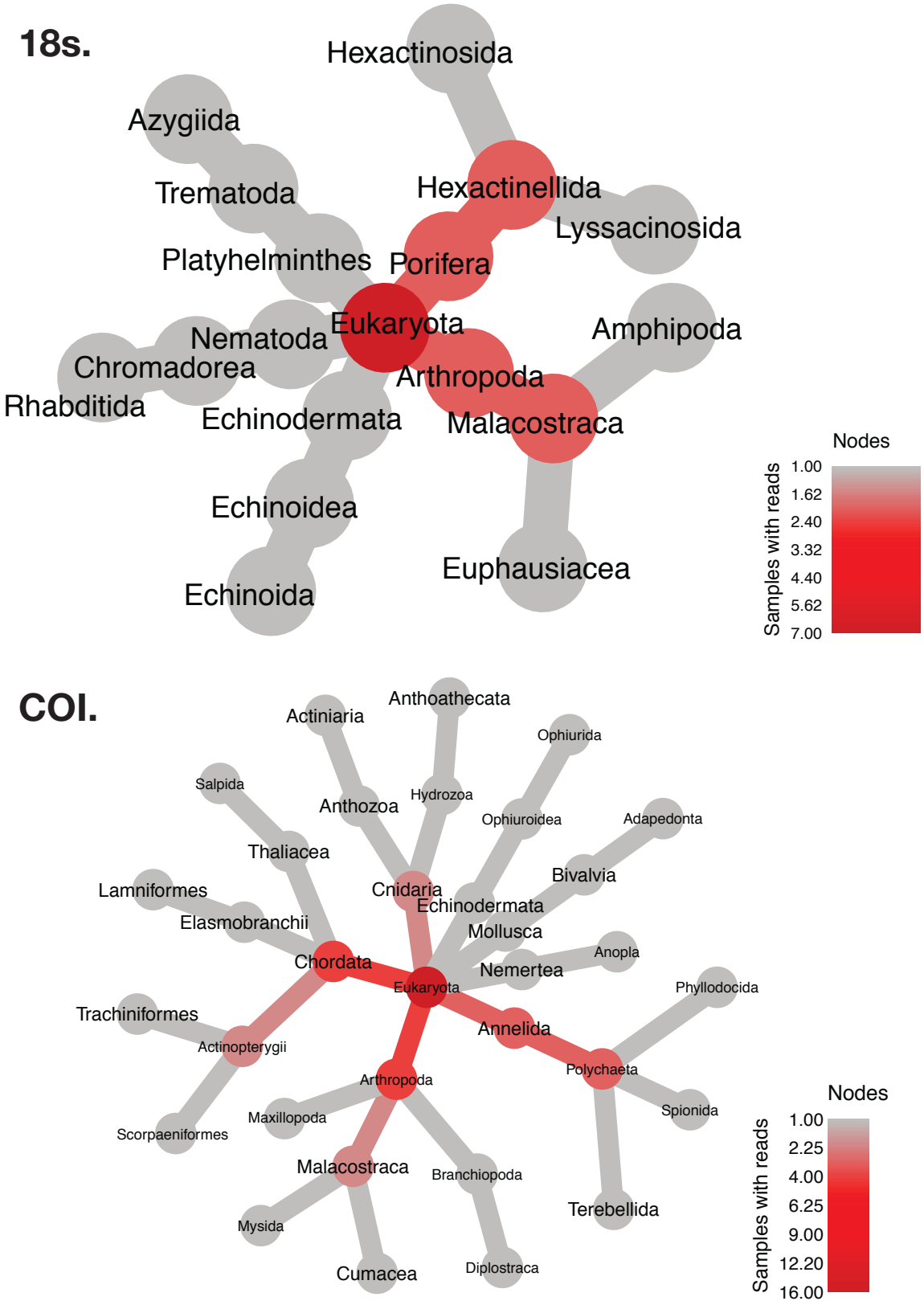

Figure 5.11. Taxa detected in Saithe stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

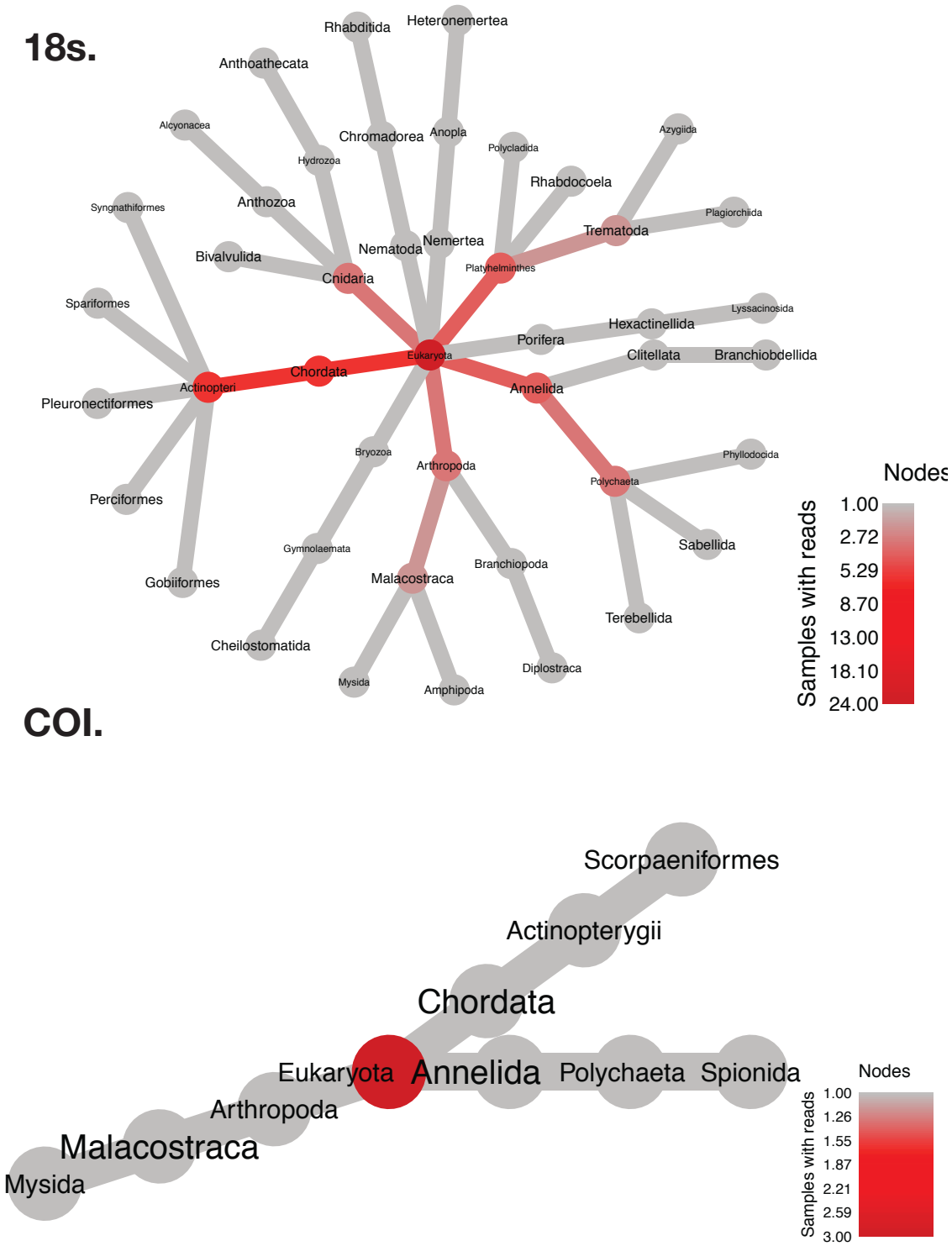

Figure 5.12. Taxa detected in whiting stomachs using 18s and COI metabarcoding. The frequency of taxa occurrence is denoted by the colour: rarely consumed taxa are shown in grey, more frequently consumed taxa are shown in red.

5.4.3 Diet partitioning

COI data did not provide an adequate sample size to be able conduct an non-metric multidimensional scaling (NMDS): stress values were less than 0.001 (indicative that sample size is too small), and no convergence could be achieved.

An NDMS on the 18s dataset using Mourisita-Horn distances (data aggregated into count distances), was conducted. Figure 5.13 highlights dietary differences between the predatory species: demersal species such as plaice, haddock, lemon sole, and to a lesser extent, whiting appear to consume similar species. Other predators had less dietary clustering.

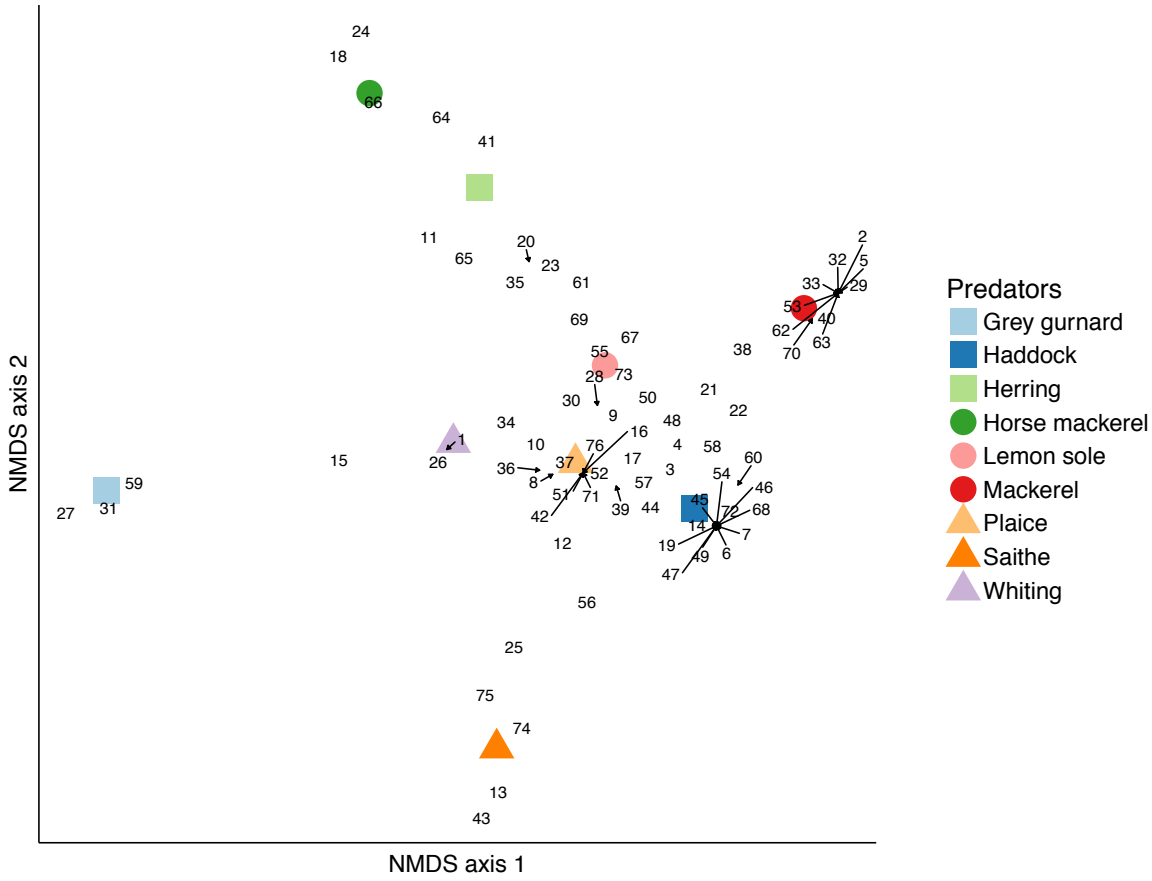

Figure 5.13. An NMDS using Morisita-Horn distances (aggregated count data) on 18s dietary data. Consumed species are shown by numbers, and are detailed in table 5.4: proximity to a predator indicates frequent predation. Clusters of consumed species, as indicated by arrows, occupy identical dietary space and may indicate secondary predation.

Table 5.4. Taxonomy and ID used to denote dietary position in figure 5.12.

ID	Phylum	Class	Order
1	Annelida	Clitellata	Branchiobdellida
2	Annelida	Clitellata	Haplotaxida
3	Annelida	Polychaeta	
4	Annelida	Polychaeta	Capitellida
5	Annelida	Polychaeta	Echiuroinea
6	Annelida	Polychaeta	Eunicida
7	Annelida	Polychaeta	Flabelligerida
8	Annelida	Polychaeta	Phyllodocida
9	Annelida	Polychaeta	Sabellida
10	Annelida	Polychaeta	Terebellida
11	Arthropoda	Branchiopoda	Diplostraca
12	Arthropoda	Malacostraca	Amphipoda
13	Arthropoda	Malacostraca	Euphausiacea
14	Arthropoda	Malacostraca	Leptostraca
15	Arthropoda	Malacostraca	Mysida
16	Arthropoda	Maxillopoda	Cyclopoida
17	Arthropoda	Ostracoda	Myodocopida
18	Arthropoda	Ostracoda	Podocopida
19	Arthropoda	Pycnogonida	Pantopoda
20	Bryozoa	Gymnolaemata	Cheilostomatida
21	Bryozoa	Gymnolaemata	Ctenostomatida
22	Bryozoa	Stenolaemata	Cyclostomatida
23	Chordata	Actinopteri	
24	Chordata	Actinopteri	Chaetodontiformes
25	Chordata	Actinopteri	Gadiformes
26	Chordata	Actinopteri	Gobiiformes
27	Chordata	Actinopteri	Perciformes
28	Chordata	Actinopteri	Pleuronectiformes
29	Chordata	Actinopteri	Scombriformes
30	Chordata	Actinopteri	Spariformes
31	Chordata	Actinopteri	Syngnathiformes
32	Chordata	Appendicularia	
33	Chordata	Ascidiacea	Enterogona
34	Cnidaria		Bivalvulida
35	Cnidaria	Anthozoa	Actiniaria
36	Cnidaria	Anthozoa	Alcyonacea
37	Cnidaria	Anthozoa	Zoantharia
38	Cnidaria	Hydrozoa	Anthoathecata
39	Cnidaria	Hydrozoa	Narcomedusae
40	Cnidaria	Scyphozoa	Semaeostomeae
41	Ctenophora	Nuda	Beroida
42	Echinodermata	Echinoidea	Clypeasteroida
43	Echinodermata	Echinoidea	Echinoida
44	Echinodermata	Echinoidea	Spatangoida
45	Echinodermata	Holothuroidea	Apodida
46	Echinodermata	Holothuroidea	Dendrochirotida
47	Echinodermata	Holothuroidea	Elasipodida
48	Echinodermata	Ophiuroidea	Ophiurida
49	Gastrotricha		Chaetonotida
50	Hemichordata	Enteropneusta	
51	Mollusca	Bivalvia	Pectinoida
52	Mollusca	Bivalvia	Veneroida
53	Mollusca	Cephalopoda	Teuthida
54	Mollusca	Polyplacophora	Neoloricata
55	Nematoda	Chromadorea	Chromadorida
56	Nematoda	Chromadorea	Rhabditida
57	Nemertea	Anopla	Heteronemertea
58	Nemertea	Palaeonemertea	
59	Platyhelminthes		Polycladida
60	Platyhelminthes		Prolecithophora
61	Platyhelminthes		Rhabdocoela
62	Platyhelminthes		Tricladida
63	Platyhelminthes	Cestoda	Phyllobothriidea
64	Platyhelminthes	Monogenea	
65	Platyhelminthes	Trematoda	Azygiida
66	Platyhelminthes	Trematoda	Plagiorchiida
67	Platyhelminthes	Turbellaria	
68	Porifera	Calcarea	Clathrinida
69	Porifera	Calcarea	Leucosolenida
70	Porifera	Demospongiae	Poecilosclerida
71	Porifera	Demospongiae	Spongillida
72	Porifera	Demospongiae	Tethyida
73	Porifera	Hexactinellida	Amphidiscosida
74	Porifera	Hexactinellida	Hexactinosida
75	Porifera	Hexactinellida	Lyssacinosida
76	Priapulida	Priapulimorpha	Priapulimorphida

5.5 Discussion

One hundred and eighty eight stomach samples collected from nine fish species were assessed for jellyfish predation using a dual amplicon HTS approach. The 18s assay yielded rich diet data for all tested species. By contrast, the COI assay detected many fewer prey items. Jellyfish consumption was detected in mackerel, lemon sole, herring, whiting, haddock, saithe, and plaice. Predation was not detected in grey gurnard or horse mackerel. In all instances of consumption, jellyfish appeared to have been fed upon as part of a diverse diet rather than specialised feeding.

5.5.1 Jellyfish predation

A variety of jellyfish species were detected in stomachs including cnidarians, ctenophores, and pelagic tunicates. Multiple species fed upon hydrozoan jellyfish and ctenophores. Consumption of scyphozoan jellyfish was limited to haddock and mackerel and was rare within those species. Mackerel have been reported predating jellyfish before[36], however this is a novel prey item for haddock. Generally these data corroborate the findings in chapter 3 that large scyphozoan species are not targeted: either because they have defensive stinging structures, or they are relatively poor source of nutrition[37] and may be acting as energy reservoirs in the ecosystem, re-routing energy pathways away from many, if not all, commercially important fish species during the time of sampling[38,39]. Alternatively, prey availability may be driving this apparent lack of consumption. However, given the majority of these samples (with the exception of herring and mackerel samples) were taken during the summer, when jellyfish biomass is normally at an annual high[40] this seems less probable.

When looking at figures 5.4 to 5.12, consumption of any given jellyfish order appears rare: most jellyfish nodes are grey. However, many organisms have evolved with a gelatinous body plan and looking at them in isolation in this manner may understate their role: figure 5.14 suggests that when treated as a single functional group a different pattern emerges and predation is relatively common.

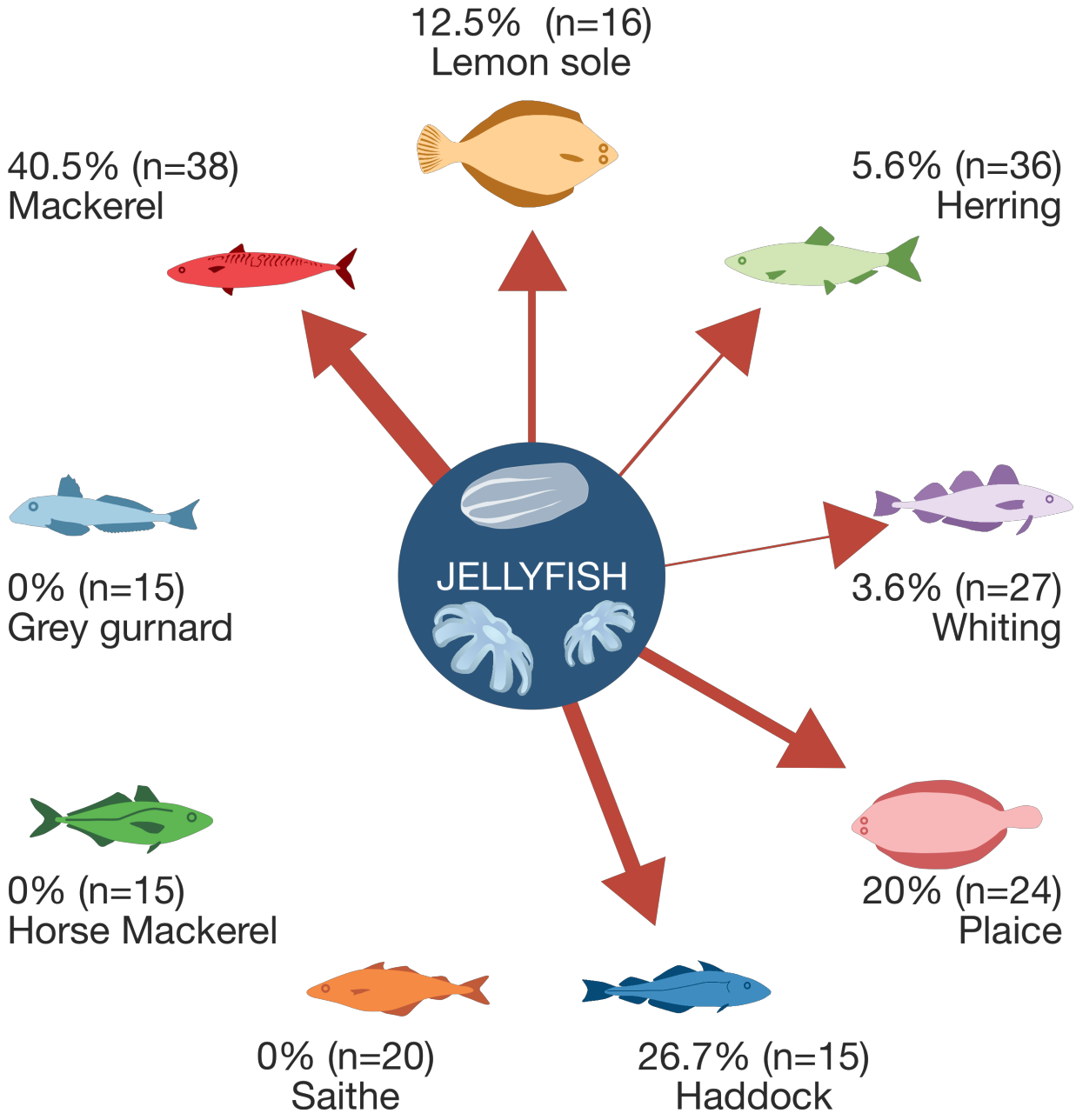

Figure 5.14. Jellyfish consumption detected using the HTS 18s assay. Thickness of arrow is proportional to the percentage of stomachs that jellyfish DNA was detected in stomachs fish (also shown as a percentage). Reported sample sizes refer to number of individuals which any DNA was detected in.

Of course, treating any broad-taxonomic group of organisms detected in these data would suggest that consumption is relatively common. Furthermore, these data are only being used to infer frequency of predation, so it is not possible to quantify the absolute importance of these trophic interactions. However, this does not detract from the fact that jellyfish play a role in these ecosystems throughout the year. Moving forward, it is worthwhile being explicit if scyphozoan, hydrozoan, ctenophores, or pelagic tunicates are being discussed. Our results suggest that mature scyphozoans

are not heavily targeted and it is easy to envisage how the perception of 'trophic dead end' arose, however other types of jellyfish are certainly involved in fish diets even late in the year. Treating this range of organisms as a single functional group is not grounded in the ecology jellyfish, and may be obscuring the different ecological roles they play.

5.5.2 A dual amplicon approach

The two genetic markers (18s and COI) here yielded very different data. We expected that the 18s primer set would uncover the invertebrate component of diet, while the COI would reveal the vertebrates component. However, the 18s assay outperformed the COI in every regard: sequences were obtained from a greater number of samples, achieved greater sequencing depth, and uncovered greater dietary diversity than the COI amplicon.

Amplicon length can affect the number of sequences produced: longer sequences are inherently more likely to be sheared during DNA degrading processes so shorter amplicons can yield higher DNA estimates[20]. However, it is unlikely the difference in performance is due to length alone as the 18s amplicons targeted a 100 bp region while the COI targeted a 130 bp region. The genetic variability of the marker is another possible driver in performance difference: COI is known for being hypervariable[41] which makes it a good candidate for assigning taxonomy, but simultaneously makes it difficult to amplify a broad array of taxa due to a high number of mismatches between target DNA and a primer set[42]. The 18s genetic region is, by contrast, very well conserved among taxa, which makes it relatively easy to amplify a broad range of organisms at the expense of taxonomic discrimination[43,44]. This wider amplification potential, coupled with the innately higher diversity of invertebrates in marine environments surrounding the British Isles may go some way towards explaining the difference. However, the dramatically lower dietary diversities in our study compared to others using the same COI primers[14] and the inability of the primer to amplify host DNA from some samples (predator DNA often overwhelms DNA recovered from stomachs[45]) from certain samples also suggest that the PCR protocol likely needs further adjustments before being used in a similar studies.

A more positive interpretation of the difference between markers highlights a hidden benefit of dual amplicon approach - redundancy. HTS studies are expensive, and the behaviour of primers in competitive PCR environments with degraded DNA is often different from single species DNA extracted from un-degraded tissue. A dual amplicon approach reduces the chances that unexpectedly poor primer performance will impact a study. Some additional costs are incurred as a result of additional PCR and cleaning in duplicate, although it should be noted that a multiplex PCR (multiple sets of primers are used simultaneously[46]) could be designed so the only extra expense would be the primers. We believe a multi-locus approach (two or more primer sets targeting different sections of DNA) should be implemented in situations where no *a priori* information on species make-up and where primer choice may be challenging. In these situations, the use of different amplicons not only increases the range of taxa that can be detected, but also improves the probability of a study yielding biologically relevant results.

5.5.3 Limitations

Although HTS studies are incredibly versatile and have opened new horizons in ecology they are not without caveats. A limitation pertinent to this study is the ability of the 18s primers to infer the vertebrate component of diet. Although vertebrates have been identified, the primers have relatively poor taxonomic discrimination abilities and were not intended to be used in this regard[22]. The taxonomic assignment of vertebrates in dietary samples should therefore be treated with a some scepticism as it possible the wrong taxonomy may have been assigned.

Another issue, discussed at length in chapter 4, but worth reiterating, is the quantitative nature of high throughput sequencing: does the number of reads obtained correspond to biomass of prey? Based on the high uncertainty uncovered in chapter 4, and the conclusions of a Deagle *et al.* that a presence / absence approach will yield less accurate data than quantitative or semi-quantitative approaches[31], a frequency of occurrence approach has been used. While a conservative approach, this does make it difficult to say how important any of the observed trophic interactions are. Using an approach like stable isotope analysis, which identifies the assimilation of isotopes into the flesh of a predator can help identify which of the observed trophic interactions are ecologically relevant[47].

Perhaps the largest unknown is the issue of secondary predation, where a consumed species' diet is erroneously assigned to the surveyed predator. A similar, but less discussed issue is the potential for eDNA to contaminate a sample[9]. Other sources of contamination, such as ingestion of species while in the net could also lead to false dietary inferences[22]. Identifying potential secondary predation could potentially be investigated using co-occurrence statistics. If consumed species never occur independently of one another this suggests that secondary predation may be present[48]. Figure 5.13 shows the dietary space consumed species occupy. Species occupying the same NDMS space co-occur frequently in stomachs, and it is possible that these cases be represent secondary predation. Focusing on gelatinous species the scyphozoan order Semaeostomeae shares dietary space with Scombriformes (mackerels and tuna-like fish). Jellyfish predation has been detected in mackerel here, and elsewhere in the literature[36] so secondary predation could have occurred.

Identifying *potential* secondary predation is relatively easy, verifying its presence is rather more difficult. It is possible to imagine a Pearson's correlation matrix, or binary equivalent (if data is treated in a non-quantitative manner), as a statistical test to establish secondary predation: If species A is never detected outside the presence of species B that may be suspicious. However, marine habitats are heterogeneous landscapes, with communities adapted to certain niches. One would expect that many species would co-occur in stomachs of predators simply because they belong to the same biological community. Of course, it is possible to assess the natural co-occurrence of organisms in the environment and then look for statistically significant differences between observed co-occurrence in the environment and stomach. Even ignoring other important factors, like temporal variation in species assemblages or the statistical headache of multiple-hypothesis testing, this level of ecological information doesn't exist in the ecosystems surveyed, or indeed, most ecosystems. Secondary predation, or other dietary contamination, is unlikely to be detected from a HTS study alone. Fortunately, other complimentary dietary assessment methodologies exist. Again, combining HTS with another technique, like stable isotope analysis, allows ecologists to identify the most important inter-specific interactions[47] assuming time and funding constraints allow two analytical techniques to be employed.

5.6 Conclusion

This study is the first to explicitly study jellyfish (cnidarian, ctenophore, or doliolid) predation using a HTS approach. Mackerel fed frequently on jellyfish and consumed the largest variety of species. Jellyfish consumption was detected in 7 of the 9 assessed species. This suggests that jellyfish remain a component of fish diet throughout the year, although the richness of other species in observed diets hints that obligate jellyfish feeders are probably rare and it remains unclear the extent to which jellyfish are predated. With the exception of mackerel, scyphozoan predation was rare: suggesting scyphozoan medusae may escape the predation seen during juveniles stages and may divert energy away from fish component of the ecosystem later in life as suggested by earlier models. Alternatively, prey availability may also contribute to the (lack of) observed predation. However, without data on the relative abundance of jellyfish at the time of sampling: disentangling these two hypotheses is challenging. The different inter-specific interactions between the different types of jellyfish highlights that although they share a similar body plan and some life-history characteristics they clearly occupy different trophic roles. Aggregating different types of jellyfish may be obscuring elements of their ecology. Although an *ad-hoc* picture of temporal predation patterns now exists, and the context in which they are consumed better understood, the actual role they play in ecosystems is still unresolved. Ecosystem models incorporating our current understanding would go some way towards addressing the trophic role they play. Ideally, a hybrid study incorporating HTS and stable isotope analysis could then be used to verify and optimise the model further.

5.7 References

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5.8 Supplementary Information

Supplementary Table 5.1. Included (marine) and excluded (terrestrial and Siluriformes) taxa in the HTS study

Taxa	Type
k__Eukaryota.p__Annelida.c__Clitellata.o__Branchiobdellida	Marine
k__Eukaryota.p__Annelida.c__Clitellata.o__Haplotaxida	Marine
k__Eukaryota.p__Annelida.c__Clitellata.o__Rhynchobdellida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Capitellida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Echiuroinea	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Eunicida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Flabelligerida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Golfingiida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Phyllodocida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Sabellida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Spionida	Marine
k__Eukaryota.p__Annelida.c__Polychaeta.o__Terebellida	Marine
k__Eukaryota.p__Arthropoda.c__Arachnida.o__Araneae	Terrestrial
k__Eukaryota.p__Arthropoda.c__Arachnida.o__Mesostigmata	Terrestrial
k__Eukaryota.p__Arthropoda.c__Arachnida.o__Opiliones	Terrestrial
k__Eukaryota.p__Arthropoda.c__Arachnida.o__Sarcoptiformes	Terrestrial
k__Eukaryota.p__Arthropoda.c__Arachnida.o__Trombidiformes	Terrestrial
k__Eukaryota.p__Arthropoda.c__Branchiopoda.o__Diplostraca	Marine
k__Eukaryota.p__Arthropoda.c__Chilopoda.o__Scolopendromorpha	Terrestrial
k__Eukaryota.p__Arthropoda.c__Collembola.o__Entomobryomorpha	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Coleoptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Diptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Embioptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Ephemeroptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Hemiptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Orthoptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Plecoptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Psocoptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Insecta.o__Thysanoptera	Terrestrial
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Amphipoda	Marine
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Decapoda	Marine
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Euphausiacea	Marine
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Isopoda	Marine
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Leptostraca	Marine
k__Eukaryota.p__Arthropoda.c__Malacostraca.o__Mysida	Marine
k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Calanoida	Marine
k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Cyclopoida	Marine
k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Harpacticoida	Marine
k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Mormonilloida	Marine
k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Poecilostomatoida	Marine

k__Eukaryota.p__Arthropoda.c__Maxillopoda.o__Siphonostomatoida	Marine
k__Eukaryota.p__Arthropoda.c__Ostracoda.o__Myodocopida	Marine
k__Eukaryota.p__Arthropoda.c__Ostracoda.o__Podocopida	Marine
k__Eukaryota.p__Arthropoda.c__Pycnogonida.o__Pantopoda	Marine
k__Eukaryota.p__Arthropoda.c__Remipedia.o__Nectiopoda	Marine
k__Eukaryota.p__Brachiopoda.c__.o__	Marine
k__Eukaryota.p__Bryozoa.c__Gymnolaemata.o__Cheilostomatida	Marine
k__Eukaryota.p__Bryozoa.c__Gymnolaemata.o__Ctenostomatida	Marine
k__Eukaryota.p__Bryozoa.c__Stenolaemata.o__Cyclostomatida	Marine
k__Eukaryota.p__Chaetognatha.c__Sagittoidea.o__Aphragmophora	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Chaetodontiformes	Marine
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k__Eukaryota.p__Chordata.c__Actinopteri.o__Gadiformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Gobiiformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Ophidiiformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Perciformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Pleuronectiformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Scombriformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Siluriformes	Excluded
k__Eukaryota.p__Chordata.c__Actinopteri.o__Spariformes	Marine
k__Eukaryota.p__Chordata.c__Actinopteri.o__Syngnathiformes	Marine
k__Eukaryota.p__Chordata.c__Appendicularia.o__	Marine
k__Eukaryota.p__Chordata.c__Ascidiacea.o__Enterogona	Marine
k__Eukaryota.p__Chordata.c__Ascidiacea.o__Stolidobranchia	Marine
k__Eukaryota.p__Chordata.c__Mammalia.o__Primates	Terrestrial
k__Eukaryota.p__Chordata.c__Thaliacea.o__Doliolida	Marine
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k__Eukaryota.p__Cnidaria.c__Hydrozoa.o__Anthoathecata	Marine
k__Eukaryota.p__Cnidaria.c__Hydrozoa.o__Leptothecata	Marine
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k__Eukaryota.p__Hemichordata.c__Enteropneusta.o__	Marine
k__Eukaryota.p__Mollusca.c__Bivalvia.o__Myoida	Marine
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k__Eukaryota.p__Mollusca.c__Bivalvia.o__Veneroida	Marine
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k__Eukaryota.p__Mollusca.c__Gastropoda.o__Thecosomata	Marine
k__Eukaryota.p__Mollusca.c__Polyplacophora.o__Neoloricata	Marine
k__Eukaryota.p__Nematoda.c__Chromadorea.o__Chromadorida	Marine
k__Eukaryota.p__Nematoda.c__Chromadorea.o__Rhabditida	Marine
k__Eukaryota.p__Nematoda.c__Enoplea.o__Dorylaimida	Marine
k__Eukaryota.p__Nematoda.c__Enoplea.o__Mononchida	Terrestrial
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k__Eukaryota.p__Nemertea.c__Enopla.o__Monostilifera	Marine
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k__Eukaryota.p__Platyhelminthes.c__.o__Prolecithophora	Marine
k__Eukaryota.p__Platyhelminthes.c__.o__Rhabdocoela	Marine
k__Eukaryota.p__Platyhelminthes.c__.o__Tricladida	Marine
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k__Eukaryota.p__Platyhelminthes.c__Monogenea.o__	Marine
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k__Eukaryota.p__Platyhelminthes.c__Turbellaria.o__	Marine
k__Eukaryota.p__Porifera.c__Calcarea.o__Clathrinida	Marine
k__Eukaryota.p__Porifera.c__Calcarea.o__Leucosolenida	Marine
k__Eukaryota.p__Porifera.c__Demospongiae.o__Axinellida	Marine
k__Eukaryota.p__Porifera.c__Demospongiae.o__Dendroceratida	Marine
k__Eukaryota.p__Porifera.c__Demospongiae.o__Poecilosclerida	Marine
k__Eukaryota.p__Porifera.c__Demospongiae.o__Spongillida	Marine
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k__Eukaryota.p__Porifera.c__Hexactinellida.o__Amphidiscosida	Marine
k__Eukaryota.p__Porifera.c__Hexactinellida.o__Hexactinosida	Marine
k__Eukaryota.p__Porifera.c__Hexactinellida.o__Lyssacinosida	Marine
k__Eukaryota.p__Priapulida.c__Priapulimorpha.o__Priapulimorphida	Marine
k__Eukaryota.p__Rotifera.c__Monogononta.o__Flosculariacea	Marine

Chapter 6: Tracking trends in the implementation of jellyfish in 30+ years of Ecopath with Ecosim models

6.1 Abstract

It has been nearly a decade since Pauly *et al.* reviewed the role of jellyfish in Ecopath with Ecosim models and concluded the manner jellyfish are incorporated was often poor. Better integration of jellyfish into fisheries research, with careful consideration of their unique biology, was advocated. Since this publication, both the fields of ecosystem modelling and jellyfish ecology have made significant progress. Here, we revisit the issue of jellyfish in Ecopath with Ecosim models to assess how the implementation of jellyfish in models has changed over the past decade and once again,highlight future areas for improvement. All Ecopath with Ecosim (EwE) models on the Ecobase repository were assessed to see how jellyfish were typically implemented in models. A greater percentage of models included jellyfish over time, however this was usually as a single functional group which may poorly reflect the functional diversity present within gelatinous zooplankton. Jellyfish were often highly linked to the wider ecosystem, with many predators, and prey, included in models. However, although the input parameters production, consumption, and biomass displayed a normal distribution across all models, ecotrophic efficiency values varied widely from model to model. Ecotrophic efficiency, a measure of how an organism is used by other species in an ecosystem, was frequently at very low values: indicative that jellyfish are still sometimes perceived to be under-utilised components of the ecosystem. When linkages between jellyfish literature, and EwE models were visualised a loose network structure between models was revealed, suggesting that although values were sometimes shared or borrowed, models were usually parameterised using independent data. Moving forward greater care should be taken to define what types of jellyfish are included in functional groups as ctenophores, cnidarians, and chordates play different functional roles. Additionally, early life stages

should be incorporated as multi-stanza groups as research becomes available to more accurately depict jellyfishes' role in marine ecosystems.

6.2 Introduction

Keeping marine ecosystems resilient and healthy is a conservation, economic development, and food security goal. The sustainability of fish stocks is not only dependant on careful management of catches, but also factors such as the preservation of food web linkages that underpin fish stocks and the ecologically-compatible implementation of other human activities in the marine environment[1]. An ecosystem approach to fisheries management (EAFM) incorporates many of these considerations, and is considered to be essential for the future health of fish stocks. Calls for an EAFM are widespread through academic and policy literature[2–4], and EAFM is a statutory requirement under the EU Common Fisheries Policy[5].

Whole-ecosystem models can play an important role towards achieving EAFM by providing a framework to quantify interspecific interactions. While they can be used to make predictions about biomass, they lack the statistical robustness and predictive power of single species models[6] and are best used as a complimentary approach if biomass predictions are desired. However, whole ecosystem models are uniquely well-suited to explore other facets of the ecosystem functions including modelling the uptake and spread of pollutants[7], exploring alternative ecosystem states, understanding the network structure of the ecosystem or the indirect consequences of exploitation [8]. Additionally, whole ecosystem models can also be used to identify knowledge gaps[8]; If a species cannot be well-parameterised in a model, this can be indicative of a uncertainty in fundamental aspects of their ecology.

Although a range of marine ecosystem modelling approaches have been developed, such as Gadget[9] and Netwrk[10], Ecopath with Ecosim[11] (EwE) remains the most pervasive in the literature[12]. Ecopath with Ecosim is a biomass compartment model which is comprised of three core elements. Ecopath is a static mass-balance model originally developed by Polvina[13]. Ecosim, developed by Walters *et al.*[14], replaces the linear equations in Ecopath with differential equations allowing dynamic temporal simulations to take place. Ecospace, the most recent addition, added the ability to run

Ecosim simulations in a grid enabling spatial analysis[15]. All EwE models must have a balanced Ecopath model as a starting condition and are parameterised by ensuring that equilibrium conditions, known as mass balance, are met for each group (species, or functional group) in the following equation[16]:

$$B_i(\frac{P}{B})_i * EE_i = \sum_j B_j * \left(\frac{Q}{B}\right)_j * DC_{ij} - C_i$$

Where B is the biomass (t km^{-2}), P/B is annual production to biomass ratio, Q/B is the annual consumption ratio, and EE is the ecotrophic efficiency: a parameter that shows the proportion of a group that is consumed by other species in the ecosystem. DC, diet composition, is the percentage that prey i accounts for in the diet of consumer j. C_i is an optional parameter showing removal of group i by fisheries. To achieve mass-balance, at least four parameters out of B, P/B, Q/B, EE and DC must be entered; EwE can calculate one unknown model parameter.

Jellyfish (cnidarians, ctenophores, and pelagic tunicates) have a notoriously poorly-understood trophic ecology[17]. To synthesise the state of the field and illustrate best practice for incorporating jellyfish in ecosystem models Pauly *et al.*[18] reviewed predators and prey of jellyfish in the online databases FishBase and SeaLifeBase, and also investigated the role of jellyfish in 23 EwE models. Jellyfish biomass was greater in disturbed ecosystems, this was thought to happen as overfishing allowed jellyfish populations to expand by feeding upon zooplankton which would otherwise be consumed by fish. A lot of variation in the parameterisation of jellyfish was present in the EwE models: in some models jellyfish were treated as trophic dead-ends, while in others they were an integral part of the ecosystem. Equally, in some ecosystems (Lancaster Sound and Chesapeake Bay) jellyfish exerted a large influence on other biota and appeared to be keystone species, however in many other systems this did not appear to be the case. It is now approaching a decade since this review[18] took place and our knowledge of jellyfish ecology, the tools available for analysis, and the number of published EwE models containing jellyfish have expanded considerably. It is worth investigating if the recommendations have been incorporated into common practice, and how the state of the field has changed.

Ecobase, a database of more than 450 EwE models and meta-data assembled by Colléter *et al.*[19] provides the basis for this study. The aim is to characterise how jellyfish are incorporated into EwE models, and the task can be broken down into the following objectives: a) quantify the prevalence of jellyfish in models (does the broader community think they play a significantly large role in models to warrant inclusion?), b) assess the connectedness of jellyfish with the rest of the ecosystem (are jellyfish always treated as trophic dead ends? Is this trend changing as more evidence about their role in the ecosystem is gathered?), c) determine the influence of key models (have parameters been copied between models? Are key publications strongly influencing the field and if so, what is the rigour of 'keystone' literature?), d) check if estimates of key parameters reflect the state of jellyfish ecology, and e) conduct an in-depth assessment of the jellyfish group incorporated in the Irish Sea EwE model[20] and offer suggestions on how to best-incorporate novel findings into the modelling framework.

6.3 Methods

6.3.1 Data collection

The primary literature (technical reports, publications, dissertations and theses) and EwE models detailed in Ecobase were first examined to ascertain whether the model included a jellyfish group. The broadest definition of jellyfish was employed, so any model containing pelagic tunicates, ctenophore, cnidarians, or some combination of all three was included in the analysis. In some instances, the primary literature could not be accessed in which case the model was assessed for jellyfish by accessing the Ecobase database using R[21] or downloading and inspecting the model directly in EwE.

Models that contained jellyfish explicitly were categorised as a '*plankton that included jellyfish*' for those that contained jellyfish in the model but only as part of a broad zooplankton group, or '*jellyfish groups'* for those that had jellyfish in their own group (jellies, gelatinous zooplankton or similar) or if they amounted for more than 75% of a zooplankton-subgroup such as carnivorous zooplankton. For all groups the geographic location of the model was extracted from Ecobase using R if possible, or georeferenced in QGIS 3.2[22] using the map section of 'Discovery Tools' on the

Ecobase website (http://ecobase.ecopath.org/). For models with explicit 'jellyfish groups', the Biomass (B); consumption to biomass ratio (Q/B); production to biomass ratio (P/B) and ecotrophic efficiency (EE) were extracted. In addition, the input parameter that was estimated by EwE via mass-balance, prey items of jellyfish, predators of jellyfish, and the literature cited for parameterising the 'jellyfish group' were recorded for each jellyfish group within a model. In some instances, models had multiple jellyfish groups, or the same model was parameterised for different years: in these instances, all jellyfish groups and years were recorded.

6.3.2 Data visualisation and statistical analysis

Comparing EE, P/B, Q/B, and B values for jellyfish between models proved to be challenging. As previously mentioned some models contained multiple groups of jellyfish or had the model parameterised for different years. Many models with 'multiple' EwE implementations for different years were created before the advent of Ecosim, and were used to conduct crude temporal analysis. To treat these models in a mode more analogous with contemporary models (that use a single Ecopath model) we used mean values of the input parameters across different time periods to give a single set of values for each jellyfish group. For models with multiple jellyfish groups, all jellyfish groups were included for analysis.

The possible link between EE and publication date (it was hypothesised that EE would increase over time, as jellyfish were considered a more important component of the ecosystem) was tested using a linear model. To assess the relative influence of models within the literature, EwE models and the corresponding cited literature, were used as nodes in a directed model in the R package 'network'[23]. Groups that jellyfish preyed upon, and groups that fed on jellyfish were visualised using 'metacoder'[24]. Since subtly different terminology (*e.g.* 'macrozooplankton' and 'zooplankton') was used to describe similar taxonomic groups, some functional groups were combined as detailed in supplementary table 6.1 and supplementary table 6.2.

6.4 Results

In total 329 models were examined, of these 211 contained no jellyfish, 32 included jellyfish as part of a zooplankton group, and 86 models included jellyfish explicitly as their own separate group(s). The inclusion of jellyfish in Ewe models has increased over time and is characterised by three periods: 1984-2000, 2000-2007, and 2007-present (Figure 6.1). Early models rarely featured jellyfish, but by the year 2000, six EwE models had explicitly featured jellyfish and total models featuring jellyfish in any way only accounted for 15.4% of models. From 2000-2007 a rapid increase in the inclusion of jellyfish then occurred, however there was an approximate 50:50 split between models including jellyfish as part of a zooplankton group and those where jellyfish were included as an explicit group. Starting in 2007, including jellyfish in EwE models as their own functional group gained traction. During this time only four models included jellyfish as part of a wider plankton group, in contrast to 56 models that featured jellyfish as their own group. Models that contain jellyfish explicitly now account for 35.8% models, of which 72.8% feature them as their own functional group. Jellyfish are represented in a wide variety of ecosystem models around the world, although there is a concentration of research in North America and Europe, with fewer models seen in the other continents (figure 6.2).

The network analysis of citations used to parameterise jellyfish components in the EwE models revealed that none of the published models are terribly influential with the most influential model only used to parameterise two other models (figure 6.3). Cited literature tells a similar story, six manuscripts were used to parameterise more than three models. Models for the most part used locally-derived independent sources of data.

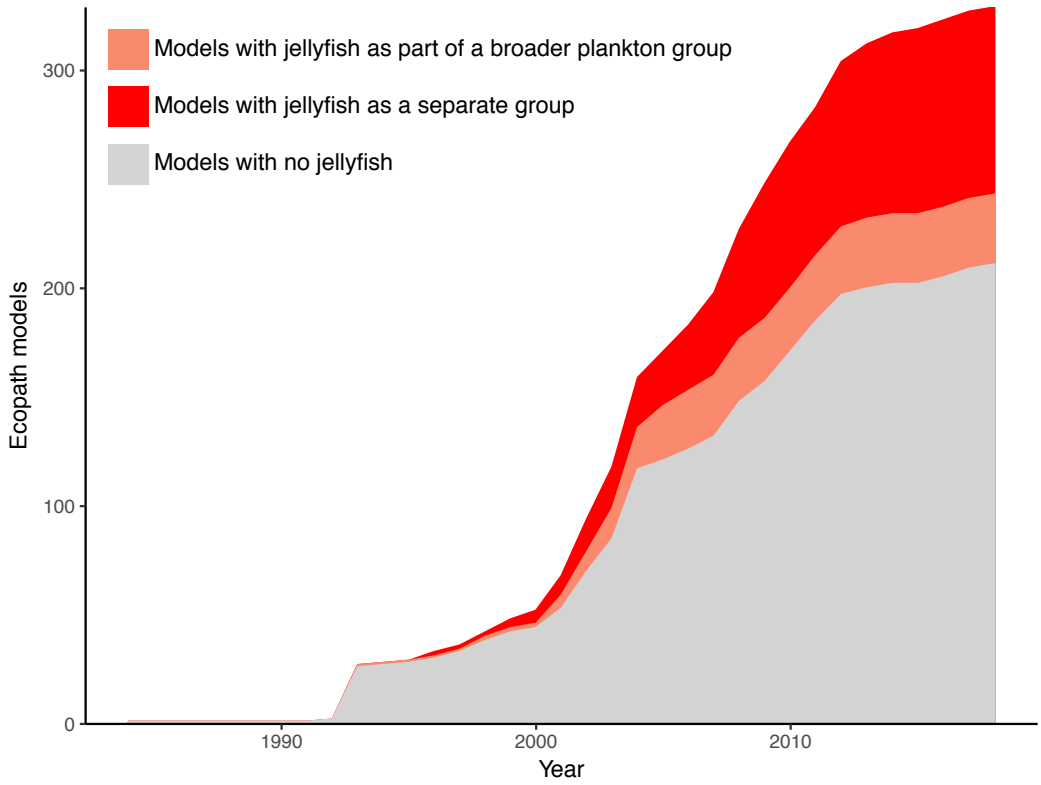

Figure 6.1. The accumulation of EwE models through time. Grey are EwE models with no jellyfish functional group included at all. Light red is models with jellyfish as part of a wider zooplankton group. Dark red, are those models with jellyfish included as their own group.

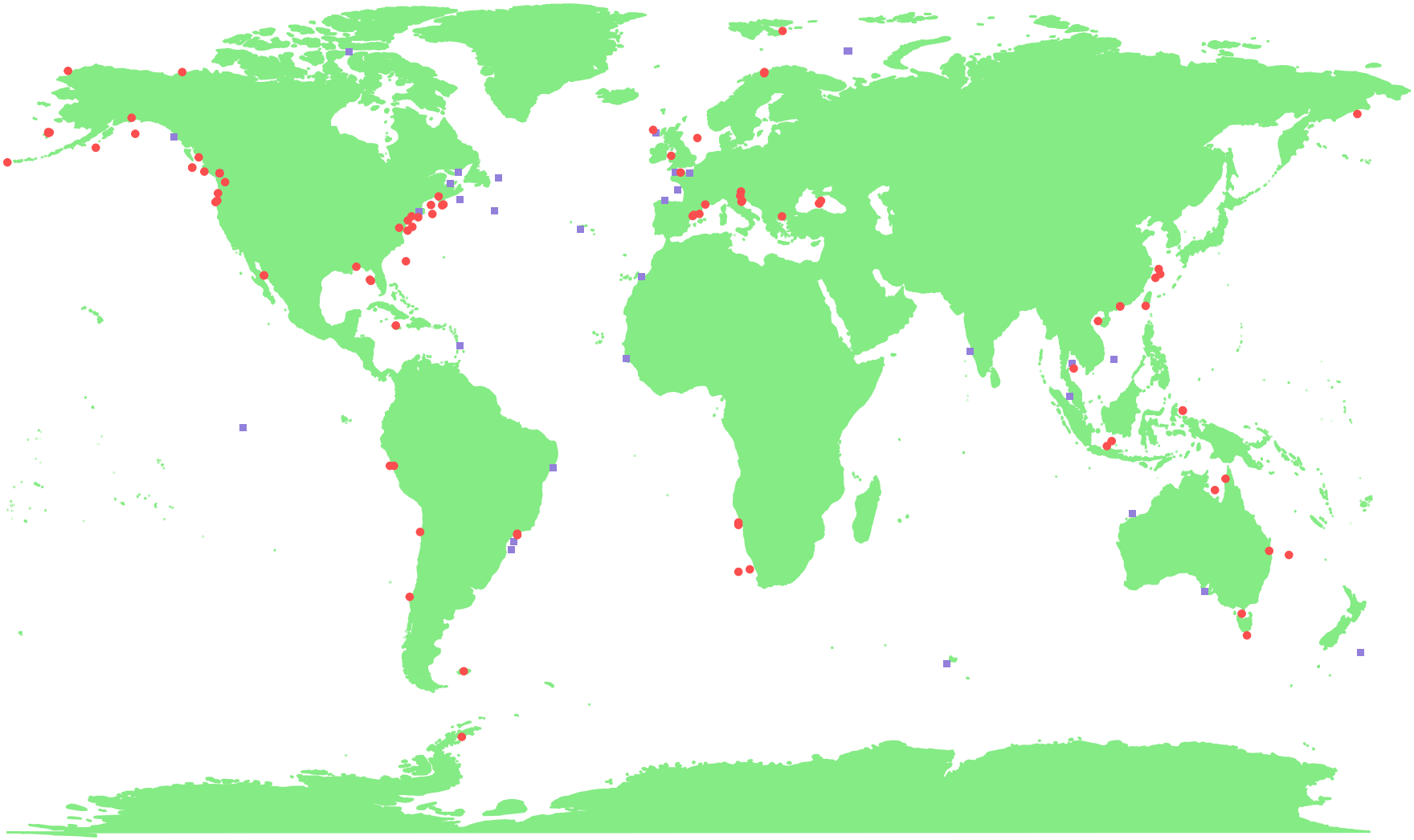

Figure 6.2. The global distribution of EwE models containing jellyfish. Groups containing jellyfish as part of a broad 'zooplankton' group are denoted by purple squares. Models containing jellyfish as explicit functional groups are shown with red circles. Models not containing jellyfish are not shown.

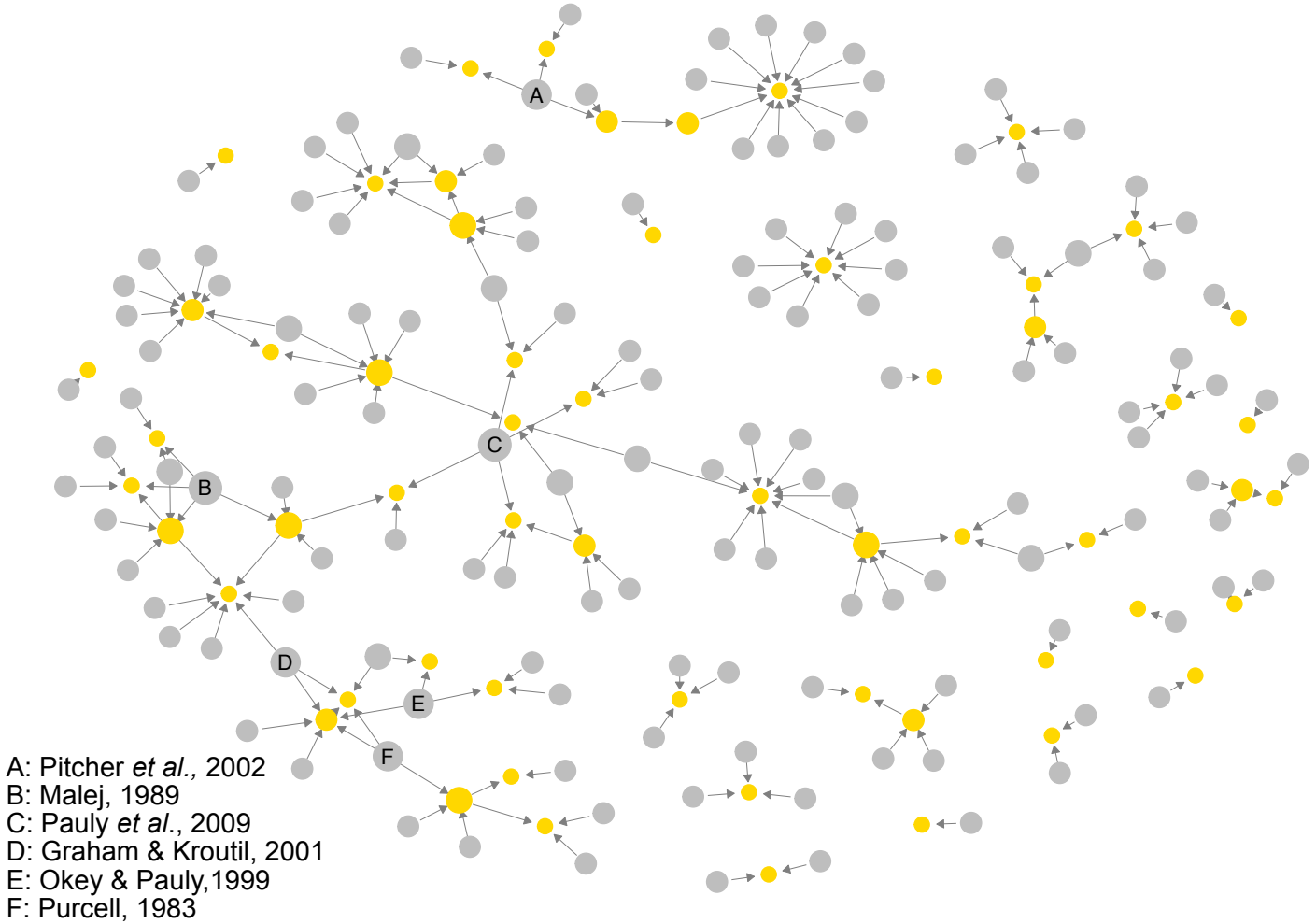

Figure 6.3. A directed citation network of EwE jellyfish models (gold nodes) and cited literature (grey nodes). The network only contains models with citations to other models or literature, models that used no other literature in parameterisation of the jellyfish group are not included. Additionally, only citations within models are included, references between literature are absent. The size of the nodes corresponds with the number of direct citations. Influential nodes, with more than 3 citations are labelled.

The B, Q/B, P/B, and EE for models containing jellyfish groups are shown in figure 6.4, full details can be found in supplementary table 6.3. Section a. shows estimates of biomass used. Most estimates cluster between 0.1 and 10 tonnes km^{-2} (although note the log scale). Two clear outliers can be seen at approximately 300 tonnes km^{-2}: these studies[25,26] both model the outbreak of *Mnemiopsis leidyi* blooms in the Black Sea which was responsible for fishery crashes and extirpation of some zooplankton and fish species[27] so extreme values are not unexpected. Values listed here are broadly consistent with those reported in the Jellyfish Database Initiative[28] (JeDI), although a true comparison is difficult as biomass is reported per volume in the database as opposed to area metrics used in EwE.

Section b. and c. (of figure 4) show the Q/B and P/B ratio respectively. A unimodal distribution of data is present in both measures, although there are outliers in Q/B ratio. It is unclear why the Q/B is set so high for the most extreme outlier[29]. The model is the Benguela upwelling, a marine ecosystem completely dominated by jellyfish after fishery collapse[30].The other outlier Q/B values belong to models of the Gulf of Maine, Mid-Atlantic Bight, Southern New England, and Georges Bank. The associated technical report[31] for all these models is unavailable so again it is unclear why such high values were chosen. P/B values are more tightly clustered, with no obvious outliers.

Section d (of figure 4), shows the distribution of EE values. Unlike, B, P/B, and Q/B a unimodal distribution is not present, instead EE has a roughly bimodal distribution with no obvious consensus and peaks at the 0 bin and just above the 0.75 bin. EE showed (figure 6.4) no significant trend through time ($p=0.88$) (Figure 6.5).

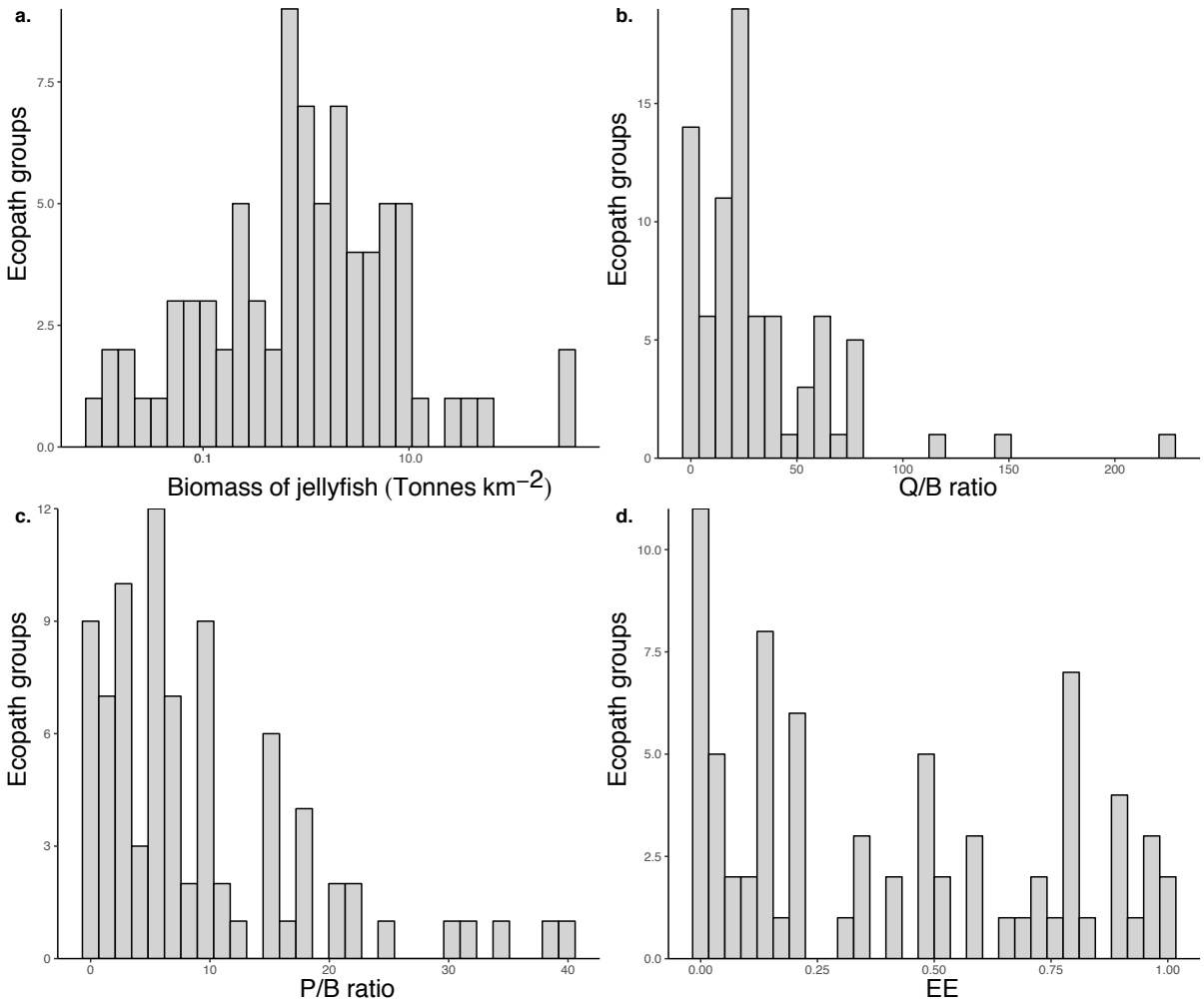

Figure 6.4. The **a.** Biomass, **b.** Q/B (consumption: biomass) ratio, **c.** P/B (production: biomass) ratio, and **d.** EE (ecotrophic efficiency) of jellyfish groups included in EwE models.

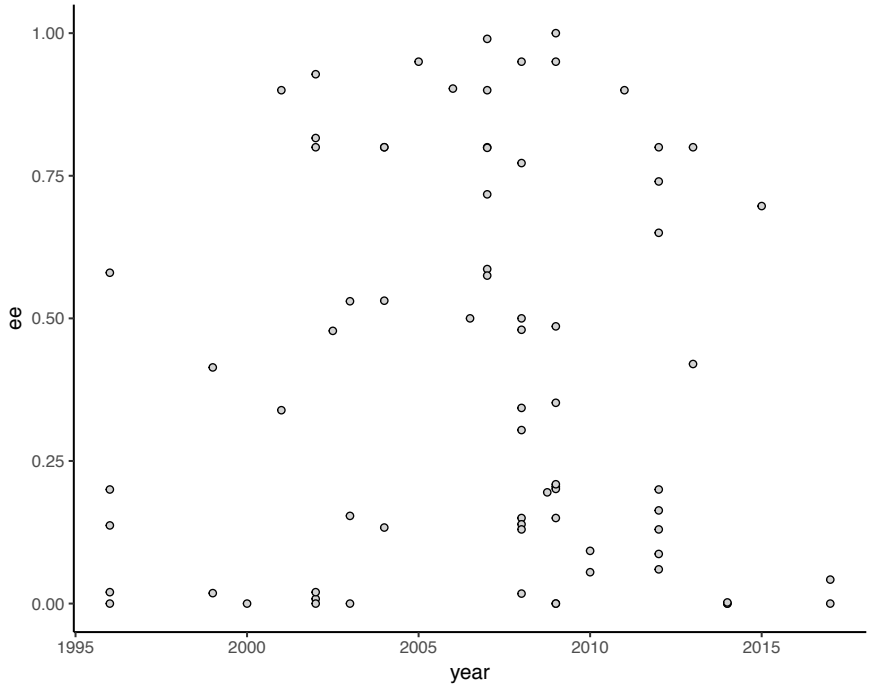

Figure 6.5. The ecotrophic efficiency reported in jellyfish groups over time.

Ecotrophic efficiency (EE) was the input parameter most frequently estimated by EwE (figure 6.6) through mass-balance. Figure 6.7 shows EE values when it was estimated, and when it was one of the input parameters. Since the data are not properly independent, nor normally distributed they did not meet the assumptions of a t-test or Mann-Whitney. However, it appears that the 0 bin is mainly populated by user-input values, while the 0.75 bin is largely comprised of EwE estimated values.

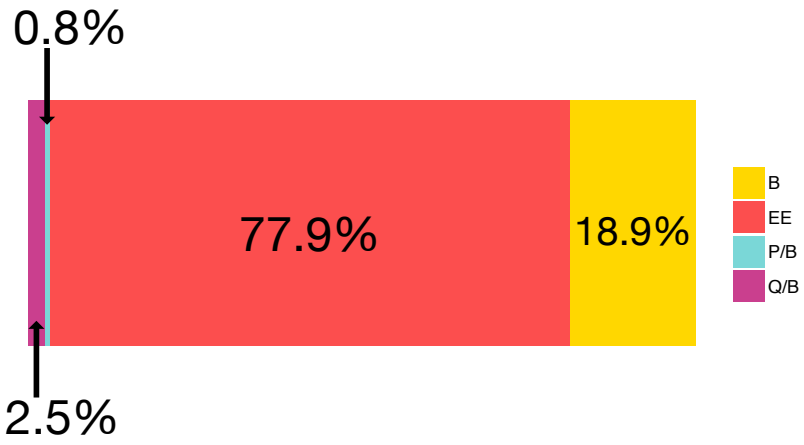

Figure 6.6. Estimated parameters in jellyfish groups (n=122).

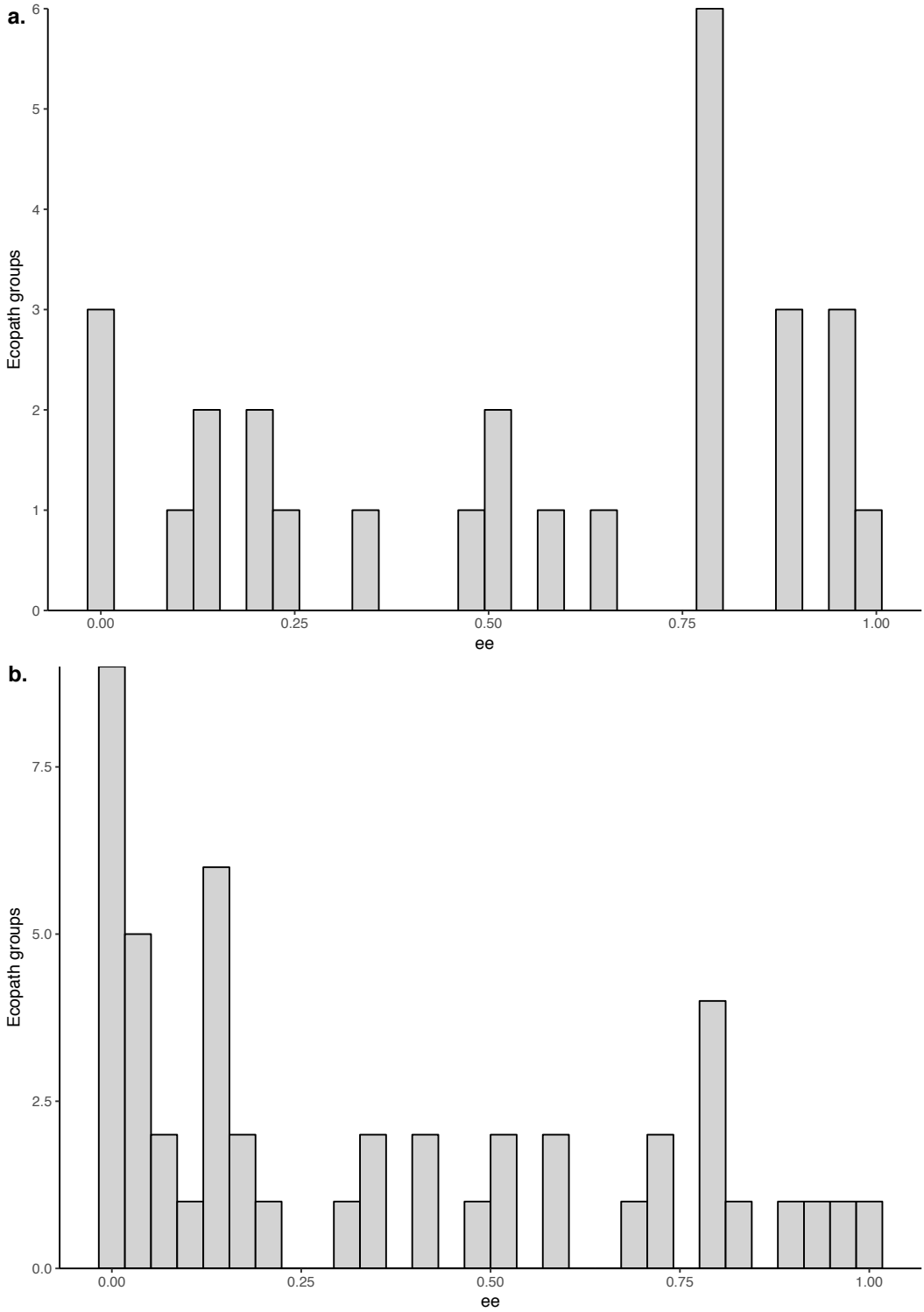

Figure 6.7. Distribution of EE values when **a.** it is estimated by the EwE software via mass-balance, and **b.** when it is directly input into the model.

The reported diet of jellyfish in models is shown in figure 6.8. Figure 6.9 details predators of jellyfish.

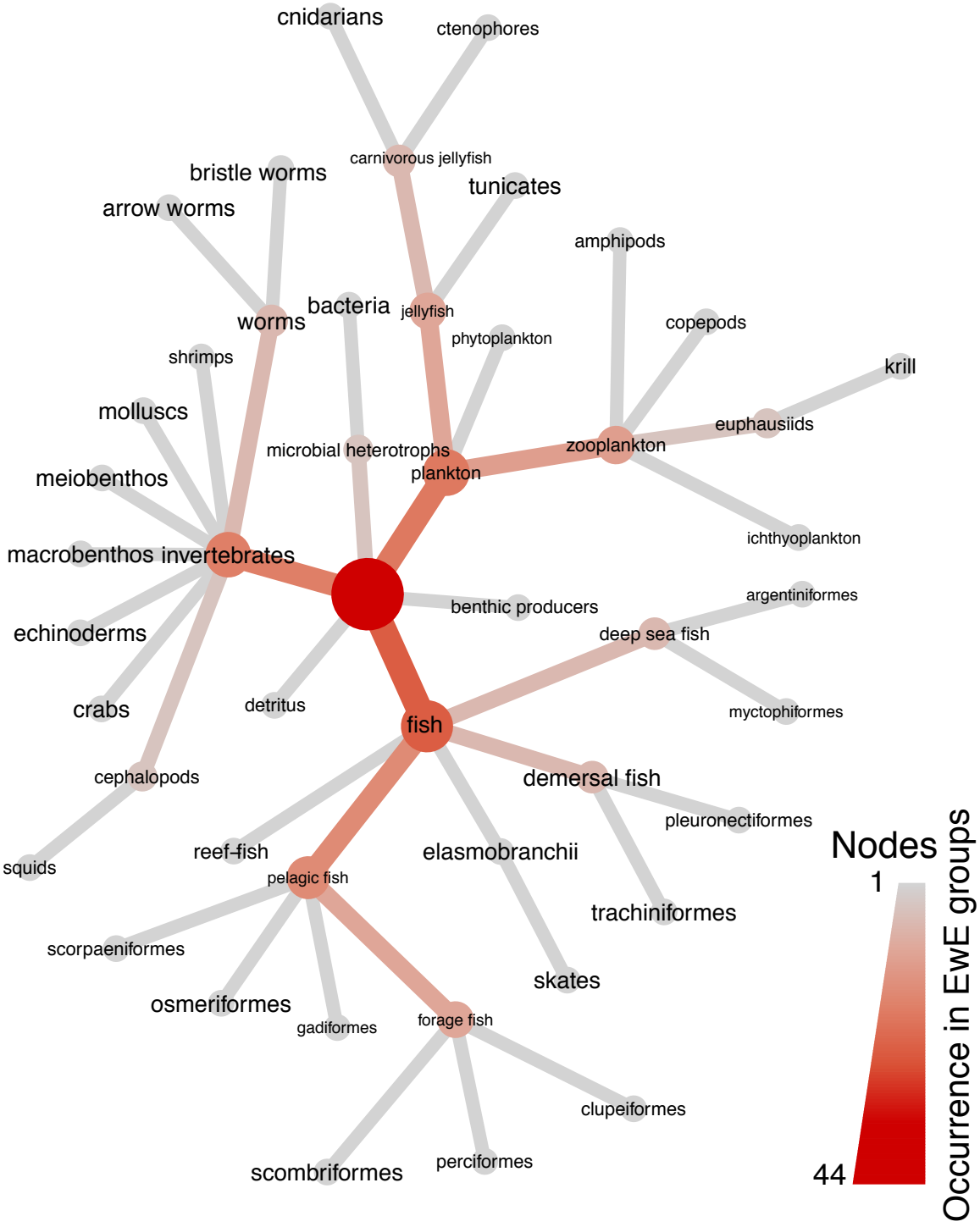

Figure 6.8. Reported prey items of jellyfish. Large nodes, and red hues, indicate prey is frequently reported in models. Smaller nodes, and tones close to grey, show that prey are infrequently listed in models. Note that the hierarchy is split into functional groups, to match the nomenclature found in models, rather than taxonomic classifications.

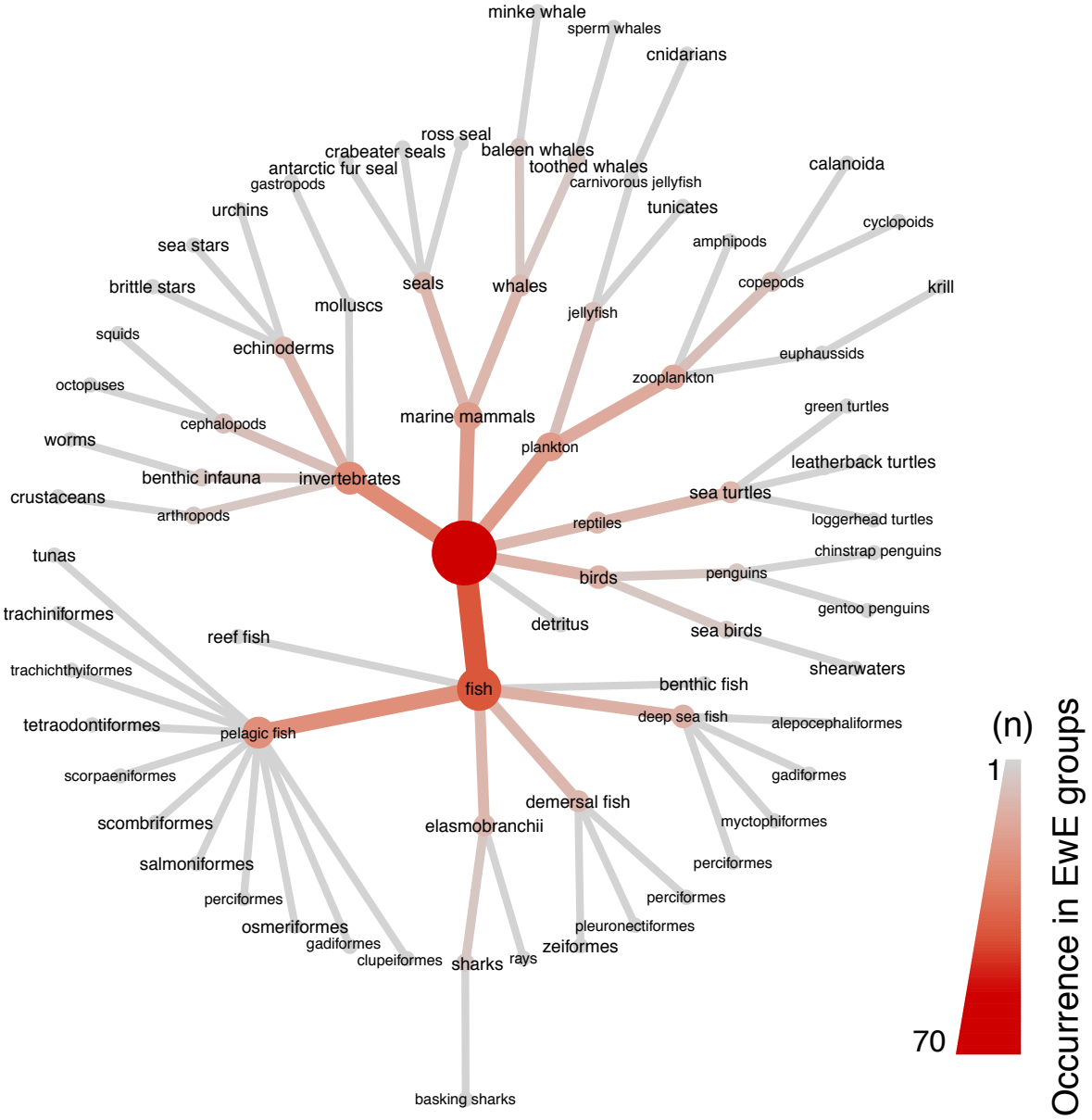

Figure 6.9. Predators of jellyfish. Large nodes, and red hues, indicate a predator is frequently reported in models. Smaller nodes, and tones close to grey, show that the predators are infrequently listed in models. Note that the hierarchy is split into functional groups, to match the nomenclature found in models, rather than taxonomic classifications.

6.5 Discussion

Three hundred and twenty nine EwE models were surveyed, of these 118 contained jellyfish, including 86 where jellyfish were treated as their own functional group. A broad geographic distribution of modelled systems was apparent, although biases towards North America and Europe where present. Taken together, a loose network structure was present, although 'keystone' literature, where a single paper or model disproportionately influences the state of the field, was not present. Values for P/B, Q/B, and B had normal distributions, while EE showed a very noisy bimodal distribution. User inputted EE values appeared to be lower than those estimated by the EwE software via 'mass-balance', although the nature of the data precluded formal statistical evaluation. A wide array of prey was indicated, mainly plankton and forage fish. Predators of jellyfish were extremely varied, although typically reflected interspecific relationships found elsewhere in the literature[32–35].

6.5.1 Prevalence of jellyfish in models

Most models do not include jellyfish (Figure 6.1). It is entirely appropriate that not every EwE model contain jellyfish: the vast majority of species are marine, and freshwater varieties are rare (although they do exist[37]) so freshwater models are unlikely to contain them. Additionally, models are created for many reasons and it is possible that jellyfish are relatively unimportant to the processes being explored in the model. However, these caveats aside, the importance of jellyfish in ecosystems is still not fully appreciated in the modelling community. Fortunately, this situation appears to be most prevalent in older models and trends are changing: an increase in the absolute, and relative, inclusion of jellyfish in EwE models was identified in figure 6.1. Increased representation in models may be influenced by two factors. First, interest and research in jellyfish has increased in recent years[38] in part due to the frequency with which observed blooms interfere with human enterprise and cause economic losses[39]. Second, the Pauly *et al.* 2009 publication *Jellyfish in ecosystems, online databases, and ecosystem models*[18] may have further accelerated uptake: the paper explicitly states that jellyfish are not being incorporated into EwE models, highlights the importance of jellyfish, and then summarises parameters used in models, making the

inclusion of jellyfish as an explicit group easier. Figure 6.3 supports this idea: as this particular review paper is the joint most-influential manuscript in the literature in terms of direct citations, however it remains the case that most models incorporating jellyfish primarily cite locally-derived literature.

6.5.2 Input parameters

Most of the parameters used in models, Q/B, P/B, and B, displayed unimodal distributions across all studies. However, it is unclear if the central tendency is an accurate portrayal of jellyfish biology and ecology. EwE was principally developed with the physiology and development of fish functional groups in mind, where energetic content of biomass is assumed to be the same across all functional groups[6]. However, jellyfish have much lower energy density than is typical for fish[40]. Modellers face a decision: should biomass be adjusted to represent relative energy flows or remain closer to values seen in the ecosystem? Further complications arise from temporal variation of jellyfish biomass, caused in part by jellyfishes' meta-genetic life cycle, where many (but not all) species spend time in the plankton, then in the benthos as small, sessile polyps until conditions favour strobilation and production of medusae[41]. No model in Ecobase has used a 'multi-stanza' approach, where different ontogenetic life stages are represented by linked groups within the model (such that population growth of adult groups is dependent on the success of juvenile life stages)[42], to simulate the functional differences seen during jellyfish development. Doing so may allow B, Q/B, and P/B values to be optimised to better reflect jellyfish ecology. If data on early life stages is difficult to obtain, it may be best to use a sine function in EwE's forcing mode to oscillate jellyfish populations through the year to produce more biologically realistic estimates.

6.5.3 Jellyfish as trophic dead-ends

Ecotrophic efficiency, the measure of what percentage of a functional group is utilised in the ecosystem is a particularly interesting as it is a measure of the trophic connectedness of a functional group. We anticipated that EE would reflect the state of the literature: older models would feature jellyfish as trophic deadends, while newer models would incorporate evidence suggesting jellyfish are more tightly integrated into wider food-webs than once thought and therefore generally have higher EE values. Unlike the other parameters which clustered around central values Figure 6.4, section

d. shows EE covers all possible values, with a rough bi-modal distribution, indicative of researchers not really knowing what an appropriate value might look like. To assess if this was an artefact of summarising many years of data, or genuine variability within the literature EE was plotted against publication year. Figure 6.5 illustrates the lack of a significant trend (p=0.88). Figure 6.6 shows which input parameters were estimated by EwE mass-balance; it was thought that perhaps user inputted values might better reflect current beliefs. However, figure 6.7 suggests this was not the case. Both sets of data were bi-modal, and had instances where featured groups originated from the same model and therefore did not meet the assumptions required for statistical testing. However, user-inputted data features the zero bin as most frequent EE suggesting many modellers were treating jellyfish as trophic dead-ends. This in-part reflects the EwE guidance for selecting values of EE. EE is very difficult to independently estimate and very few estimates exist. Low values are often associated with organisms that simply die-off following blooms (less than 0.5)[16]. However, even taking this guidance into account the values ascribed to jellyfish seem extreme. EE is very often used by researchers constructing an EwE model as a diagnostic index to determine whether a model is properly balanced. Most researchers are simply content, during the balancing process, if they can get the EEs of all groups to be below 1, without worrying too much about whether or not this makes sense physiologically. Therefore it is doubtful whether any ecologically useful insights can be inferred from the observed EE values that end up being used for jellyfish in many models.

Changing EE to match the current state of jellyfish ecology should be a priority as poorly optimised models will give erroneous predictions. It can be exceedingly difficult to measure EE in the 'wild'. Some authors such as Van Rooij *et al.*[43] have attempted this using exhaustive analysis of observational data, but this is not commonplace. Model specific EE for jellyfish could be more easily achieved either using quantitative diet assessment techniques such as stable isotope analysis[34] or ensuring the quality of other input parameters is sufficient to generate a biologically plausible EE.

6.5.4 The trophic ecology of jellyfish

Many species (or functional groups) included in models were consumed by jellyfish (figure 6.8). The reported diet of jellyfish contained few surprises, zooplankton and other jellyfish (frequent prey items described in the literature[44–46]) were common

prey items in models. Fish, particularly forage fish, were frequently listed as a prey item. However, in many instance it was unclear if this corresponded to ichthyoplankton and juvenile stages, or fully grown fish. Crabs appear like unlikely prey items: the mechanics of how a pelagic soft-bodied predator would eat clawed, hard-bodied, benthic prey are difficult to imagine. Three models included crabs as prey: Aydin *et al*[47], Lee *et al.*[48], and Trites *et al*.[49]. The cited literature for the Trites *et al.* model could not be accessed (old and out of print books). The Aydin and Lee models contain no references for prey items, and both list jellyfish as having very rich diets, 24 and 27 dietary items respectively, with very low frequency of predation listed. Although only the authors could say, these dietary items may have been included to help balance the model, rather than reflecting genuine inter-specific link, or it could reflect crab larvae that exist as part of the zooplankton. Phytoplankton was an unexpected prey item as cnidarian jellyfish and ctenophores are predators and are not normally associated with phytoplankton (but see[50]). Phytoplankton is consumed by herbivorous salps and tunicates[51,52], however even when only cnidarian jellyfish were included, phytoplankton was still observed as a prey item. The inclusion of phytoplankton can be attributed to a variety of factors. In one instance[53] it was a result of parameterising a model using a value from another model, the model which was cited[54] used a reference which could not be accessed. Akoglu[55] *et al.* cited two references, one which contained no reference to phytoplankton and another, a ctenophore paper, which was not accessible. Aydin *et al.*[56] provided no references. It appears Mackinson *et al.*[57] included phytoplankton to achieve Ecopath model balance (i.e. to remove excessive predation pressure on other groups and hence reduce their estimated EE values). Avoiding the use of broad jellyfish groups has been repeatedly advocated[18,58], in part, to avoid situations such as this where questionable assumptions about the basic ecology of jellyfish are made. To improve clarity on interspecific interactions moving forward, if a broad functional group must be used it would be best to carefully define exactly what species are included and which references correspond to which input parameters.

A wide variety of species predated jellyfish in models (figure 6.9). Many of the listed functional groups have been linked with jellyfish predation elsewhere in the literature including fish[34,59,60], sea birds[33,61] and penguins[62,63], turtles[64], other jellyfish[46], and benthic invertebrates[65–67]. Interestingly, many groups that jellyfish

feed upon, for example forage fish, jellyfish, and zooplankton, are themselves predators of jellyfish. This suggests that the ontogenetic dietary reversals, discussed in chapter three may be relatively common for jellyfish. However, with no model having multi-stanza jellyfish groups it is impossible to say if this is the mechanism responsible.

6.5.5 Citation network

Citations, used inappropriately, can propagate bias and erroneous findings throughout the literature[68]. Previously, Sanz-Martin *et al.* used network analysis to demonstrate that the perception that jellyfish populations were increasing was mainly a result of inappropriate citation practices throughout jellyfish literature[69]. Figure 6.3 shows a citation network of Ecopath models containing jellyfish groups. A loose network is formed, however the references used for parameterising jellyfish groups appear to be largely independent from one another. Eight four point seven % of nodes are cited only once, 11.3% cited twice. Papers with more than two citations are labelled: four papers were cited three times, and only Pauly *et al*[18] and Malej's[70] papers were cited four times.

The citations for Malej (1989) appear appropriate: the original study focused on *Pelagia noctiluca* in the Adriatic Sea. Barausse *et al*[53]., Coll et al.[71], and Libralato et al.[72] were all modelling the Adriatic Sea so the shared citation is unsurprising. The other model by Coll et al.[71] is in the Catalan Sea, but features the same jellyfish as the original study. Data from the Pauly *et al.*[18] manuscript was used to verify that a P/B value from a local source was biologically plausible[73], assign diet based on other studies[74], assign trophic level[75], and in one instance was used to borrow values from a model[71] in the same ecosystem.

Investigating the studies with three citations[76–79] revealed more questionable use of published data. Values were often borrowed or derived from very different ecosystems. For example a jellyfish group on the Falkland Islands[80] was parameterised with data obtained from Hong Kong harbour[78]. Similarly, jellyfish data from Alaska[77] was used to parameterise a jellyfish group in the Gulf of Carpentaria, Australia. We did not assess how frequently non-local data, or data derived from different species was used to parameterise jellyfish in all models. Fortunately, the constructed citation network suggests that only a loose network exists so questionable parametrisation of jellyfish

does not proliferate throughout the entire field, but instead remains in the original model for the most part. However, since direct references of models were used in network construction, (citations between references were not included) this may underestimate the extent to which knowledge is shared within the network. Due diligence to assess the quality of the original source of the data from a model should be undertaken, and results interpreted appropriately in-light of the quality and relevance of the original data.

6.5.6 Applying recommendations to Irish Sea model

Building upon data summarised in this chapter, and using data generated for this thesis it should be possible in improve the quality of the gelatinous zooplankton group in the Irish Sea EwE model[20]. The current biomass estimate of 0.605 tonnes km^{-2} for jellyfish comes from an estimate derived for the English Channel. However, sufficiently little bibliographic data is provided that the original document cannot be found. Assessment is impossible, and it is unclear the quality of the original biomass estimate of the first model. The Q/B estimate is based on values from the British Columbia shelf model, although the same reference as the 'English Channel' model is given within the technical report. Assessing the validity of the estimate is therefore challenging, as the Q/B does not match the Q/B associated with other British Columbia EwE models[81,82]. The diet composition of jellyfish is assumed to be entirely zooplankton and was derived from the aforementioned inaccessible English Channel model . Ecotrophic efficiency is set at 0.99, however no justification is given. This value seems too high: this value would be more indicative of an organism at the bottom of the food web, like phytoplankton. It seems improbable that 99% of the jellyfish biomass will be consumed within the ecosystem, especially as when the model was produced very few jellyfish predators were known in the system. The production: biomass ratio (P/B) was estimated by the model.

Table 6.1. Suggested input parameters for gelatinous zooplankton in the Irish Sea EwE model

	Name	B	Q/B	P/B	EE
Current Values	Gelatinous zooplankton	0.605	24.99	7.5	0.99
Suggested values	Scyphozoan jellyfish	5.65	24.99	7.5	-

Ideally scyphozoan jellyfish and ctenophores would be separated into different functional groups. Cnidarian jellyfish would be split into a multi-stanza group to reflect

different life history stages of polyps, ephyrae, and medusae. Parameterising 'multi-stanza' groups requires Von Bertalanffy Growth functions (VGBF) to model the transition from one stanza to the next. VGBFs for jellyfish are available[83], however data required to better parameterise the other inputs for the functional groups with this level of detail are currently unavailable. Using a simplified approach, using currently available data, the Irish Sea biomass (Q/B) can be derived: Lynam *et al*. landed a mean value of 555 g of scyphozoan jellyfish per 4,113 m^3 of the Irish Sea sampled over 16 years[84]. Assuming the Irish Sea has a volume of 2,430 km^3 [85], this would suggest the Irish Sea has 32,899.3 tonnes of jellyfish. Using a surface area of 58,000 km^2 [20], a biomass of 5.65 tonnes km^{-2} cnidarian jellyfish can be derived. This suggests the biomass estimate for jellyfish in the existing model[20] is a considerable underestimate of the real value.

Deriving values for consumption and production requires a knowledge of the jellyfish composition in the Irish Sea. In the Lynam *et al.* study *Aurelia aurita* was the most-caught jellyfish in the Irish Sea, accounting for 81.33% catch by weight, 16.66% was accounted for by *Cyanea* spp.[84]. Given, over 80% of jellyfish by weight are estimated to be *Aurelia aurita*, and since we are unaware of easily obtainable *Cyanea* spp. estimates, consumption and production, could be well approximated by a well-supported *Aurelia aurita* estimate from another model: Daskalov *et al*.[86] derived a Q/B and P/B of 29.2 and 10.95 respectively using four peer-reviewed *Aurelia aurita* studies. These values were derived for the Black Sea, a considerably warmer sea, so higher metabolic rates could be expected. We would expect the Irish Sea values be a bit lower. The existing values in the Irish Sea model reflect this, and are a good starting place for the group, although deriving new values from fresh data would yield more accurate estimates. Ecotrophic efficiency could be estimated by EwE.

Diet studies of jellyfish predators now exist for the Irish Sea[60]: therefore the functional groups that correspond to mackerel, herring, whiting, grey gurnard, dover sole, lesser-spotted dogfish, sprat, dragonet, poor cod, dab, and squid should be updated to reflect predator status. The diet of jellyfish seems broadly appropriate, and with no Irish Sea study of jellyfish diet, the only suggested amendment would be to include ichthyoplankton as these are common prey item of jellyfish[17] and it is difficult to envisage why this would not also be the case in the Irish Sea.

6.6 Conclusion

A detailed look at jellyfish groups in EwE models reveals a mixed picture. They are recognised as an important part of marine ecosystems around the world, and feature in a growing array of EwE models. Additionally, analysis suggests that while some network structure is present the models are parameterised somewhat independently and the spread of blanket values is limited. Yet, contrary to calls in the literature, jellyfish are often represented as a single functional group and in some of the examined cases the group has been parameterised in a questionable manner. Additionally, in no instance were the functionally diverse life stages of cnidarian jellyfish incorporated into a model. However, many of these issues can be attributed to lack of available data. Entire regions lack any ecosystem models incorporating jellyfish. Ongoing monitoring of wild populations of medusae is required to fill in data gaps, and further reduce uncertainty. Furthermore, future research is sorely needed to characterise the basic ecology and functional role of early jellyfish life stages in the ecosystem.

6.7 References

Some of the included references[25,31,47,56] had data extracted from Ecobase. Complete bibliographic details could not be obtained, as such the references are presented here with all available information.

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6.8 Supplementary information

Supplementary table 6.1 Taxonomic groups listed as jellyfish prey in the models (spellings are as they appear in models), and the aggregated groups they correspond to in figure 6.8

Group in model	Aggregated group
zooplankton	diet;plankton;zooplankton
jellyfish	diet;plankton;jellyfish
phytoplankton	diet;plankton;phytoplankton
reef-associated fish	diet;fish;reef-fish
microzooplankton	diet;plankton;zooplankton
mesozooplankton	diet;plankton;zooplankton
pico-nanophytoplankton	diet;plankton;phytoplankton
copepods	diet;plankton;zooplankton;copepods
carnivorous jellyfish	diet;plankton;jellyfish;carnivorous jellyfish
benthic producer	diet;benthic producers
_Chaetognatha	diet;invertebrates;worms;arrow worms
calanoids	diet;plankton;zooplankton;copepods
T.lepturus	diet;fish;pelagic fish;forage fish;perciformes
small copepods	diet;plankton;zooplankton;copepods
Capelin	diet;fish;pelagic fish;osmeriformes
macrozooplankton	diet;plankton;zooplankton
near omni-zooplankton	diet;plankton;zooplankton
large phytoplankton	diet;plankton;phytoplankton
krill	diet;plankton;zooplankton;euphausiids;krill
euphausiids	diet;plankton;zooplankton;euphausiids
micro-zooplankton	diet;plankton;zooplankton
juvenile pollock	diet;fish;pelagic fish;gadiformes

juvenile butterfish	diet;fish;pelagic fish;forage fish;scombriformes
herbivorous zooplanktons	diet;plankton;zooplankton
zooplankton	diet;plankton;zooplankton
small jellies	diet;plankton;jellyfish
marine zooplankton	diet;plankton;zooplankton
carnivorous zooplankton	diet;plankton;zooplankton
pollock	diet;fish;pelagic fish;gadiformes
bay anchovy	diet;fish;pelagic fish;forage fish;clupeiformes
sharks	diet;fish;elasmobranchii;sharks
detritus	diet;detritus
carnivorous jellies	diet;plankton;jellyfish;carnivorous jellyfish
jacks	diet;fish;pelagic fish;forage fish;perciformes
large zooplankton	diet;plankton;zooplankton
large herbivorous zooplankton	diet;plankton;zooplankton
small herbivorous zooplankton	diet;plankton;zooplankton
gelatinous plankton	diet;plankton;jellyfish
gelatinous zooplankton	diet;plankton;jellyfish
macro-zooplankton	diet;plankton;zooplankton
salps	diet;plankton;jellyfish;tunicates
small zooplankton	diet;plankton;zooplankton
meiobenthos	diet;invertebrates;meiobenthos
ciliates	diet;plankton;jellyfish;carnivorous jellyfish
littoral forage fish	diet;fish;pelagic fish; forage fish
microphytoplankton	diet;plankton;phytoplankton
detritus	diet;detritus
euphausiid(larvae)	diet;plankton;zooplankton;euphaussids
cyclopoids	diet;plankton;zooplankton;copepods
S.niphonius	diet;fish;pelagic fish;forage fish;scombriformes
other mesozooplankton	diet;plankton;zooplankton
small pelagic fish	diet;fish;pelagic fish
bacteria	diet;microbial heterotrophs;bacteria
omni-zooplankton	diet;plankton;zooplankton
micro and mesozooplankton	diet;plankton;zooplankton
small phytoplankton	diet;plankton;phytoplankton
juvenile herring	diet;fish;pelagic fish;forage fish;clupeiformes
juvenile large pelagic	diet;fish;pelagic fish
carnivorous zooplanktons	diet;plankton;zooplankton
Ichthyoplankton	diet;plankton;zooplankton; Ichthyoplankton
appendicularians	diet;plankton;jellyfish;tunicates
detritivorous polychaeta	diet;invertebrates;worms;bristle worms
estuarine zooplankton	diet;plankton;zooplankton
other zooplankton	diet;plankton;zooplankton
herbivorous zooplankton	diet;plankton;zooplankton
P.cod	diet;fish;pelagic fish;gadiformes

decapterus maruadsi	diet;fish;pelagic fish;forage fish;perciformes
shrimps	diet;invertebrates;shrimps
shallow demersals	diet;fish;demersal fish
pacific cod	diet;fish;pelagic fish;gadiformes
micro-zooplankton'cryptophytes	diet;plankton;zooplankton
worms	diet;invertebrates;worms
ctenophores	diet;plankton;jellyfish;carnivorous jellyfish;ctenophores
small gelatinous zooplankton	diet;plankton;jellyfish
large pelagic fishes	diet;fish;pelagic fish
harpacticoids	diet;plankton;zooplankton;copepods
benthic producers	diet;benthic producers
Stromateoidae	diet;fish;pelagic fish;forage fish;scombriformes
near herbi-zooplankton	diet;plankton;zooplankton
scyphomedusae	diet;plankton;jellyfish;carnivorous jellyfish;cnidarians
scyphomeduase	diet;plankton;jellyfish;carnivorous jellyfish;cnidarians
pelagic detritus	diet;detritus
krill larvae	diet;plankton;zooplankton;euphaussids;krill
squids	diet;invertebrates;cephalopods;squids
juvenile medium pelagic	diet;fish;pelagic fish
microbial heterotrophs	diet;microbial heterotrophs
noctituc	diet;plankton;zooplankton
mollusca	diet;invertebrates;molluscs
marine ichthyoplankton	diet;plankton;zooplankton;icthyoplankton
marine ichthyoplnakon	diet;plankton;zooplankton;icthyoplankton
pelagic microbes	diet;microbial heterotrophs
P.halibut	diet;fish;demersal fish;pleuronectiformes
ctenophores	diet;plankton;jellyfish;carnivorous jellyfish;ctenophores
trichiurus haumela	diet;fish;pelagic fish;forage fish;perciformes
chaetognaths	diet;invertebrates;worms;bristle worms
pacific halbut	diet;fish;demersal fish;pleuronectiformes
small zooplankon	diet;plankton;zooplankton
omniovorus zooplankton	diet;plankton;zooplankton
pleurobachia pileus	diet;plankton;jellyfish;carnivorous jellyfish;ctenophores
mesozooplankton	diet;plankton;zooplankton
small jellyfish	diet;plankton;jellyfish
small pelagic fishes	diet;fish;pelagic fish
copepodites	diet;plankton;zooplankton;copepods
E.japonicus	diet;fish;pelagic fish;forage fish;clupeiformes
macrobenthos	diet;invertebrates;macrobenthos
microflora	diet;plankton;phytoplankton
herbi-zooplankton	diet;plankton;zooplankton
chaetognaths	diet;invertebrates;worms;bristle worms
carniv-zoops	diet;plankton;zooplankton
bathylagidae	diet;fish;deep sea fish;argentiniformes

juvenile small pelagic	diet;fish;pelagic fish
peneida&caridae	diet;invertebrates;shrimps
marine microbial heterotrophs	diet;microbial heterotrophs
Arrowtooth flounder	diet;fish;demersal fish;pleuronectiformes
psenopsis anomala	diet;fish;pelagic fish;forage fish;clupeiformes
greenland turbot	diet;fish;demersal fish;pleuronectiformes
diatoms	diet;plankton;phytoplankton
Sagitta setosa	diet;invertebrates;worms;arrow worms
euphasiid(eggs)	diet;plankton;zooplankton;euphaussids
larvae	diet;plankton;zooplankton;icthyoplankton
large copepods	diet;plankton;zooplankton;copepods
near phytoplankton	diet;plankton;phytoplankton
mesok	
other large zooplankton	diet;plankton;zooplankton
myctophidae	diet;fish;deep sea fish;myctophiformes
juvenile lare reef associated	diet;fish;pelagic fish;forage fish
water column detritus	diet;detritus
other detritus benthos	diet;detritus
estuarine phytoplankton	diet;plankton;phytoplankton
estuarine microbial heterotrophs	diet;microbial heterotrophs
Small flatfish	diet;fish;demersal fish;pleuronectiformes
upeneus bensasi	diet;fish;pelagic fish;forage fish;perciformes
crab larvae	diet;invertebrates;crabs
ice algae	diet;plankton;phytoplankton
cryptophytes	diet;plankton;phytoplankton
fish larvae	diet;plankton;zooplankton;icthyoplankton
fish eggs	diet;plankton;zooplankton;icthyoplankton
offshore phytoplankton	diet;plankton;phytoplankton
capeline	capelin
juvenile medium reef associated	diet;fish;pelagic fish;forage fish
echinodermata	diet;invertebrates;echinoderms
marine water-column detritus	diet;detritus
marine phytoplankton	diet;plankton;phytoplankton
skates	diet;fish;elasmobranchii;skates
other pelagics	diet;fish;pelagic fish
seaweeds	diet;plankton;phytoplankton
flathead sole	diet;fish;demersal fish;pleuronectiformes
other phytoplankton	diet;plankton;phytoplankton
water column detritus	diet;detritus
detritus	diet;detritus
detritus	diet;detritus
mespelagics	diet;fish;pelagic fish
mesopelagics	diet;fish;pelagic fish
sand lance	diet;fish;demersal fish;trachiniformes

juvenile small reef associated	diet;fish;pelagic fish;forage fish
sculpins	diet;fish;pelagic fish;scorpaeniformes
yellowfin sole	diet;fish;demersal fish;pleuronectiformes
larval juvenile fish	diet;plankton;zooplankton;icthyoplankton
eulachon	diet;fish;pelagic fish;osmeriformes
juvenile large demersal	
detached estuarine macrophytes	
sablefish	diet;fish;pelagic fish;scorpaeniformes
small pelagics-commercial	diet;fish;pelagic fish
small pelagics-other	diet;fish;pelagic fish
small pelagics-squid	diet;invertebrates;cephalopods;squids
small pelagics-anadromous	diet;fish;pelagic fish
small pelagics-commerical	diet;fish;pelagic fish
managed forage	diet;fish;pelagic fish; forage fish
other pelagic smelt	diet;fish;pelagic fish
pelagic gelatinous filter feeder	diet;plankton;jellyfish;tunicates
juvenile small demersal	
juvenile large planktivore	diet;fish;pelagic fish; forage fish
juvenile small planktivore	diet;fish;pelagic fish; forage fish
juvenile anchovy	diet;fish;pelagic fish;forage fish;clupeiformes
juvenile deepwater fish	diet;fish;deep sea fish
juvenile macro algal browsing	diet;fish;pelagic fish; forage fish
juvenile eroding grazers	diet;fish;reef-fish
juvenile scraping grazers	diet;fish;reef-fish
estuarine sediment detritus	diet;detritus
rockfish	diet;fish;reef-fish
macrouridae	diet;fish;pelagic fish;gadiformes
zoarcidae	diet;fish;pelagic fish;forage fish;perciformes
tanner crab	diet;invertebrates;crabs
snow crab	diet;invertebrates;crabs
king crab	diet;invertebrates;crabs
benthic amphipods	diet;plankton;zooplankton;amphipods
P.herring	diet;fish;pelagic fish;forage fish;clupeiformes
cephplapods	diet;invertebrates;cephalopods;squids
forage fish	diet;fish;pelagic fish; forage fish
alaska placie	diet;fish;demersal fish;pleuronectiformes
saketes	diet;fish;elasmobranchii;skates
macrouridate	diet;fish;pelagic fish;gadiformes
shrimp	diet;invertebrates;shrimps
pacific herring	diet;fish;pelagic fish;forage fish;clupeiformes
cephalpods	diet;invertebrates;cephalopod
Forage fish	diet;fish;pelagic fish; forage fish
norway pout	diet;fish;demersal fish;pleuronectiformes
other gadoids	diet;fish;pelagic fish;gadiformes

small mobile epifauna	diet;invertebrates;worms
planktonic microflora	diet;plankton;phytoplankton

Supplementary table 6.2 Taxonomic groups listed as jellyfish predators in the models (spellings are as they appear in models), and the aggregated groups they correspond to in figure 6.9

Group in model	**Aggregated group**
macrourids	pred;fish;deep sea fish;gadiformes
snappers	pred;fish;pelagic fish;perciformes
sea birds	pred;birds;sea birds
leatherback turtles	pred;reptiles;sea turtles;leatherback turtles
macro-zooplankton	pred;plankton;zooplankton
jellyfish	pred;plankton;jellyfish
sea nettles	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians
gelatinous zooplankton	pred;plankton;jellyfish
atlantic mackerel	pred;fish;pelagic fish;scombriformes
seabirds	pred;birds;sea birds
horse mackerel	pred;fish;pelagic fish;perciformes
filefish	pred;fish;pelagic fish;tetraodontiformes
predatory gastropods	pred;invertebrates;molluscs;gastropods
small gelatinous zooplankton	pred;plankton;jellyfish
Appendicularians	pred;plankton;jellyfish;tunicates
cyclopoids	pred;plankton;zooplankton;copepods;cyclopoids
sharks	pred;fish;elasmobranchii;sharks
marine mammals	pred;marine mammals
rays and skates	pred;fish;elasmobranchii;rays
other demersal fish	pred;fish;demersal fish
Turtles	pred;reptiles;sea turtles
krill	pred;plankton;zooplankton;euphaussids;krill
large copepods	pred;plankton;zooplankton;copepods
large pelagic	pred;fish;pelagic fish
spiny dogfish	pred;fish;elasmobranchii;sharks
squid	pred;invertebrates;cephalopods;squids
Sandeels	pred;fish;pelagic fish;trachiniformes
gelatinous zooplankton	pred;plankton;jellyfish
scyphomedusa	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians
scyphomedusae	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians
tuna billfishes	tuna & billfishes
norway pout	pred;fish;pelagic fish;gadiformes
pelagic fish	pred;fish;pelagic fish
rorcuals	pred;marine mammals;whales;baleen whales
salmon	pred;fish;pelagic fish;salmoniformes
Benthic infauna	pred;invertebrates;benthic infauna
misc. shallow fish	pred;fish;pelagic fish
scyphozoid jellies	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians

green turtles	pred;reptiles;sea turtles;green turtles
ilex squid	pred;invertebrates;cephalopods;squids
carnivorous benthos	pred;invertebrates;benthic infauna
Large-mouth sculpin	pred;fish;pelagic fish;scorpaeniformes
large pelagics	pred;fish;pelagic fish
Pitcher2002	
chum	pred;fish;pelagic fish;salmoniformes
Sea turtles	pred;reptiles;sea turtles
misc. pelagics	pred;fish;pelagic fish
leiognathids	pred;fish;pelagic fish;perciformes
shallow sharks	pred;fish;elasmobranchii;sharks
baleen whales	pred;marine mammals;whales;baleen whales
ross seal	pred;marine mammals;seals;ross seal
aurelia aurita	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians
billfish tuna	tuns & billfishes
hake	pred;fish;pelagic fish;gadiformes
common mora	pred;fish;pelagic fish;gadiformes
other tuna	pred;fish;pelagic fish;tunas
dogfish	pred;fish;elasmobranchii;sharks
other gadiformes	pred;fish;pelagic fish;gadiformes
other small pelagics(fish)	pred;fish;pelagic fish
large jellyfish	pred;plankton;jellyfish;carnivorous jellyfish;cnidarians
chinook	pred;fish;pelagic fish;salmoniformes
small fish	pred;fish;pelagic fish
small mouthed flatfish	pred;fish;demersal fish
marine turtles	pred;reptiles;sea turtles
Copeoda calanoida	pred;plankton;zooplankton;copepods;calanoida
decapterus maruadsi	pred;fish;pelagic fish;perciformes
sharks and rays	pred;fish;elasmobranchii;sharks
elasmobranchs	pred;fish;elasmobranchii
medium sciaenids	pred;fish;pelagic fish
lgoceplank	pred;fish;pelagic fish
amphipods	pred;plankton;zooplankton;amphipods
cod	pred;fish;pelagic fish;gadiformes
butterfishes	pred;fish;pelagic fish;perciformes
sea turtle	pred;reptiles;sea turtles
sablefish	pred;fish;pelagic fish;scorpaeniformes
demersal fishes	pred;fish;demersal fish
large zooplankton	pred;plankton;zooplankton
demersal sharks	pred;fish;elasmobranchii;sharks
other gadoids(small)	pred;fish;pelagic fish;gadiformes
red snapper	pred;fish;pelagic fish;perciformes
Notothenia rossii	rock cod (perciformes)
atka mackerel	pred;fish;pelagic fish;scorpaeniformes

other sebastes	pred;fish;pelagic fish;scorpaeniformes
dusky rockfish	pred;fish;pelagic fish;scorpaeniformes
squids	pred;invertebrates;cephalopods;squids
oceanic turtles	pred;reptiles;sea turtles
patagonian squid	pred;invertebrates;cephalopods;squids
reef sharks	pred;fish;elasmobranchii;sharks
ponyfishes,pinkies and trumpters	ponyfishes,pinkies and trumpters
demersal fish	pred;fish;demersal fish
echinodermata	pred;invertebrates;echinoderms
benthic-feeding fish	pred;fish;benthic fish
squids and cuttlefish	pred;invertebrates;cephalopods
small benthopealgic invert feeders	pred;invertebrates
misc demersals	pred;fish;demersal fish
other rays	pred;fish;elasmobranchii;rays
cluepoids	pred;fish;pelagic fish;clupeiformes
small pelagics	pred;fish;pelagic fish
small demersals	pred;fish;demersal fish
coryphanoides L	pred;fish;deep sea fish;gadiformes
basking sharks	pred;fish;elasmobranchii;sharks;basking sharks
crabeater seal	pred;marine mammals;seals;crabeater seals
sperm whales	pred;marine mammals;whales;toothed whales;sperm whales
oceanic small pelagics	pred;fish;pelagic fish
large sharks	pred;fish;elasmobranchii;sharks
juvenile pollock	pred;fish;pelagic fish;gadiformes
medium mesopelagic fishes	pred;fish;pelagic fish
forage fisih	pred;fish;pelagic fish
smelt	pred;fish;pelagic fish;osmeriformes
other salmon	pred;fish;pelagic fish;salmoniformes
sea stars	pred;invertebrates;echinoderms;sea stars
macrozooplankton	pred;plankton;zooplankton
large croakers	pred;fish;pelagic fish;perciformes
Copepoda cyclopoida	pred;plankton;zooplankton;copepods;cyclopoids
trichiurus haumela	pred;fish;pelagic fish;perciformes
S. lalandi	pred;fish;pelagic fish;perciformes
pelocejelly/eaters	pred
small pelagics-commercial	pred;fish;pelagic fish
micronekton	pred;invertebrates
pollock	pred;fish;pelagic fish;gadiformes
forage fish	pred;fish;pelagic fish
demersal invertebrate-eaters	pred;fish;demersal fish
salmon fry	pred;fish;pelagic fish;salmoniformes
loggerhead turtle	pred;reptiles;sea turtles;loggerhead turtles
mesopk	pred;plankton;zooplankton
alepocephalids	pred;fish;deep sea fish;alepocephaliformes

fish larvae	pred;fish;pelagic fish
juv rock	pred;fish;pelagic fish;scorpaeniformes
sharpchin rockfish	pred;fish;pelagic fish;scorpaeniformes
rougheye rockfish	pred;fish;pelagic fish;scorpaeniformes
sabelfish	pred;fish;pelagic fish;scorpaeniformes
sculpins	pred;fish;pelagic fish;scorpaeniformes
adult snappers	pred;fish;pelagic fish;perciformes
codling	pred;fish;pelagic fish;gadiformes
squid and cuttlefishes	pred;invertebrates;cephalopods;squids
octopus	pred;invertebrates;cephalopods;octopuses
piscivorous rays	pred;fish;elasmobranchii;rays
sessile epibethos	pred;invertebrates;benthic infauna
squids and cuttlefishes	pred;invertebrates;cephalopods
carnivorous jellies	pred;plankton;jellyfish
orange roughy	pred;fish;pelagic fish;trachichthyiformes
juvenile haddock	pred;fish;pelagic fish;gadiformes
sperm whale	pred;marine mammals;whales;toothed whales;sperm whales
gentoo penguins	pred;birds;penguins;gentoo penguins
coastal omnivores	pred
mesopelagic crustaceans	pred;invertebrates;arthropods;crustaceans
large pelagic (fish)	pred;fish;pelagic fish
transient salmon	pred;fish;pelagic fish;salmoniformes
piscivorous fish	pred;fish;pelagic fish
benthopelagic fish	pred;fish;pelagic fish
small pelagic (fish)	pred;fish;pelagic fish
eulachon	pred;fish;pelagic fish;osmeriformes
benthic fish	pred;fish;benthic fish
mackerel	pred;fish;pelagic fish;clupeiformes
anchovy	pred;fish;pelagic fish;clupeiformes
small demersal fish	pred;fish;demersal fish
small demersal fish	pred;fish;demersal fish
upeneus bensasi	pred;fish;pelagic fish;perciformes
S. niphonius	pred;fish;pelagic fish;scombriformes
sessile epibenthos	pred;invertebrates;benthic infauna
demersals-benthivores	pred;fish;demersal fish
medium pelagics	pred;fish;pelagic fish
small pelagics-other	pred;fish;pelagic fish
L benth	pred;fish;benthic fish
benthic carnivores	pred;fish;benthic fish
warty oreo	pred;fish;demersal fish;zeiformes
shrimp	pred;invertebrates;arthropods;crustaceans;shrimps
sand demersals	pred;fish;demersal fish
yellowtail	pred;fish;pelagic fish;scorpaeniformes
large demersals	pred;fish;demersal fish

pacific ocean perch	pred;fish;pelagic fish;scorpaeniformes
shortspine thornyhead adult	pred;fish;pelagic fish;scorpaeniformes
rock cod	pred;fish;pelagic fish;gadiformes
large jellies	pred;plankton;jellyfish
bairds smoothhead	na
chinstrap pengins	pred;birds;penguins;chinstrap penguins
flying birds	pred;birds;sea birds
detritus	pred;detritus
large reef associates (fish)	pred;fish;reef fish
juvenile fish	pred;fish
medium pelagic fishes	pred;fish;pelagic fish
juvenile salmon	pred;fish;pelagic fish;salmoniformes
mesopelagics	pred;fish;pelagic fish
carnivorous jellyfish	pred;plankton;jellyfish
juvenile pink salmon	pred;fish;pelagic fish;salmoniformes
pomfrets	pred;fish;pelagic fish;perciformes
other pelagics	pred;fish;pelagic fish
Stromateoidae	pred;fish;pelagic fish;scombriformes
demersals-omnivores	pred;fish;demersal fish
small pelagics-squid	pred;invertebrates;cephalopods;squids
skates	pred;fish;elasmobranchii;rays
urchins	pred;invertebrates;echinoderms;urchins
other oreos	pred;fish;demersal fish;zeiformes
small mobile epifauna	pred;invertebrates;benthic infauna
reef demersals	pred;fish;reef fish
black	pred;fish;pelagic fish;scorpaeniformes
electrona antarica	pred;fish;deep sea fish;myctophiformes
arrowthooth flounder	pred;fish;demersal fish;pleuronectiformes
yellowfin sole	pred;fish;demersal fish;pleuronectiformes
adult large sharks	pred;fish;elasmobranchii;sharks
snoek juvenile	pred;fish;pelagic fish;scombriformes
bulls eye black cardinalfish	pred;fish;deep sea fish;perciformes
epifaunal macro-benthos	pred;invertebrates;benthic infauna
flying birds	pred;birds;sea birds
cephalopods	pred;invertebrates;cephalopods
medium reef associates (fish)	pred;fish;reef fish
large pelagic fishes	pred;fish;pelagic fish
juvenile rockfish	pred;fish;pelagic fish;scorpaeniformes
demersals-piscivores	pred;fish;demersal fish
macro zoobenthos	pred;invertebrates;benthic infauna
depth demersals	pred;fish;demersal fish
sloperock	pred;fish;pelagic fish;scorpaeniformes
ssthorny	pred;fish;pelagic fish;scorpaeniformes
A.gazella	pred;marine mammals;seals;antarctic fur seal

flathead sole	pred;fish;demersal fish;pleuronectiformes
manta ray	pred;fish;elasmobranchii;rays
southern blue whiting	pred;fish;pelagic fish;gadiformes
benthic teleosts	pred;fish
infaunal macro-benthos	pred;invertebrates;benthic infauna
other icefish	pred;fish;demersal fish;perciformes
large planktivore	pred;fish;pelagic fish
juvenile fish	pred;fish
rockfish	pred;fish;pelagic fish;scorpaeniformes
juvenile wild salmon	pred;fish;pelagic fish;salmoniformes
sharks-pelagics	pred;fish;elasmobranchii;sharks
sharks-coastal	pred;fish;elasmobranchii;sharks
redfish	pred;fish;deep sea fish;myctophiformes
transitory squid	pred;invertebrates;cephalopods;squids
zooplankton	pred;plankton;zooplankton
small flat	pred;fish;demersal fish;pleuronectiformes
lsthory	pred;fish;pelagic fish;scorpaeniformes
L. carcinophagus	pred;marine mammals;seals;crabeater seals
northern rockfish	pred;fish;pelagic fish;scorpaeniformes
rock sole	pred;fish;demersal fish;pleuronectiformes
alaska plaice	pred;fish;demersal fish;pleuronectiformes
juvenile medium pelagic	pred;fish;pelagic fish
hoki	pred;fish;pelagic fish;gadiformes
cephalopods	pred;invertebrates;cephalopods
infaunal mesobenthos	pred;invertebrates;benthic infauna
toothfish	pred;fish;demersal fish;perciformes
small planktivore	pred;fish;pelagic fish
coho	pred;fish;pelagic fish;salmoniformes
wild salmon	pred;fish;pelagic fish;salmoniformes
HMS	
A plaice	pred;fish;demersal fish;pleuronectiformes
coast soft micro carn telo	pred;fish
non-migratory small fish	pred;fish;pelagic fish
shearwater	pred;birds;sea birds;shearwaters
juvthorny	pred;fish;pelagic fish;scorpaeniformes
birds	pred;birds
alaska plaice	pred;fish;demersal fish;pleuronectiformes
arrowtooth flounder	pred;fish;demersal fish;pleuronectiformes
adult small pelagic	pred;fish;pelagic fish
basking shark	pred;fish;elasmobranchii;sharks;basking sharks
gelatinous plankton	pred;plankton;jellyfish
cephlapods	pred;invertebrates;cephalopods
large nototheniidae	pred;fish;demersal fish;perciformes
deepwater fish	pred;fish;deep sea fish

juvenile hatchery salmon	pred;fish;pelagic fish;salmoniformes
flounders	pred;fish;demersal fish;pleuronectiformes
coast sm dem reef plk telo	pred;fish
rex	pred;fish;demersal fish;pleuronectiformes
B.acutorostrata	pred;marine mammals;whales;baleen whales;minke whale
misc. deepfish	pred;fish;deep sea fish
trubot	pred;fish;demersal fish;pleuronectiformes
halibut	pred;fish;demersal fish;pleuronectiformes
juvenile small pelagic	pred;fish;pelagic fish
small nototheniidae	pred;fish;demersal fish;perciformes
hatchery salmon	pred;fish;pelagic fish;salmoniformes
odontocetes	pred;marine mammals;whales;toothed whales
haddock	pred;fish;pelagic fish;gadiformes
coast sch carn pel telo fl	pred;fish
junvenile smallpealgic	pred;fish;pelagic fish
toothed whales, dolphins & porpoises	pred;marine mammals;whales;toothed whales
carnivorous zooplankton	pred;plankton;zooplankton
deep demersals large	pred;fish;demersal fish
other pelagic	pred;fish;pelagic fish
oceanic planktivores	pred;fish;pelagic fish
alaska plaice	pred;fish;demersal fish;pleuronectiformes
adult large reef associated	pred;fish;reef fish
seals and sea lions	pred;marine mammals;seals
shallow demersals	pred;fish;demersal fish
deep demersals small	pred;fish;demersal fish
sm pel plk telo	pred;fish
turbot	pred;fish;demersal fish;pleuronectiformes
juvenile large reef associated	pred;fish;reef fish
deep demersals large	pred;fish;demersal fish
flatfih(benthic)	pred;fish;demersal fish;pleuronectiformes
flatfish(small)	pred;fish;demersal fish;pleuronectiformes
sm pel carn tel fl	pred;fish
mig mesopelagic	pred;fish;pelagic fish
whitebl skate	pred;fish;elasmobranchii;rays
mud skate	pred;fish;elasmobranchii;rays
alakan skate	pred;fish;elasmobranchii;rays
adult small reef associated	pred;fish;reef fish
juvenile small reef associated	pred;fish;reef fish
adult large planktivore	pred;fish;pelagic fish
juvenile large planktivore	pred;fish;pelagic fish
adult small planktivore	pred;fish;pelagic fish
adult anchovy	pred;fish;pelagic fish;clupeiformes
juvenile anchovy	pred;fish;pelagic fish;clupeiformes
adult deepwater fish	pred;fish;deep sea fish

juvenile deepwater fish	pred;fish;deep sea fish
adult scraping grazers	pred;fish
juvenile scraping grazers	pred;fish
jellyfish and hydroids	pred;plankton;jellyfish
deep demerals small	pred;fish;demersal fish
myctophids	pred;fish;deep sea fish;myctophiformes
champsocephalus gunnari	pred;fish;demersal fish;perciformes
pleuragramma antarcticum	pred;fish;demersal fish;perciformes
notothenia gibberifrons	pred;fish;demersal fish;perciformes
cnidaria	pred;plankton;jellyfish
arthropod other	pred;invertebrates;arthropods
worms	pred;invertebrates;benthic infauna;worms
asteroidae	pred;invertebrates;echinoderms;sea stars
pleuragramm antarcticum	pred;fish;demersal fish;perciformes
arhropod crustecea	pred;invertebrates;arthropods;crustaceans
echinoidea	pred;invertebrates;echinoderms
ophiuroidea	pred;invertebrates;echinoderms;brittle stars
spur-dog	pred;fish;elasmobranchii;sharks

Supplementary table 6.3 Input parameters of jellyfish functional groups in the models investigated

First Author	Year	B	P/B	Q/B	EE	Estimated input	Name of group	Model start Year	Reference
Tecchio	2013	0.08	22.00	56.00	0.42	EE	gelatinous zooplankton	2009	[87]
	2007	0.22	10.23	26.46	0.98	EE	jellyfish & hydroids	1990	
Pitcher	2007	0.30	10.23	26.46	0.45	EE	jellyfish & hydroids	2005	[88]
Li	2010	12.50	9.60	20.00	na	EE	jellyfish	2005	[89]
Griffiths	2010	2.12	32.00	70.00	0.06	EE	gelatinous zooplankton	2004	[90]
Ruzicka	2012	23.30	15.00	60.00	0.06	EE	gelatinous zooplankton	2006	[91]
Tsagarakis	2010	2.48	4.84	12.09	0.09	EE	jellyfish & M.leidyi	2003	[74]
		0.58	5.00	20.00	0.00	EE;Q/B	sea nettles	1950	
Christensen	2009	3.40	8.80	35.20	0.21	EE;Q/B	ctenophores	1950	[92]
		26.07	0.44	1.47	0.50	EE	gelatinous zooplankton	1600	
		20.11	0.44	1.47	0.50	EE	gelatinous zooplankton	1900	
		28.47	0.44	1.47	0.50	EE	gelatinous zooplankton	1967	
		245.66	0.44	1.47	0.15	EE	gelatinous zooplankton	1990	
		5.00	0.58	1.67	0.17	EE	gelatinous zooplankton	1900	
		5.00	0.58	1.67	0.17	EE	gelatinous zooplankton	1600	
Watermeyer	2008	5.00	0.58	1.67	0.15	EE	gelatinous zooplankton	1960	[93]
Bānaru	2012	0.04	18.00	38.00	0.20	dc	gelatinous zooplankton	2000	[94]
		3.08	9.00	30.00	0.90	B	small jellyfish	2000	
		0.86	15.00	60.00	0.86	EE	large jellyfish	2000	
		8.95	9.00	30.00	0.90	B	small jellyfish	2000	
Ruzicka	2007	3.27	15.00	60.00	0.29	EE	large jellyfish	2000	[95]
Ainsworth	2002	3.00	18.00	60.00	0.69	EE	carnivorous jellyfish	2000	[81]

		3.00	18.00	60.00	0.67	EE	carnivorous jellyfish	1950	
		3.36	18.00	60.00	0.95	EE	carnivorous jellyfish	1900	
		4.63	18.00	60.00	0.95	EE	carnivorous jellyfish	1750	
Arancibia	2008	7.77	0.58	1.42	0.15	B	jellies	2000	[96]
Cheng	2009	0.68	5.00	20.00	0.35	EE	jellyfish	2000	[97]
		0.16	2.06	10.30	0.10	B	tunicates	2000	
		6.62	9.00	30.00	0.80	B	small gelatinous zooplankton	2000	
		8.26	3.00	11.50	0.50	B	jellyfish	2000	
		0.18	2.06	10.30	0.08	B	tunicates	1981	
		8.25	9.00	30.00	0.80	B	small gelatinous zooplankton	1981	
Harvey	2012	10.41	3.00	11.50	0.80	B	jellyfish	1981	[98]
Li	2009	2.15	5.00	25.05	0.95	EE	jellyfish	2000	[75]
		0.81	8.80	35.20	0.90	B	ctenophores	1880	
		0.13	8.80	35.20	0.90	B	ctenophores	1930	
		0.28	8.80	35.20	0.90	B	ctenophores	1980	
Nuttall	2011	0.15	8.80	35.20	0.90	B	ctenophores	2000	[99]
		0.02	0.30	0.79	0.02	EE	ctenophores	1999	
Pavés	2008	0.02	0.30	0.73	0.02	EE	ctenophores	1999	[100]
Lin	2008	na	na	na	na	na	na	1999	[101]
		1036.00	4.34	14.45	0.49	none	lavaceans	1999	
Persad	2009	20859.00	0.10	0.33	0.00	none	carnivores	1999	[102]
		0.77	5.00	25.00	0.25	B	jellyfish	1981	
Duan	2009	1.53	5.00	25.00	0.05	EE	jellyfish	1998	[103]
Hong	2008	2.25	5.01	25.05	0.34	B	large jellyfish	1997	[104]
Chen	2008	1.12	6.12	25.05	0.14	B	jellyfish	1997	[105]
Duan	2009	1.53	5.01	25.04	0.30	EE	jellyfish	1997	[106]
		0.02	0.58	2.92	0.95	Q/B	gelatinous zooplankton	1995	
Tam	2008	0.00	0.58	2.92	0.95	Q/B	gelatinous zooplankton	1997	[107]
Okey	2002	0.27	40.00	80.00	0.93	EE	carnivorous jellyfish	1997	[108]
Dommasnes	2001	4.00	3.00	10.00	0.34	EE	jellyfish	1997	[109]
		41.09	40.00	143.08	0.90	EE	gelatinous zooplankton (George's Bank)	1996	
		42.91	35.00	146.00	0.91	EE	gelatinous zooplankton (Gulf of Maine)	1996	
		37.72	40.00	146.00	0.90	EE	gelatinous zooplankton (Mid-Atlatntic Bight)	1996	
Link	2006	40.42	40.00	146.00	0.90	EE	gelatinous zooplankton (Southern New England)	1996	[31]
Barausse	2009	1.02	14.81	44.44	0.15	EE	jellyfish	1996	[53]
		0.52	15.51	62.05	0.21	EE	gelatinous zooplankton	1995	
Araújo	2012	0.52	15.51	62.05	0.14	EE	gelatinous zooplankton	1995	[73]

		0.52	15.51	62.05	0.14	EE	gelatinous zooplankton	1995	
		0.03	11.00	29.20	0.00	EE	aurelia	1960	
		0.82	11.00	29.20	0.00	EE	mnemopsis	1960	
		0.11	11.00	29.20	0.00	EE	aurelia	1960	
Akoglu	2014	0.48	11.00	29.20	0.00	EE	aurelia	1960	[55]
Okey	2001	0.07	18.25	80.00	0.90	B	Jellies	1995	
Okey	2001	0.27	40.00	80.00	0.95	B	jellies	1995	[110]
		1.10	7.00	23.33	0.38	EE	Carnivorous zooplankton	1995	
Stanford	2004	1.10	7.00	23.33	0.68	EE	Carnivorous zooplankton	1973	[111]
Okey	2004	6.39	5.00	29.41	0.01	EE	jellies	1994	[112]
Coll	2006	0.39	28.51	50.48	0.22	EE	jellyfish	1994	[71]
Watson	2013	4.77	9.20	10.00	0.80	B	gelatinous zooplankton	1993	[113]
Pedersen	2008	0.01	6.50	23.50	0.13	EE	Scyphomedusae	1993	[114]
Falk-Petersen	2004	0.72	6.50	17.33	0.13	EE	scyphomedusae	1993	[115]
Bulman	2002	8.00	6.00	22.00	0.80	none	gelatinous zooplankton	1992	[116]
Mackinson	2008	0.07	2.86	6.35	0.77	EE,Q/B	gelatinous zooplankton	1973	[57]
Fondo	2015	1.08	0.09	0.40	0.70	EE	jellyfish	1990	[117]
		0.08	5.00	15.00	na	EE	gelatinous zooplankton	1990	
		0.18	5.00	20.00	na	EE	gelatinous zooplankton	1990	
Althauser	2003	1.50	2.50	10.00	na	EE	gelatinous zooplankton	1990	[118]
Díaz-Uribe	2007	2.05	30.70	118.45	0.80	EE	gelatinous zooplankton	1990	[119]
		1.03	3.00	12.00	0.80	none	large jellies	1990	
		1.11	9.00	30.00	0.80	none	small jellies	1990	
		1.17	3.00	12.00	0.80	EE	large jellies	1960	
Field	2004	1.34	9.00	30.00	0.80	EE	small jellies	1960	[120]
Cornejo-Donoso	2008	1.00	3.60	12.00	0.48	EE	salps	1990	[121]
		0.11	0.88	3.00	0.61	EE	scyphozoid jellies	1990	
		0.34	0.88	3.00	0.66	EE	scyphozoid jellies	1990	
		0.11	0.88	3.00	0.48	EE	scyphozoid jellies	1990	
		3.16	5.48	15.64	0.80	EE	gelatinous filter feeders	1990	
		0.70	5.48	15.64	0.80	EE	gelatinous filter feeders	1990	
Aydin	2007	0.94	5.48	15.64	0.80	EE	gelatinous filter feeders	1990	[56]
Ainsworth	2005	0.22	10.23	26.46	na	EE	jellyfish and hydroids	1990	[122]
Cheung	2005	1.85	3.43	13.73	0.95	EE	jellyfish	1990	[80]
		1.61	10.00	39.20	na	EE	gelatinous organisms	1955	
		214.00	10.00	39.20	na	EE	gelatinous organisms	1990	
Gucu	2002	952.00	10.00	39.20	na	EE	gelatinous organisms	1980	[26]
		0.10	30.00	80.00	0.50	B	large jellies	1990	
Okey	2007	0.18	40.00	80.00	0.50	B	small jellies	1990	[123]
Orek	2000	315.00	0.50	2.00	0.00	Q/B	jellies	1990	[25]
Coll	2007	4.00	14.60	50.48	0.17	EE	jellyfish	1990	[54]

		0.25	1.00	3.30	na	EE	cnidaria	1990	
Rocha	2007	0.51	1.00	3.30	na	EE	cnidaria	1990	[124]
		0.02	40.00	80.00	0.50	B	large jellies	1986	
Okey	2006	0.03	0.03	80.00	0.50	B	small jellies	1986	[125]
Whitehouse	2014	0.66	0.88	3.00	0.00	EE	jellyfish	1985	[126]
Mackinson	1996	15.00	3.00	12.00	0.14	EE	jellies	1982	[127]
		0.05	0.88	2.00	0.00	EE	jellyfish	1980	
Aydin	2002	1.40	1.50	3.00	0.02	EE	jellyfish	1980	[47]
		5.29	16.20	35.00	0.04	EE	ctenophores	1981	
Vasslides	2017	0.35	13.00	20.00	0.00	EE	sea nettles	1981	[128]
Wang	2012	1.07	5.01	25.04	0.74	EE	jellyfish	1981	[129]
		8.00	9.00	30.00	0.00	EE	salps and gelatinous herbivore	1980	
Polovina	1996	9.10	3.00	10.00	0.02	EE	jellies	1980	[130]
		6.19	7.00	23.30	0.20	not clear	salps	1983	
Pauly	1996	5.00	3.00	12.00	0.58	not clear	carnivorous jellies	1983	[82]
Díaz-Uribe	2012	0.02	6.80	40.00	0.13	EE	jellyfishes	1980	[131]
Lee	2010	0.05	0.88	2.00	na	EE	jellyfish	1979	[48]
		0.05	0.88	2.00	0.02	EE	jellyfish	1980	
Trites	1999	0.05	0.88	2.00	0.02	EE	jellyfish	1950	[49]
Nurhakim	2003	0.10	5.01	25.05	0.53	EE	jellyfishes	1979	[132]
		4.55	0.58	1.67	0.16	EE	gelatinous zooplankton	1990	
Shannon	2003	5.00	0.58	1.67	0.15	EE	gelatinous zooplankton	1980	[133]
Coll	2013	0.33	13.87	50.48	0.22	EE	jellyfish	1978	[134]
Coll	2009	2.17	14.60	50.48	0.17	EE	jellyfish	1975	[135]
Buchary	1999	0.10	5.01	25.05	0.41	EE	jellyfish	1974	[136]
Heymans	2009	na	2.86	na	0.95	B;Q/B	gelatinous plankton	1974	[137]
Lees	2007	5.44	7.50	25.00	0.99	Q/B	gelatinous zooplankton	1973	[20]
Vibunpant	2003	2.00	5.00	20.00	0.00	EE	jellyfish	1973	[138]
Hoover	2013	na	na	na	na	not clear	jellies	2013	[139]
		2.50	3.00	12.00	0.21	EE;Q/B	salps	1970	
Hoover	2009	1.53	0.25	na	1.00	EE	cnidaria	1970	[140]
		0.02	10.95	29.20	0.02	EE	Pleurobrachia pileus	1960	
		0.02	20.00	100.00	0.02	EE	Pleurobrachia pileus	1960	
		0.03	10.95	29.20	0.00	EE	Aurelia aurita	1960	
Daskalov	2002	0.03	20.00	100.00	0.00	EE	Aurelia aurita	1960	[86]
Chagaris	2002	0.22	20.08	80.00	na	EE	carnivorous jellyfish	1950	[141]
Christensen	2015	0.50	10.00	20.00	na	EE	carnivorous jellyfish	1950	[142]
		na	na	na	na	not clear	jellyfish	NA	
Libralato	2010	na	na	na	na	not clear	jellyfish	NA	[72]
Heymans	2000	0.60	25.00	225.00	na	EE	jellyfish	NA	[29]

6.9 Supplementary References

Some of the included models in Ecobase had incomplete bibliographic information, in instances where the source document could not be found the reference is presented as listed in Ecobase.

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Chapter 7: General discussion and concluding remarks

7.1 Summary

Recent evidence suggests that populations of jellyfish in the Irish Sea have proliferated over the past two decades[1] leading to positive effects, such as the establishment of a jellyfish fishery[2], and negative effects such fish mortality in aquaculture [3]. However, the interactions between jellyfish and other biota in the ecosystem is largely unknown. Despite being one of the most well studied populations of gelatinous zooplankton, the trophic ecology of jellyfish in the Irish Sea was poorly characterised. In this thesis, molecular tools were developed and used in an attempt to address this knowledge gap.

In chapter two, we developed a molecular assay targeting the 16s region of the mitochondrial genome to facilitate rapid, and inexpensive, screening of marine organisms to detect jellyfish predation[4]. We demonstrated the effectiveness of this assay by using it in conjunction with DNA extracted from the stomachs of 2513 marine organisms to show moon jellyfish and mauve-stinger jellyfish are consumed by a variety of species including herring, whiting, grey gurnard, dover sole, lesser-spotted dogfish, sprat, dragonet, poor cod, dab, and squid during spring. In chapter three, we demonstrated temporal variability in jellyfish predation by documenting consumption of mauve stinger, and *L. tetraphylla* by mackerel, pilchard, and sprat in October. Moon jellyfish were not detected at this time, since they were caught alongside the other marine organisms during the survey, we considered prey unavailability was unlikely to explain the lack of predation. Instead, we hypothesised that they may be escaping predation through somatic growth and the development of stinging tentacles.

In Chapter five we wished to explore the trophic ecology of Irish Sea fishes in further detail: while the molecular approach developed in Chapter two could be conducted rapidly and inexpensively - only presence-absence data was yielded. To gain deeper

understanding, a high throughput sequencing (HTS) approach was adopted in chapter 5 to capture the broader diet of jellyfish predators. There is ongoing debate on the correct way to interpret HTS results: whether to use presence-absence data only, semi-quantitative approaches, or a pure quantitative approach[5,6]. To review the methods used in published studies that used HTS in diet studies, a structured review and meta-analysis was conducted in the fourth chapter. The influence of the number of species included, type of sequencer used, and starting material on the quantitative ability of HTS was assessed by building a two-level hierarchical meta-analysis model. No significant explanatory variables were revealed, although the analysis suggested that a quantitative signal was present. Using a semi-quantitative approach is likely to yield more accurate results than strict presence / absence.

In chapter five, a dual amplicon (18s and COI) HTS assessment on stomachs of fish species collected around the British Isles was conducted. Hydrozoans were the most frequently consumed jellyfish and were consumed by mackerel, lemon sole, herring, whiting, plaice, haddock and saithe. Haddock and whiting had also preyed upon ctenophores, while scyphozoan predation was only observed in mackerel. Again, we hypothesised that the rare cnidarian predation was rare due to jellyfish being too large, and well defended, to be easily digested by a wide variety of species.

Finally, in chapter six, we examined how the findings of the thesis, and the wider literature corresponded with jellyfish groups within published ecosystem models in the Ecobase database. Increasing interest in jellyfish is reflected: an increasing proportion of EwE models contained jellyfish as their own separate group. Network analysis showed a loose structure between the models, suggesting that while values are often borrowed between models, the influence of any given model on the state of knowledge is somewhat limited. The trophic ecology (both as predators and as prey) of jellyfish seemed well represented, and is broadly in line with the literature. However, ecotrophic efficiency (a measure of how integrated into the ecosystem a species is) suggested that the community is undecided on how important trophic links are: values ranged from 0 (completely distinct from the rest of the ecosystem) to 1 (very tightly coupled with the rest of the ecosystem). Lastly, we examined an Irish Sea ecosystem model to see how the knowledge gained in the thesis, and the literature, could be used. The model had been parameterised based on values borrowed from English Channel and

British Columbia EwE models. The model was likely underestimating the prevalence of jellyfish, based on Irish Sea specific estimates in the literature. However, consumption and production were judged to be sound estimates. We are not aware of any experimentally derived value for ecotrophic efficiency in jellyfish, however using an updated diet composition using predators found in this thesis, EwE can calculate an EE. In this manner, all derived values would now be supported by peer-reviewed literature.

7.2 Synthesis

Taken together chapters two, three, and five all address the broad question: which predatory species eat jellyfish? Prior to this research, many studies had identified species that prey upon jellyfish[7–15]. However, this was normally limited to just one or two species and the role within any given ecosystem essentially remained unknown. All three chapters looked at ecosystem level predation of jellyfish, at a broad taxonomic scale not conducted before. However, when considered together more information can be gleaned.

The most obvious outcome is the replication of results: both temporally, spatially, and with different analytical approaches. The detection of jellyfish consumption from many sites around the UK demonstrated that this phenomenon appears to be widespread, rather than a sea-specific or time-specific phenomenon. Furthermore, mackerel samples from chapter three were resampled in chapter five. In truth, this was done as we wanted to look at mackerel diet in depth, and mackerel were not caught in the sampling for chapter five. However, by using the same samples, the replicability of results can be tested. Pleasingly, both methodologies (diagnostic PCR and HTS) detected hydrozoan and scyphozoan jellyfish sequences. As such, the robustness of results, appears stronger when the chapters are considered in direct relation to one another. Although, *ad hoc* sampling was conducted across multiple years, some evidence about the temporal elements of trophic interactions was revealed. Sampling

in February and March revealed, a wide assortment of jellyfish predators, however sampling in July, August, September, and October indicated predation was much rarer. Throughout the thesis, we hypothesised that somatic growth and the development of stinging tentacles led to Moon jellyfish escaping from predation. The continued predation of smaller jellyfish adds more weight to this hypothesis. However, inter-annual differences may be at play too. To test this hypothesis formally, repeated sampling at a site would be required (see future directions).

Chapter four, the meta-analysis of HTS studies, provides context for chapter five, the HTS analysis. The meta-analysis, suggests that sequencing platform, starting material, and number of samples included does not have a significant effect on the quantitative ability of the study. The analysis also suggested that HTS generally has a quantitative signature, albeit not a perfect one to one relationship. The findings of chapter five, were treated in a semi-quantitative way, as chapter four had suggested using a detected versus not detected approach was overly conservative.

Finally, the conclusions of chapter six can be augmented when considering the first four data chapters. Although, an EwE model was not constructed for this thesis, the findings of the thesis undoubtedly have modelling implications. A quantitative diet composition matrix is required for each functional group included in an EwE model. Obtaining this data is difficult, particularly for species like jellyfish.
The meta-analysis suggests that models using HTS data in a quantitative way will likely perform better than those using a frequency of occurrence or detected / not detected approach. This could be key to improving the parameterisation of species with poorly documented trophic ecologies. As such chapter five could be used to create a quantified diet composition matrix. Results from chapter two could also be used to help parameterise an ephyrae stanza (juvenile jellyfish age group), bringing greater biological realism to the EwE model than has hither-to been possible.

7.3 Reflections and future research directions

Overall, the findings presented here have improved our understanding of the ecology of jellyfish in the Irish Sea considerably. Upon starting the thesis, our understanding of interactions between jellyfish and other biota, aside from rare predators like sunfish[16]

and turtles[17], was very limited. The discovery they play a role in common fish species' diet was therefore unexpected. This initial discovery also received considerable public interest – especially in Japan: the publication was tweeted about over one thousand times with an Altmetric score of 343, and a blog article I wrote covering the subject was reprinted in *the Independent*, and *Scientific American* amassing at least 15,000 reads. In the literature, our results have joined a growing body of evidence from other ecosystems demonstrating that jellyfish play an integral part of marine food webs[7,9,11,14,15,18]. We identified the shortcomings of EwE models handling of jellyfish and demonstrated the applicability a variety of molecular techniques to provide further insight into the ecology of these animals. Here, we discuss how the findings of this thesis could be built upon further in future research.

7.3.1 Seasonal fluctuations in the predation of jellyfish

Chapters two, three, and five taken together show strong evidence that jellyfish predation changes with the season, both in terms of the predator species targeting jellyfish and the jellyfish species targeted. However, inter-annual and spatial differences are entangled in these data. To address this issue, future research could be directed towards repeated sampling. Figure 7.1 provides a schematic overview to illustrate the key components of this research.

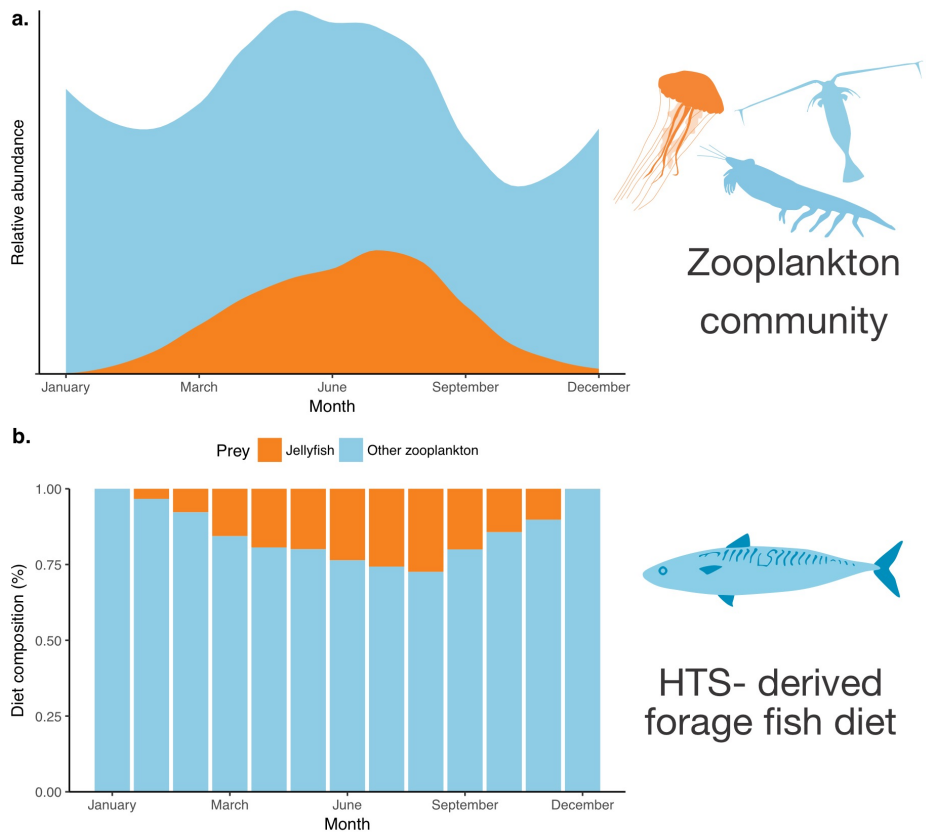

Figure 7.1. Proposed study (using made-up data for illustrative purposes) to determine if jellyfish predation is a result of specialist diet or general predation. **a.** Shows the absolute abundance of jellyfish **b.** shows the potential frequency of occurrence in forage fish diet.

Over the course of one year, monthly sampling would take place. Forage fish species of interest would be sampled (sample size as large as funding permits, ideally over 50 per sample), taking morphometric measurements, and extracting DNA from the stomachs. At the same time, standardised plankton sampling would take place using ring nets (or similar) giving a quantified breakdown of the plankton community using morphometric or molecular approaches as appropriate. The diet of the forage fish could be ascertained using the HTS protocol described in chapter five. At each site, for each month data would exist for the species of jellyfish present, the stage of the life cycle and, the relative abundance of jellyfish relative to other zooplankton. Using this data, it would be possible to show if changes in predation are linked to the life stage of the jellyfish, or the availability of jellyfish. Selectivity indices could be calculated using classic approaches[19,20] or novel network-based analyses using the econullnetr R package[21]. Morphometric measurements could see if any functional traits of the fish such as gape size were influencing the propensity to consume jellyfish.

This research could be improved even further, albeit it at greater cost, by incorporating other dietary study techniques such as stable isotope analysis (SIA). Combining SIA and HTS in a single study has two key benefits: first, the use of independent techniques can increase confidence in the results if it is detected in both assays. Second, since SIA provides quantitative data at a very poor taxonomic resolution and HTS can provide very high taxonomic resolution (if appropriate primers are used), Bayesian mixing-models could be used to consider these data sources together and obtain high-taxonomic resolution quantitative data[22].

7.3.2 Quantitative performance of HTS

The meta-analysis in chapter four addressed the question: to what extent is HTS quantitative? Ultimately, many of the tested variables: sequencer type, starting material, and number of samples in a trial did not yield significant relationships with per species sequence abundance. The factor(s) driving quantitative performance appear to lie elsewhere in the procedure. We hypothesised that nucleotide diversity of the tested community at the primer binding sites may be responsible. Figure 7.2 provides a diagrammatic representation of the experiment that could be used to test this. In this case the same primers would be used, on communities of four species. In each community, the biomass percentage would be 10%, 20%,30%,40%. Each community would be replicated, such that every possible combination of biomass composition for the species given is tested (24 replicates). Across the mock-communities different variations of nucleotide diversity at the primer binding sites would be used. The mock communities would be sequenced, and the number of sequences matching the original species in the mock communities recorded. For each sample, the original biomass (%), the proportion of reads produced (%), and the nucleotide pairwise diversity of the mock community at the binding site would be known. Therefore, for each mock community the slope between original biomass and proportions of reads could be calculated.

Using all data, a GLM could then be fitted to see if the quantitative relationship (slope) is a function of the nucleotide pairwise diversity of the sampled mock community. If the experiment yields significant results, it should be possible, in actual trials to gain an estimate of the quantitative nature of the assay *after* the experiment has been

conducted. In theory, past studies could be analysed in a quantitative manner. If funding permits, the findings of the experiment could be assessed by quantifying an unknown mock community (e.g. provided by a different lab group, or complied without the primary researcher knowing the composition).

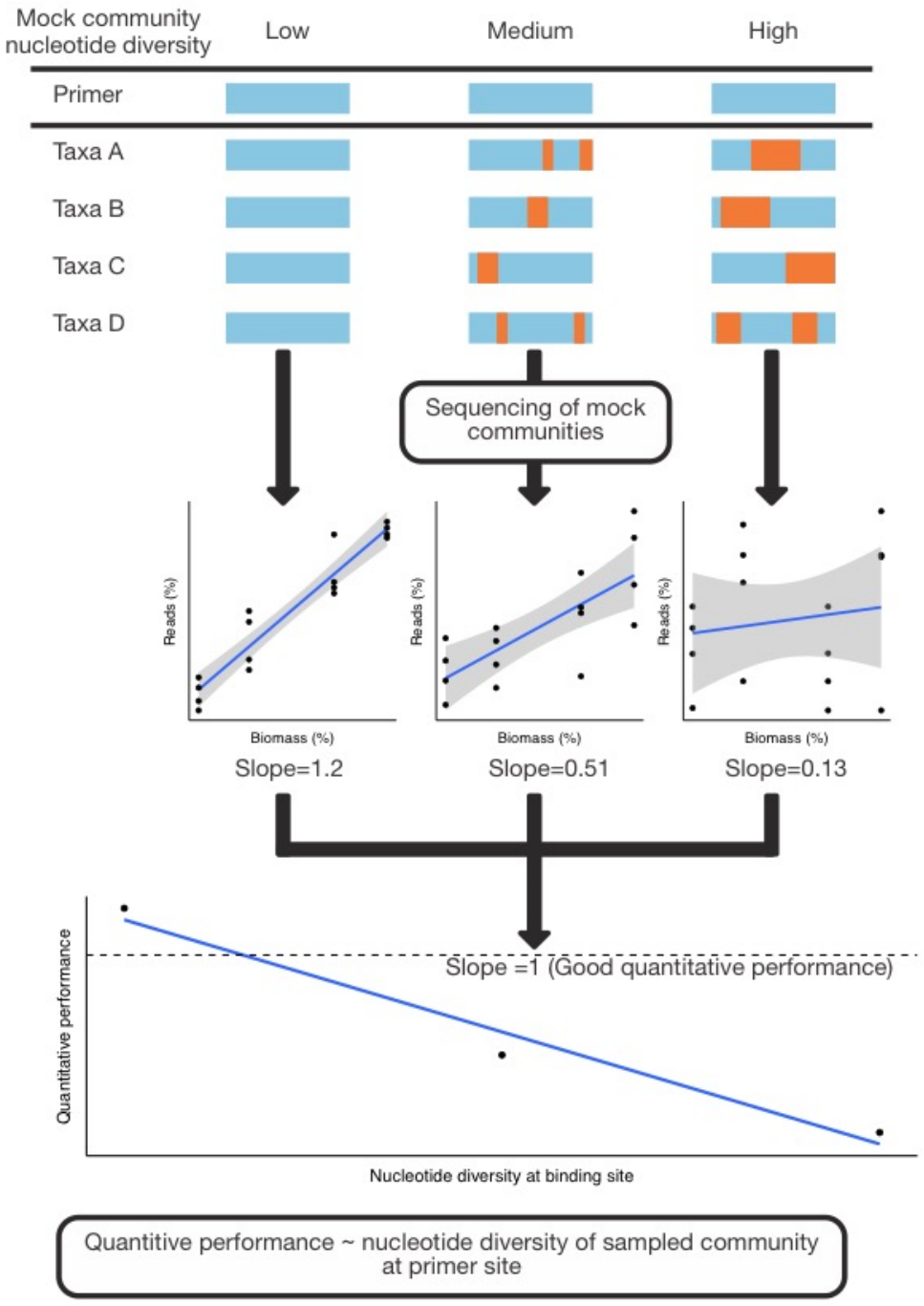

Figure 7.2. Proposed experiment ((using made-up data for illustrative purposes)) to determine if the quantitative performance is a function of the nucleotide diversity at primer binding site.

7.3.3 **Uncovering the trophic role of jellyfish**

We were motivated to undertake this line of research to try and ascertain what role jellyfish play in marine ecosystems around the British Isles. However, the state of the field at present is such that much of the PhD was spent answering more basic ecological questions. Moving forward, our findings could be incorporated into the EwE model described in chapter six to quantify the strength of the discovered interactions, and further explore the trophic ecology of jellyfish. Jellyfish could be split into cnidarian, ctenophore, and tunicate varieties and the life cycle better represented by a multi-stanza groups[23]. If the quantitative performance of the HTS work described above is successful, it might be possible to obtain the biomass of functional groups existing in the environment by means of an eDNA survey, otherwise data from the annual fisheries surveys that take place in the Celtic and Irish Sea under the auspices of Cefas (England & Wales), AFBI (Northern Ireland) and DARD (Republic of Ireland). Diet composition could be estimated through a HTS molecular gut contents analysis of functional groups. Q/B, and P/B would be obtained from peer-reviewed literature as available. At this point network properties could be explored, MTI could be used to quantify the role of jellyfish in the Ecopath model, and the contributions of jellyfish to the ecosystem visualised. Ecosim could then be used to 'game' such that: jellyfish biomass could be increased in line with the climate-jellyfish relationship established by Lynam *et al.*[1] and climate change predictions to see how the ecosystem might respond. Would this lead to an increase in jellyfish predators? Or would jellyfish outcompete other fish, leading to fish stock collapse and simplification of the system? Equally, elements of the ecosystem could be manipulated to see if any impact is made on jellyfish. For example, what is the role the predatory species play in controlling jellyfish populations.

7.4 References

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