Trophic structure of mesopelagic species in the Northeast Atlantic Ocean based on stable isotopes of carbon and nitrogen

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ABSTRACT

The mesopelagic zone is one of the largest, yet among the least explored habitats of the planet. Possible estimates of fish living in this zone range from around 1 to 15 billon tones of biomass which is 10 to 15 times that of the annual global capture fisheries production. Utilizing this untapped resource can help satisfy a growing demand for food in the world. Mesopelagic species do also play an important role in the "biological pump" by transferring of organic material from the surface water to depth. This among other reasons makes this group of species an important link in the open ocean food- webs and knowing more about the trophic structure of this species will be important. This study addresses the estimation of the trophic level as well as investigate isotopic niches and intraspecific diet pattern for mesopelagic species in the Northeast Atlantic Ocean using nitrogen and carbon stable isotope analyses. Species was collected from a transect from the Canary Islands to the Bay of Biscay. Determinations of $\delta^{15}N$ and $\delta^{13}C$ values were made on a total of 48 mesopelagic species including both small and large specimens. Their where six crustacean species: *Acanthephyra* quadrispinosa, *Gennadas valens*, *Oplophorus spinosus*, *Systellaspis debilis*, *Robustosergia robusta* and *Eucopia sculpticauda* and 42 mesopelagic fish species from seven families (Platytroctidae, Serrivomeridae, Myctophidae, Eurypharyngidae, Gonostomatidae, Sternoptychidae and Stomiidae). Isotope analyses on the seston was done as well to obtain an isotopic baseline for the trophic level calculations. The result shows that the mesopelagic species spans over three trophic levels from TL 1.5 to 3.2 and suggesting that the families can be coupled into three isotopic groups. As well did most species have a significant relationship between size and $\delta^{15}N$ and $\delta^{13}C$ which suggests that several of the species might change diet or parts of their diet as they grow. Additionally, were local environmental conditions found to be a significant predictor of $\delta^{15}N$ and $\delta^{13}C$ values in mesopelagic species in the Northeast Atlantic Ocean. This study also illustrates the importance of an appropriate baseline in trophic levels estimates.

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1. INTRODUCTION

1.1 Life in the Mesopelagic Zone

The mesopelagic zone is one of the largest, yet among the least explored habitats of the planet (Webb et al., 2010). By volume, it accounts for almost 20% of the living space in the ocean (Proud et al., 2017). The mesopelagic zone is the depth layer found between the epipelagic zone, which is the uppermost sunlit (= euphotic) zone of ocean were the sunlight is strong enough for phytoplankton to perform photosynthesis, and the bathypelagic zone were surface light does not reach (Salvanes & Kristoffersen, 2001). Hence, the mesopelagic zone is also known as the "ocean twilight zone" (Kaartvedt et al., 2019). Commonly, the mesopelagic zone is defined as the layer between 200 and 1000 meter depth (Robinson et al., 2010; Salvanes & Kristoffersen, 2001). However, an ecologically more meaningful definition might be in terms of absolute light intensities ranging from 10^{-9} to 10^{-1} μmol quanta m^{-2} s^{-1}, roughly corresponding to the visual threshold of the small, but abundant fish that have found a niche in this twilight environment (Kaartvedt et al., 2019). Fish living in the mesopelagic zone is found in all oceans, but the greatest abundance is found in subtropical and tropical oceans (Gjøsæter & Kawaguchi, 1980).

Around 100 families of fish are known to live in the aphotic environment at depth below 200 m. Around 30 of this is normally found in the mesopelagic zone (Gjøsæter & Kawaguchi, 1980). One of the most frequent and abundant families are Myctophidae in the order myctophiforms, and the families Gonostomatidae and Sternoptychidae in the order stomiiforms (Bernal et al., 2014; Davis et al., 2016; Gjøsæter & Kawaguchi, 1980). The family Myctophidae, also known as lanternfishes, is the most diverse at the genus level with around 33 genera and approximately 250 species (Catul et al., 2011). True to their name, lanternfishes have non-bacterial bioluminescent organs called "photophores", which are ventrally arranged and species-specific (Catul et al 2011). Bioluminiscence is also found in many other species in the mesopelagic zone, for instance in species in the families Gonostomatidae and Sternoptychidae (Davis et al., 2016).

Many mesopelagic species perform diel vertical migration (DVM) (Bianchi & Mislan, 2016; Gjøsæter & Kawaguchi, 1980; T. T. Sutton, 2013). DVM is the biggest animal migration in terms of biomass and numbers on earth and it occurs every day (Hays, 2003). At dusk hundreds and thousands of individuals rise to the surface from the mesopelagic zone. They feed in surface waters before migrating back to the twilight zone at dawn. When they are in the epipelagic

zone, mesopelagic organism prey on plankton and each other, while at daytime they hide from predators and digest at great depths until nightfall before they migrate up to the surface to feed again (Brierley, 2014; Salvanes & Kristoffersen, 2001). DVM is suggested to be a strategy that maximize the trade-off between the risk of predation and foraging success (Hays, 2003; Pinti & Visser, 2018). Globally, on average roughly 50% of all species forming dense acoustic scattering layers at mesopelagic depth migrate daily although there are considerable differences in migrating proportions between areas (ranging from 20% to 90%) (Klevjer et al., 2016). Several of the species in the families Myctophidae, Gonostomatidae, Sternoptychidae, Stomiidae are found to preform DVM (T. T. Sutton, 2013).

This migration is a part of the "biological pump" where carbon is exported from the surface to the deep ocean. The biological pump has a passive and active way of transporting carbon to the deep. The passive way is when organic material sinks down through the water column. The active pathway is when carbon-containing compounds are physically transported by animals as they migrate daily or seasonally between the surface were they feed and the depth where they digest and release the organic matter (Davison et al., 2013; Falkowski et al., 2003). It is estimated that mesopelagic fish contribute 14–17% of total active carbon export (Davison et al., 2013; Pinti et al., 2021).

Even though the mesopelagic zone is an enormous habitat, it has been under-represented in global databases of marine biological records. The reasons for the under-representation are uncertain, but is most likely due to either under-sampled midwater zones (because they are mostly far from land and costly to sample,) or because they harbor low biomass (Webb et al., 2010). The latter seems unlikely considering that the most recent biomass estimates based on acoustics(Irigoien et al., 2014; Proud et al., 2019) suggested that there may be significantly more biomass in the mesopelagic zone than previous estimations using trawl catches (Gjøsæter & Kawaguchi, 1980; Kaartvedt et al., 2012). Possible estimates range from around 1 to 15 billon tones of biomass (Gjøsæter & Kawaguchi, 1980; Irigoien et al., 2014; Proud et al., 2019).

The human population keeps increasing and with it the demand for food. Given current trends, the world's population is projected to reach 9.8 billion by 2050, increasing total food demand bit about 60% (SAPEA, 2017). This, together with the growing concern about overfishing and the increased need for aquaculture feeds have caused a renewed interest in mesopelagic fisheries (St. John et al., 2016). Although the biomass estimates of mesopelagic fish are still uncertain (Proud et al., 2019) there are huge amounts, even if we assume the lowest estimate of

1 billion tons to be true. In comparison, this is about 10 times that of the annual global capture fisheries production, which has stagnated at around 80-90 million tons since the 80's (FAO, 2020). There is however a need for better knowledge of the biodiversity, food web structure (e.g., mesopelagic fish may be an important food source for many of the commercially exploited epipelagic fish stocks), and the role of mesopelagic migrators in carbon sequestration, before we can sustainably utilize this untapped resource.

1.2 Trophic relationship

Studying the trophic relationships between species provide a good starting point when attempting to get a better understanding of the organization in the mesopelagic zone. The organisms in an ecosystem can be classified into different trophic levels. Trophic level is defined as the position of an organism in the food chain (Pavluk & bij de Vaate, 2008). The concept of organizing species in to different trophic level is useful as it gives information about the energy flow in the system as well as it is a universal concept that can be applied to all ecosystems (Yodzis, 2001). Alongside with the tropic level, species can be organized into different niches. Over the years many definitions of niche have been described (Moore, 2013; Newsome et al., 2007). One way to describe a niche is that it characterizes the position of a species within an ecosystem, comprising both the habitat requirements and the functional role of a species (Polechová & Storch, 2019). All species are naturally affected by environment and other organisms. Having species organized into niches can help getting an ecological overview if the presence of a species is determined by the presence of other species (food sources, competitors, predators, etc.) (Polechová & Storch, 2019).

Trophic level usually ranges from a value of 1 to 5 in the marine ecosystem (Pavluk & bij de Vaate, 2008). Primary producers, such as algae and phytoplankton, are at trophic level 1. Organisms that feed on the algae like zooplankton, mussels along with certain fish are at level 2 and are called primary consumers. Organisms that in turn feed on these primary consumers are at trophic level 3 and so on. Organisms at the highest trophic level are top predators, like marine mammals (Pavluk & bij de Vaate, 2008). Comparing the food chain in the ocean and on land shows that marine food chain is generally longer. The trophic position in agriculture compared to fisheries products, reveal that for instance herring in the ocean occupies the same trophic level as a wolf (level 3) on land. Tuna or other top predators in the ocean don’t have any comparable predator on the land that occupies the same trophic level (Duarte et al., 2009).

All organisms depend on the energy made available through photosynthesis by the primary producers at trophic level one. As one organism feeds on another, the energy is passed along the food chain from one trophic level to the next. However, with each trophic level (= step in the food chain) most of the energy, in the range of 80-90%, is lost to heat. That means that the biomass at each trophic level gets smaller the higher you get in the food chain. This is the reason why there are fewer top predators e.g., tuna, and more copepods in the ocean. For example, 1000 kilos of phytoplankton will be able to give about 100 kilos of zooplankton, which can give 10 kilos of krill, which can give 1 kilo of capelin, which in turn can give 0.1 kilos of cod (Semb-Johansson et al., 2019). There are however some uncertainties around how much energy is lost to heat. In mesopelagic ecosystems Irigoien et al., (2014) suggested that energy loss from phytoplankton to mesopelagic fishes in the open ocean is lower than what is normally assumed as they may be respiring approximately 10% of the primary production.

1.3 Methodological approach

Trophic levels and niche segregation are based on what a species eats and where they feed (Moore, 2013; Yodzis, 2001). Trophic level can be estimated from stomach content analysis or stable isotope analysis (Hussey et al., 2014). Historically the most common way to make an estimate of the trophic level was to look at the stomach content. This was done by categorizing the identified prey in the stomach into broad functional trophic level groups to provide an aggregate trophic level for the consumer (Hussey et al., 2011). The disadvantage of this method is that it requires the dissection of many individuals of a species to get enough stomach content to make a representative characterization of their diet. Stomach content analysis is also biased by how recent the meals are ingested and what type of prey is consumed. Some prey is digested faster than others, for instance the contribution of gelatinous organisms has been hard to quantify (Arai et al., 2003). Also, methods where prey is grouped into broad trophic level groups do not give an optimal estimate of the trophic level of the consumers since the broad functional prey groups do not necessary reflect the true range of the trophic levels of the preys (Hussey et al., 2014). To avoid this problems, stable isotope analysis has become increasingly common to estimate the tropic level. While stomach content analysis provide a snapshot of and individuals diet, the isotope fingerprint in the tissue of the consumer reflects their diet integrated through time and space, thus helping us to better understand long term feeding habits (Hussey et al., 2014; Post, 2002).

Stable isotopes are a powerful tool used in many fields and has for instance in paleoceanographic and paleoclimatic studies become one of the most important tools to

reconstruct the past climatic and oceanographic changes using the stable isotopes of oxygen, carbon, and nitrogen (Tiwari et al., 2015). It is also described as an extremely useful tool in forensics applications (Chesson et al., 2014), in geochemistry to get a greater understanding of ore-forming processes (Pat Shanks, 2014), in archaeology and anthropology to reconstruct diets of modern and ancient animals including humans (Sponheimer & Cerling, 2014) and in many more fields. When it comes to marine biology, stable isotopes are most often used to study trophic interactions in marine organisms (Bailey et al., 2019). Stable isotope analyses has been found to be a powerful tool when estimating the trophic positions of an organism (Post, 2002), and a natural and perhaps crucial tool in contemporary studies of the ecological niche (Newsome et al., 2007).

Isotopes are different variants of an element with different numbers of neutrons, but the same numbers of protons meaning that it has the same atomic number but different mass numbers. There are two types of isotopes: unstable isotopes (also called radioisotopes), and stable isotopes. Unstable isotopes have unstable nuclei with too much energy. To regain stability, the extra energy is released as radiation called radioactive decay. Each radioisotope has a unique decay period and is measured in half-life. For instance, the unstable isotope ^{14}C that are commonly used to decide the age of organic material, has a half-time of 5730 years. On the contrary, stable isotopes do not have a decay period (Ellam, 2016). The most common isotopes of carbon is the stable isotope ^{12}C which makes up 98,9% of all carbon, next is ^{13}C with 1,1 % occurrence (Holtebekk Trygve et al., 2019). There are two stable isotopes of nitrogen, the most common being ^{14}N with 99.6 % occurrence and ^{15}N with 0.4% occurrence (Kofstad & Pedersen, 2021).

Isotope studies of marine organisms usually use stable isotope ^{13}C and ^{15}N. The containment of ^{13}C and ^{15}N in an organisms' tissue can reveal at which trophic level the organism feeds and can also give an indication towards if the organism feeds in-shore, off-shore or in oligotrophic or eutrophic waters (Bailey et al., 2019). The measurement of isotopes uses the notation "δ" to signify the difference to standards during the analysis. The international Reference Standards used to calculate $\delta\ ^{13}C$ is the "PeeDee Belemnite" (PDB) (Fry, 2006). PDB is a cretaceous belemnite sample from the Peedee formation in South Carolina in USA (Wieser & Brand, 1999). It has a ratio between heavy and light isotopes ($^{13}C/^{12}C$) of 0.011180, where 1.1% is ^{13}C and 98.9% ^{12}C. The international Reference Standards used to calculate $\delta\ ^{15}N$ is air which has a $^{15}N/^{14}N$ ratio of 0.0036765, where 0.37% is the heavy isotope ^{15}N and 99.6 lighter isotope ^{14}N. The measurement between the sample and the standard is very small. Because of this, when

calculating the δ, a final multiplication by 1000 is performed, making the unit of δ per mil (‰). δ ^{13}C for fish are normally a negative number meaning that there is relatively less heavy isotope(^{13}C) in the animal tissue than in the standard PDB. δ ^{15}N in fish are normally a positive number and have relatively more heavy isotopes (^{15}N) than in air (atmospheric nitrogen) (Fry, 2006).

Earlier studies have shown that naturally occurring stable carbon and nitrogen are conserved when an animal is feeding (Minagawa & Wada, 1984; Peterson & Fry, 2003; Rounick & Winterbourn, 1986). Nitrogen isotopes in the organism's tissue have shown to be more fractionated during the feeding process than carbon isotope and has therefore commonly been used to calculate the trophic level of an organism (Rounick & Winterbourn, 1986). The fractionation of the stable isotopes of nitrogen is due to the discrimination of the heavier isotope (^{15}N) in the metabolism compared to the lighter isotope ^{14}N. This means that after the excretion, the animal is left with a higher δ^{15}N value (Fry, 2006). The higher trophic level and organism, the higher δ^{15}N value are found in their tissue. In Minagawa & Wada (1984), which is one of the oldest and most cited studies on enrichment of δ^{15}N along food chains, it was found that for each trophic level δ^{15}N increases with between 1.3 to 5.3 ‰ in the consumer compared to its prey. The average of +3.4 ‰ have commonly been used as the trophic enrichments factor in studies when calculations of trophic level is estimated relative to a baseline. 3.4 ‰ is however just an average and several studies have estimated other trophic enrichment factors for specific species, habitats, body sizes, tissue origin and so on (McCutchan et al., 2003; McMahon et al., 2015; MILL et al., 2007; Sweeting et al., 2007; Zanden & Rasmussen, 2001). Sweeting et al. (2007) does however suggest that if there is no specific trophic enrichment factor available for a specific species of fish, a δ^{15}N trophic enrichment factor of 3.2 ‰ (Between muscle and prey) and 2.9‰ (between whole fish and prey) should be applied when calculating the trophic level.

Stable isotopes of carbon are also useful in trophic studies of fish. While stable nitrogen isotopes can serve as an indicator for trophic level, stable isotopes of carbon vary little throughout the food chain. δ^{13}C values are used to determine the primary producers in the bottom of the food chain (Cherel & Hobson, 2007; McCutchan et al., 2003). In marine environments this can give an indication on the habitat an organism is feeding in which is useful when organizing species into niches (Newsome et al., 2007). It has been shown that δ^{13}C values can be a good indicator if an organism prefer to feed inshore, off-shore, pelagic or benthic as there are found differences in δ^{13}C in animals along latitude gradient (Cherel & Hobson, 2007; Hobson et al., 1994). This can be determined because different primary producers can have a distinct δ^{13}C signature based

on their photosynthetic pathway. Marine phytoplankton has for instance a $\delta^{13}C$ signature that is significantly lighter than that of many inshore plants (e.g., seagrasses) because phytoplankton discriminate ^{13}C more in the CO_2 fixation than inshore plants (Kelly, 2000). Higher latitude plankton are also found to be more enriched in ^{13}C compared to plankton found in lower latitude. With an underlying assumption that the $\delta^{13}C$ signature of the primary producer in a food chain can reflect on an organism with a higher trophic level, the habitats and feeding preference of a given consumer can be more easily determined (Cherel & Hobson, 2007).

1.4 Study Area

The study area is the Northeast Atlantic. More specific along a transect from just south of the Canary Islands to the Bay of Biscay. The southern part of the study area is affected by a persistent coastal upwelling on the continual slope of northwest Africa (Marcello et al., 2011) and the Canary currents which is one of the most productive areas in the world (Carr, 2001; Demarcq & Somoue, 2015). The northern part of the study area is affected by overflowing water coming through the strait of Gibraltar from the Mediterranean (Baringer & Price, 1999) and a intergyre zone with weak circulation in the bay of Biscay (Pollard et al., 1996). García-Seoane et al. (2021) have documented the oceanographic features and meso- and bathypelagic fish assemblages along the transect using data from the same cruise that samples in this study is received from. Their research show that temperature, salinity, and oxygen vary along the transect. In the surface layer (0-200m depth) along the transect going northwards there was a general decline in temperature and salinity and an increase in oxygen. In the upper layer (300-700m depth) there was little variation, except the oxygen saturation which had a small increase. In the intermediate layer (700–1200 m depth) there was an increase with increasing latitude in all three variables. In term of the mesopelagic fish assemblages it was found a significantly higher biomass and species richness in the southern and middle part of the transect compared with the northmost area.

1.5 Trophic studies in the Northeastern Atlantic

Few studies have addressed the trophic relationship between the mesopelagic species and their position in the food web in the northeast Atlantic. There have mostly been studies investigating patterns of vertical migration as well as species diversity and composition (Domanski, 1984; García-Seoane et al., 2021; Roe et al., 1984; Roe & Badcock, 1984; Siegelman-Charbit & Planque, 2016; Tuset et al., 2014). Research has also been done on the area, looking into microplastic interactions with mesopelagic fish (Lusher et al., 2016) and the potential for a

commercial fishery on northeast Atlantic mesopelagic species (Grimaldo et al., 2020; Standal & Grimaldo, 2020). When it comes to stable isotopes studies on mesopelagic species in this area, there are fewer results. There has been a trophic position study on deep-sea fish in the Porcupine Seabight (Stowasser et al., 2009) and on mesopelagic crustacean west of Spain (Rau et al., 1989).

The closest and most complex stable isotopes studies done on mesopelagic fish are in the western Mediterranean (Valls et al., 2014) and in the tropical and equatorial Atlantic (Olivar et al., 2019). The area for the northernmost sampling stations in Olivar et al. (2019) study overlapping the area for the most southern sampling station on the transect in this study. However, while Olivar et al. (2019) only investigated the $\delta^{15}N$ and $\delta^{13}C$ values in 20 species in the myctophid family, this study will look at 48 species in total from 12 different mesopelagic families.

1.6 Objectives

In this Master project, $\delta^{15}N$ and $\delta^{13}C$ values in mesopelagic species collected in the Northeast Atlantic will be investigated. This region is interesting in regard to the study of mesopelagic fish due to the variation of environmental factors and geographic properties.

Considering the lack of isotope studies for mesopelagic fish in this area while knowing the importance of their part in the biological pump along with their potential in fishery to help satisfy a growing demand for food in the world, there is no doubt that more knowledge of the species in this area will be useful. To uncover a small part of the deep-sea mystery and contribute to reveal more of the secrets from these creatures hiding in the dark deep the aim of this study is to get a better knowledge of the trophic interaction in the mesopelagic zone based on stable isotopes of nitrogen and carbon. Thus, the main objective of this thesis is:

(1) To investigate and compare the trophic level and isotopic niches of mesopelagic species in the Northeast Atlantic Ocean using carbon and nitrogen stable isotope analysis.
(2) To investigate if there is a relationship between the size of mesopelagic species and values of $\delta^{15}N$ and $\delta^{13}C$.
(3) To investigate the effects geographical and environmental factors on the $\delta^{15}N$ and $\delta^{13}C$ values in mesopelagic species in Northeast Atlantic Ocean

2. MATERIAL AND METHODS

2.1 Cruise

Samples used in this study were collected on board *R.V.* "Kronprins Haakon" during a multidisciplinary cruise between 02 and 22 May 2019 along a transect in the eastern north Atlantic from the Canary Island to Bay of Biscay (**Feil! Fant ikke referansekilden.**). For this study, samples from 15 out of a total of 18 trawl stations were selected. The stations are referred from 4604 to 4618. At each station, CTD equipped with Niskin bottles was deployed to examine the oceanographic characteristics (temperature, salinity, fluorescence and oxygen) and to collect samples for seston analysis. The trawl stations were divided into 9 different areas based on latitude and proximity to the shelf. For this study we use areas 3, 5, 6, 7 and 9 (Tab. 1)

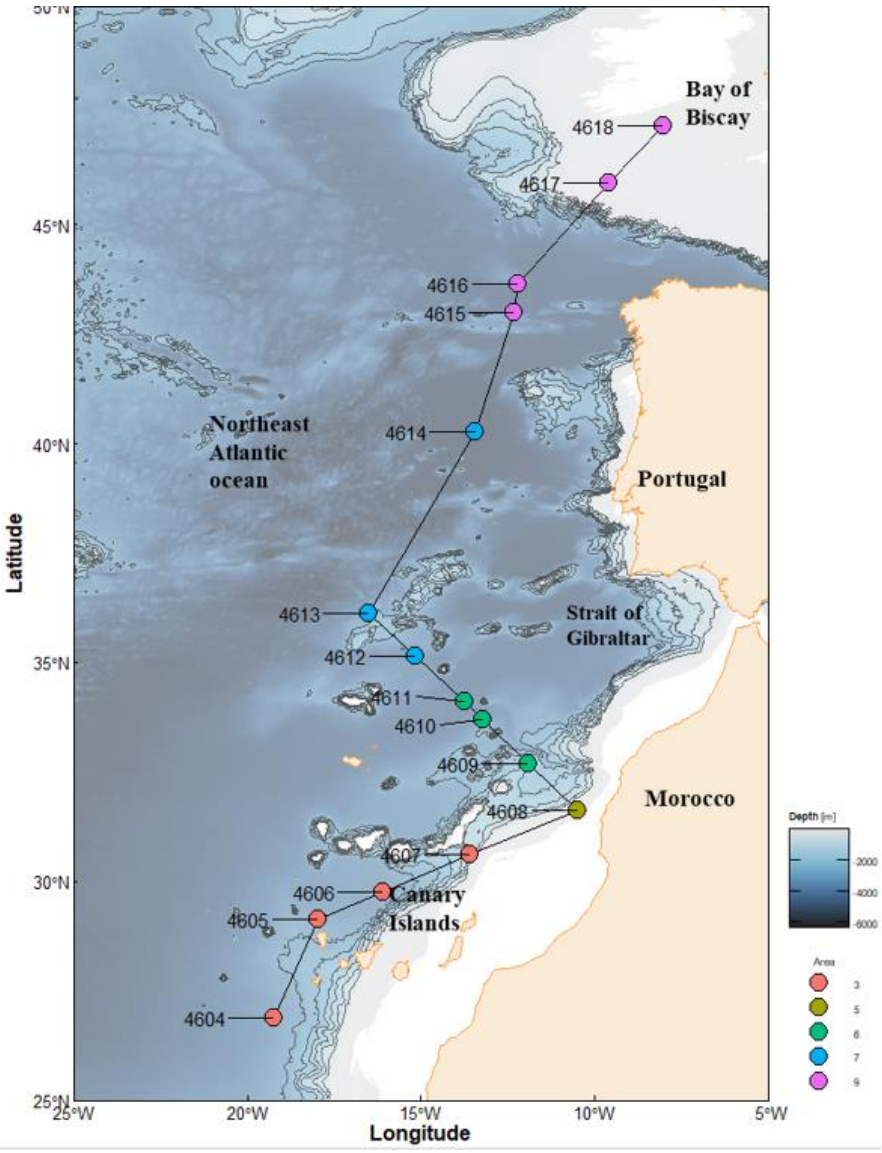

Figure 1. Map of the study area showing locations of the 15 trawl stations used in the analysis. The colors represent the areas the trawl station was divided into

During the cruise, samples of seston, crustacean and mesopelagic fish were all collected and frozen at -20°C for later use in the stable isotopes analysis.

2.2.1 Seston

Seston is defined as material moving in the water and includes organisms such as plankton and non-living matter such as decaying algae or kelp. In this study, seston is used as a baseline in the food chain. Seston was collected filtering water samples taken at the Deep Chlorophyll Maximum at each station using Niskin bottles. The water was pre-filtered with a sieve of 90 µm mesh-size and then subsequently filtered through glass fiber filters (GF/F, Whatman) which

were first treated by heating them to 450 ° C for 5h. For each station, 2 samples of seston were collected and the filters were subsequently rinsed with freshwater and stored frozen at -20 °C until the stable isotope analysis.

2.2.1 Trawl sampling

During the cruise, two types of trawls were used. A Macroplankton trawl with a 6 x 6 m trawl opening and mesh size of 8 mm was used at most of the stations (12 of the 15 stations selected). On the remaining three stations, a pelagic fish trawl Multpelt 380 was used. The Multpelt had a trawl opening with 40 m height and 55 m width with an 8mm mesh size (Tab. 1). The Multpelt 380 was used to catch larger organisms to see what may be avoiding the macroplankton trawl. In all trawl stations, except for station 4611 and 4616, the trawl was lowered from the surface down to a maximum depth of 1200m. At station 4611 and station 4616 the trawl was conducted at night and at a shallower depth (84 m and 290 m depth, respectively) because most mesopelagic fish are known to migrate closer to the surface at night.

Table 1. Overview of trawl-sampling used in this study during the cruise from Canary Island to Bay of Biscay.

Trawl Station	Area	Trawl type	Date	Time (UTC)	Latitude (°N)	Longitude (°W)	Max depth
4604	3	Multpelt	07.05.2019	09:49:00	26.89873	-19.231931	1200
4605	3	Macroplankton	08.05.2019	09:22:51	29.14031	-17.965428	1200
4606	3	Multpelt	09.05.2019	08:28:37	29.76686	-16.087191	1200
4607	3	Macroplankton	10.05.2019	07:55:25	30.61229	-13.589995	1200
4608	5	Macroplankton	11.05.2019	14:37:09	31.63383	-10.510127	1200
4609	6	Macroplankton	12.05.2019	08:18:09	32.69978	-11.935774	1200
4610	6	Macroplankton	13.05.2019	08:20:03	33.69493	-13.231978	1200
4611	6	Macroplankton	14.05.2019	03:02:46	34.09725	-13.75908	84
4612	7	Macroplankton	15.05.2019	08:22:33	35.14885	-15.169759	1200
4613	7	Macroplankton	16.05.2019	09:49:34	36.11405	-16.494473	1200
4614	7	Multpelt	18.05.2019	07:44:10	40.28236	-13.432552	1200
4615	9	Macroplankton	19.05.2019	09:26:13	42.98223	-12.31804	1200
4616	9	Macroplankton	20.05.2019	00:52:41	43.63405	-12.227826	290
4617	9	Macroplankton	21.05.2019	08:00:52	45.9535	-9.588206	1200
4618	9	Macroplankton	22.05.2019	07:13:09	47.2549	-8.034269	1200

All the individuals used in this study, except for the genus Sternoptyx where multiple species were unidentified, was identified onboard the boat. For the sake of simplicity *Sternoptyx spp.* will be referred to as a species, but keep in mind that these species were only identified to genus.

Whenever possible, six individuals per species for each area (covering small and large individuals from the size range) were taken for isotope sampling and preserved frozen at -20 °C for later processing in the laboratory onshore. The final selection of species for this work was based on the abundance and frequency of the species in the catch data and the availability of the frozen samples.

2.3 Laboratory work

In the laboratory, a total of 885 individuals from 48 species were prepared for stable isotope analysis (Tab. 2). 42 of these species were fish and six crustaceans. A total of 30 seston samples (2 replicates per station) were also processed. When possible, six individuals per species, three small and three large ones, were selected for each area (Fig.2 a). In cases when there were less than six specimens available per area, all of them were processed. The final selection of species for this work was based on the abundance and frequency of the species in the catch data and the availability of the frozen samples. In appendix A there is an overview of how many samples and specimens that was prepared for stable isotope analysis for each area and station in this study.

Table 2. List of taxa from the northeast Atlantic ocean used in this study

Group	Order	Family	Species
Fish	Anguilliformes	Serrivomeridae	*Serrivomer beanii*
	Scopharyngiformes	Eurypharyngidae	*Eurypharynx pelecanoidid*
	Alepocephaliformes	Platytroctidae	*Searsia koefoedi*
	Stomiiformes	Gonostomatidae	*Bonapartia pedaliota*
			Cyclothone braueri
			Cyclothone microdon
			Cyclothone pseudopallida
			Sigmops elongatus
		Sternoptychidae	*Argyropelecus aculeatus*
			Argyropelecus gigas
			Argyropelecus hemigymnus
			Maurolicus muelleri
			Sternoptyx spp.
			Valenciennellus tripunctulatus
			Vinciguerria poweriae
		Stomiidae	*Chauliodus sloani*
			Photostomias guernei
	Myctophiformes	Myctophidae	*Benthosema glaciale*
			Benthosema suborbitale
			Bolinichthys indicus
			Ceratoscopelus warmingii
			Diaphus mollis
			Diaphus rafinesquii

			Diogenichthys atlanticus
			Gonichthys cocco
			Hygophum benoiti
			Hygophum hygomii
			Hygophum reinhardtii
			Hygophum taaningi
			Lampanyctus alatus
			Lampanyctus crocodilus
			Lampanyctus cuprarius
			Lampanyctus pusillus
			Lepidophanes gaussi
			Lobianchia dofleini
			Lobianchia gemellarii
			Myctophum punctatum
			Nannobrachium atrum
			Notolychnus valdiviae
			Notoscopelus kroyeri
			Notoscopelus resplendens
			Symbolophorus veranyi
Crustacea	Decapoda	Acanthephyridae	*Acanthephyra quadrispinosa*
		Benthesicymidae	*Gennadas valens*
		Oplophoridae	*Oplophorus spinosus*
			Systellaspis debilis
		Sergestidae	*Robustosergia robusta*
	Lophogastrida	Eucopiidae	*Eucopia sculpticauda*

For all fish species, except *Serrivomer beanie* and *Eurinphax pelecanoides*, standard length to the nearest mm was measured. For *S. beanie* and *E. pelecanoides* a total length was measured. Total weight was measured for all fish to the nearest 0.001 g (Fig. 2 e). For fish ≥60 mm samples of muscle tissue were taken (Fig. 2 h), however for *Chauliodus sloani*, *Eurypharynx pelecanoides*, *Photostomias guernei*, *Serrivomer beanie* and *Sigmops elongatus,* the spine was occasionally included due to the difficulty of separating the muscle from the spine. Fish <60 mm was kept whole expect for myctophid, where the stomach was removed for another project and only the eviscerated fish was preserved. The samples were put into separate glass containers and kept frozen for later processing.

For crustaceans, carapace length (from the inside of the eye socket to the posterior margin of the carapace) was measured and the total weight was recorded. The whole individual was kept frozen in a glass container for later processing. In some cases the females had roe and the roe was included in the samples as well. Figure 2 illustrated some of the species that were processed in this study. More pictures of species can be found in appendix C.

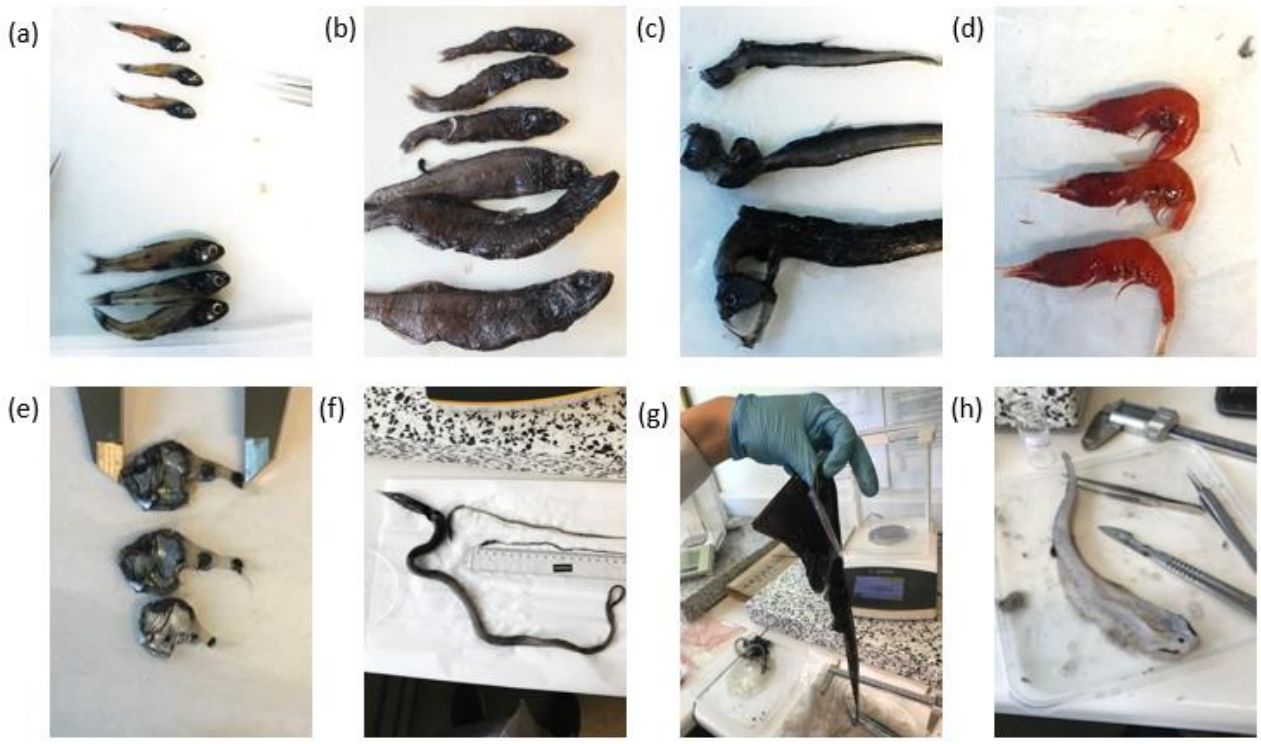

Figure 2. (a) Three small and three big individuals of *Ceratoscopelus warmingii*. (b) *Searsia koefoedi*, (c) *Chauliodus sloani*. (d) *Systellaspis debilis*. (e) Standard length measurements of *Argyropelecus hemigymnus* (f) *Serrvomier beanii*. (g) The mouth of *Eurypharynx pelecanoidid*. (h) Separation of muscles tissue for of *Searsia koefoedi*

2.3.1 Stable isotope analyses

All samples (crustaceans, fish, and fish muscle tissue) were freeze-dried. Before freeze-drying, each glass container was covered with parafilm with small holes to prevent the sample material from being sucked from the containers due to the vacuum pressure in the freeze dryer. The samples were freeze-dried in a Labconco *FreeZone 12 Liter Console* Freeze Dry System (Labconco; Kansas City, MO, USA) at a collector temperature of -50 ºC for 75 hours (Fig. 3 a). After 75 hours, the samples were grounded with a mortar and pestle into a fine homogeneous powder (Fig. 3 b). Between 0.5 mg and 1.0 mg of the powder was loaded into an 8 x 5 mm tin capsule. The tin capsules were carefully packed and stored in 96-well trays (Fig.3 c). The analysis of carbon and nitrogen stable isotopes were performed at the Stable Isotopes and Instrumental Analysis Facility at the University of Lisbon, Portugal (LIE-SIIAF).

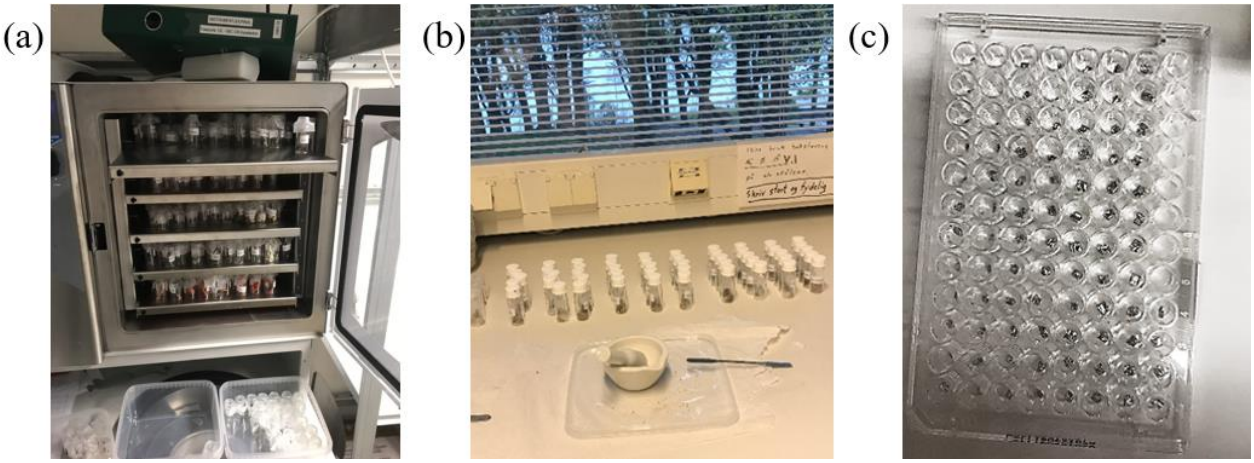

Figure 3. Pictures taken during laboratory work. (a) freeze dryer with samples in, (b) Grounding with a mortar and pestle, (c) Tray with samples packed in tin capsules

At LIE-SIIAF the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios ($\delta^{15}N$ and $\delta^{13}C$) were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Isoprime (GV, UK)(Preston & Owens, 1983) stable isotope ratio mass spectrometer, coupled to an EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion.

Expressed as parts per thousand (‰), $\delta^{15}N$ and $\delta^{13}C$ were calculated as described by Fry (2006):

$$\delta^{H}X = [(R_{sample}/R_{standard}-1)]*1000 \quad \textbf{(Equation 1)}$$

Where δ is the measure of heavy to light isotopes in the sample, ^{H}X is either ^{13}C or ^{15}N, R_{sample} is the isotope ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and, $R_{standard}$ is the isotope ratio in the standard. The standards used for nitrogen isotope ratio were IAEA-N1 and IAEA-600, also referred to as air. Standards used for carbon isotope ratio were IAEA-CH6 and IAEA-CH7 or IAEA-600, also referred to as PeeDee Belemnite. Precision of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standard material interspersed among samples in every batch analysis, was ≤0.2‰.

2.4 Data analysis

R (version 4.0.4) was used to do the data analysis (R Core Team, 2021) and the package ggplot2 (Wickham, 2016) to make the figures. Se appendix B for list of rest of the R packages used.

The classical additive model (Post, 2002)was used to estimate the trophic level (TL) for the species in this study:

$$\mathbf{TL = \delta^{15}N_{consumer} - \delta^{15}N_{mean\ baseline} / 2.9 + 2} \qquad \textbf{(Equation 2)}$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value for the individual, and $\delta^{15}N_{mean\ baseline}$ is the $\delta^{15}N$ value for the baseline organism, which in this case is the mean $\delta^{15}N$ value of the seston from station where the individual was sampled. A value of 2.9‰ is assumed to be the average ^{15}N trophic enrichment factor between whole fish and its food (Sweeting et al., 2007). The addends 2 denotes the assumed trophic level of the baseline organism, in this case the seston which here was assumed to be mainly primary consumers like zooplankton.

A linear regression model was used to predict the value of the stable isotopes based on length measurements for the species and to look for positive, negative or no relationship between the variables.

Package 'lmerTest' (Kuznetsova et al., 2017) was used to check the effect of environmental features on $\delta^{15}N$ and $\delta^{13}C$. A linear mixed model was used to predict the effect on $\delta^{15}N$ and $\delta^{13}C$ with the variables: Distance to shore (km), the temperature maximum, salinity maximum and oxygen minimum. This data was collected at each location. The model included species as random effect. Standardized parameters were obtained by fitting the model on a standardized version of the dataset. 95% Confidence Intervals (CIs) and p-values were computed using the Wald approximation.

3. RESULT

There was a great variation in both the carbon and nitrogen isotope in the samples. The maximum and minimum $\delta^{15}N$ values in individual samples ranged from 1.91‰ to 13.2 ‰, while the $\delta^{13}C$ values ranged from -27.01‰ to -17.08‰.

The species that was found to be most enriched with ^{13}C with a $\delta^{13}C$ average of -20‰ was *Serrivomer beanie* (family Serrivomeridae), *Photostomias guernei* (Stomiidae), *Chauliodus sloani* (Stomiidae), *Eurypharynx pelecanoides* (Eurypharyngidae), *Searsia koefoedi* (Platytroctidae) and Sigmops elongatus (Gonostomatidae) representing 5 different families (Tab. 3). The two species *Benthosema glaciale* and *Lampanyctus crocodilus* from the Myctophidae family was found to have a -20‰ $\delta^{13}C$ average as well. The specimen with the highest $\delta^{13}C$ was of the species *Lampanyctus crocodilus*, which was also one of the species with greatest range of stable isotope values (Appendix H) along with a large length range (Tab. 3). Species in the family Gonostomatidae, Sternoptychidae, along with species in the crustacean families Sergestidae, Acanthephyridae, Oplophoridae and certain species within

Myctophidae had an average $\delta^{13}C$ at -21‰. Lower enrichment in ^{13}C with a $\delta^{13}C$ average of -23‰ was measured in two myctophids; *Diogenichthys atlanticus*, *Ceratoscopelus warmingii* and crustacean *Eucopia sculpticauda* from the family Eucopiidae. Last was the seston samples found to have a distinctly lower $\delta^{13}C$ value than the fish and crustacean with a value of -25‰.

The five species found to be the most enriched in ^{15}N (mean $\delta^{15}N$) was *Searsia koefoedi, Lobianchia gemellarii, Lobianchia dofleini, Chauliodus sloani* and *Diaphus rafinesquii,* having an average $\delta^{15}N$ values between 11‰ and 9.6‰. *Lobianchia ssp* and *D. rafinesquii* belongs to the Myctophidae family (Tab.3). *Searsia koefoedi* was the only representative from the family Platytroctidae, while *Chauliodus sloani* was one of two members represented from the family Stomiidae. Species in the families Gonostomatidae, Eurypharyngidae (*Eurypharynx pelecanoides*) and Serrivomeridae (*Serrivomer beaniie)* was found to have relatively high $\delta^{15}N$ values (9.4 ‰ to 8.5‰) compared to the other species in the study. The exception is *Cyclothone braueri* (Gonostomatidae) which had a distinctly lower $\delta^{15}N$ value (7.9‰) than the other members of the family, along with a lower mean length. Myctophids was found to have $\delta^{15}N$ values evenly distributed between the maximum and minimum values of $\delta^{15}N$ found in this study. The family had a mean $\delta^{15}N$ value ranging from 11‰ to 6.6‰. Sternoptychids also had significant variation with a mean $\delta^{15}N$ value ranging from 8.8 ‰ to 6.7‰. The crustaceans had δ15N values inside the lower part of the max-min rang in this study with a mean δ15N value from 8‰ (Eucopia sculpticauda) to 6.3 ‰ (Gennadas valens). Seston was found to have a relatively low average δ15N (6.6‰) value compared to most fishes, but two crustaceans (Systellaspis debilis and Gennadas valens) as well as the Myctophid *Diogenichthys atlanticus* was found to have a lower mean $\delta^{15}N$ than seston. $\delta^{15}N$ values for the Seston samples did however vary a lot (Fig 4.) ranging from 1.9‰ to 10.4‰.

Table 3. Stable isotope values (mean values ± s.d for δ13C and δ15N) and trophic level estimates (TL) for mesopelagic species sampled from the eastern north Atlantic. Species are sorted from high to low mean trophic level inside the families to exhibit the trophic structure. n is total number of samples analyzed. The length are the mean body lengths, standard length for all fish (except Serrivomer beanie and Eurinphax pelecanoides which are measured in total length) and carapace length for crustaceans. Weight is the mean total weight in grams

Group	Family	Species	n	Length(mm)	Weight (g)	Length range (mm)	$\delta^{15}N$(‰)	$\delta^{13}C$(‰)	TL
Seston	Seston	Seston	30				6.6 ± 1.9	-25 ± 0.95	2 ± 0.33
Crustaceans	Acanthephyridae	*Acanthephyra quadrispinosa*	30	15	2,1	9-24	7.2 ± 0.72	-21 ± 1.2	2.3 ± 0.54
	Benthesicymidae	*Gennadas valens*	11	12	0,79	9-16	6.3 ± 1.3	-22 ± 0.69	1.8 ± 0.37
	Oplophoridae	*Oplophorus spinosus*	18	12	0,98	6-16	7.9 ± 0.76	-21 ± 1	2 ± 0.37
		Systellaspis debilis	29	11	1	4-17	6.5 ± 0.76	-21 ± 0.83	1.9 ± 0.53
	Sergestidae	*Robustosergia robusta*	16	20	2,2	14-29	7.7 ± 1	-21 ± 0.58	2 ± 0.27
	Eucopiidae	*Eucopia sculpticauda*	12	12	0,48	8-16	8 ± 0.44	-23 ± 0.74	2.6 ± 0.96
Fish	Platytroctidae	*Searsia koefoedi*	20	53	3,9	20-140	11 ± 0.9	-20 ± 0.84	3.3 ± 0.53
	Serrivomeridae	*Serrivomer beanii*	26	360	15	200-670	8.5 ± 0.97	-20 ± 0.72	2.5 ± 0.47
	Myctophidae	*Lobianchia gemellarii*	14	60	4,1	30-86	11 ± 0.97	-21 ± 1.2	3 ± 0.37
		Lampanyctus crocodilus	12	92	12	36-150	9.3 ± 2	-20 ± 1.7	2.9 ± 0.88
		Diaphus mollis	9	42	1,2	32-49	8.5 ± 0.63	-21 ± 0.72	2.9 ± 0.7
		Lampanyctus cuprarius	22	61	1,6	36-74	8.9 ± 0.74	-21 ± 0.63	2.9 ± 0.46
		Nannobrachium atrum	30	72	3,9	27-117	9.2 ± 1.3	-21 ± 0.98	2.9 ± 0.46
		Lampanyctus pusillus	12	30	0,31	22-35	9.1 ± 0.7	-21 ± 0.66	2.9 ± 0.37
		Lobianchia dofleini	24	27	0,4	18-35	9.6 ± 0.9	-21 ± 0.89	2.8 ± 0.62
		Diaphus rafinesquii	9	58	3,2	50-67	9.6 ± 0.53	-21 ± 0.82	2.8 ± 0.32
		Lampanyctus alatus	14	47	1,1	35-55	8.7 ± 0.51	-21 ± 0.53	2.7 ± 0.35
		Hygophum reinhardtii	9	32	0,56	17-45	7.2 ± 0.82	-21 ± 0.9	2.6 ± 0.32
		Benthosema suborbitale	12	23	0,21	17-28	7.6 ± 0.7	-21 ± 0.62	2.6 ± 0.3
		Benthosema glaciale	12	32	0,48	17-57	8.3 ± 1.5	-20 ± 0.94	2.5 ± 0.68
		Notolychnus valdiviae	24	21	0,074	17-24	7.9 ± 0.59	-22 ± 0.45	2.5 ± 0.29
		Notoscopelus resplendens	18	38	1,2	24-78	8.3 ± 1.2	-21 ± 0.64	2.2 ± 0.68
		Bolinichthys indicus	18	35	0,7	27-45	8.1 ± 0.6	-21 ± 0.44	2.2 ± 0.51
		Lepidophanes gaussi	18	38	0.5	31-46	8.3 ± 0.51	-22 ± 0.47	2.2 ± 0.32
		Hygophum hygomii	18	36	0,99	18-54	8 ± 0.66	-21 ± 0.55	2.1 ± 0.45

		Diogenichthys atlanticus	12	18	0,07	14-21	6.6 ± 0.63	-23 ± 0.19	2.1 ± 0.31
		Hygophum taaningi	14	32	0,6	19-47	7.8 ± 0.47	-21 ± 0.51	2 ± 0.38
		Notoscopelus kroyeri	16	30	0,26	21-39	7 ± 1.6	-21 ± 0.61	1.9 ± 0.68
		Myctophum punctatum	20	31	0,75	18-72	7.4 ± 1.3	-22 ± 0.66	1.9 ± 0.67
		Ceratoscopelus warmingii	11	44	1,2	35-55	7.5 ± 0.42	-23 ± 0.8	1.9 ± 0.28
		Hygophum benoiti	12	29	0,5	13-45	7.6 ± 0.97	-22 ± 0.6	1.7 ± 0.54
		Symbolophorus veranyi	11	40	1,8	22-105	7.5 ± 1.3	-22 ± 0.5	1.6 ± 0.61
		Gonichthys cocco	12	25	0,24	16-51	6.8 ± 0.87	-22 ± 0.64	1.5 ± 0.37
	Eurypharyngidae	*Eurypharynx pelecanoides*	24	290	10	84-520	9.2 ± 1.1	-20 ± 0.83	2.8 ± 0.75
	Gonostomatidae	*Cyclothone pseudopallida*	30	36	0,16	25-57	8.8 ± 0.59	-21 ± 0.74	3.1 ± 0.38
		Cyclothone microdon	24	39	0,28	24-55	9.1 ± 1.2	-21 ± 0.66	2.8 ± 0.55
		Bonapartia pedaliota	21	53	1,3	29-71	9.4 ± 0.68	-21 ± 0.6	2.8 ± 0.53
		Cyclothone braueri	30	27	0,07	15-34	7.9 ± 0.71	-21 ± 0.56	2.6 ± 0.6
		Sigmops elongatus	23	110	8,3	38-208	9.2 ± 0.87	-20 ± 0.93	2.7 ± 0.58
	Sternoptychidae	*Valenciennellus tripunctulatus*	21	24	0,13	19-29	8.8 ± 0.89	-21 ± 0.63	2.9 ± 0.43
		Vinciguerria poweriae	24	26	0,22	20-33	8 ± 0.78	-21 ± 0.68	2.4 ± 0.67
		Argyropelecus gigas	18	54	9,3	14-102	8.2 ± 0.97	-21 ± 0.74	2.3 ± 0.74
		Argyropelecus hemigymnus	18	25	0,38	15-35	7.7 ± 0.74	-21 ± 0.62	2.2 ± 0.5
		Argyropelecus aculeatus	24	28	1,8	9-68	7.3 ± 1.1	-21 ± 0.62	2.1 ± 0.49
		Sternoptyx sp.	23	20	0,64	7-36	6.7 ± 1.3	-21 ± 0.79	2.1 ± 0.48
		Maurolicus muelleri	12	37	0,86	15-49	7.3 ± 1.6	-21 ± 0.71	1.8 ± 0.58
	Stomiidae	*Chauliodus sloani*	24	150	14	57-260	9.6 ± 0.83	-20 ± 0.74	2.7 ± 0.51
		Photostomias guernei	24	84	2,5	40-126	9.2 ± 0.79	-20 ± 0.73	2.8 ± 0.53

3.1 Baseline identification

Carbon and nitrogen stable isotope values for the seston varied (Fig. 4) with a generally larger variation across stations than within stations. The variation within stations was largest in Area 3 (water samples from station 4604-4607) where the spread between the two filters from one water sample is visibly larger than those from the other areas. The two filters from the water sample from station 4607 have two significantly different $\delta^{15}N$ values, while the water sample from station 4605 shows large variation in $\delta^{13}C$ values. The $\delta^{15}N$ values varied from 1.9‰ (Station 4606) to 10.4‰ (station 4607) and the $\delta^{13}C$ values from -27‰ (station 4605) to -23.2 ‰ (station 4618). The $\delta^{15}N$ mean of the two seston values at each station was used as the baseline to calculate trophic levels for individuals caught at that station. The highest $\delta^{15}N$ mean was found in station 4612 (9.2‰), 4610 (8.5‰) and 4618 (7.9‰) and the lowest in 4605 (4.5%), 4617 (3.7‰) and 4606 (3.2‰). The $\delta^{15}N$ mean of all seston samples was 6.6‰ and $\delta^{13}C$ mean -25‰.

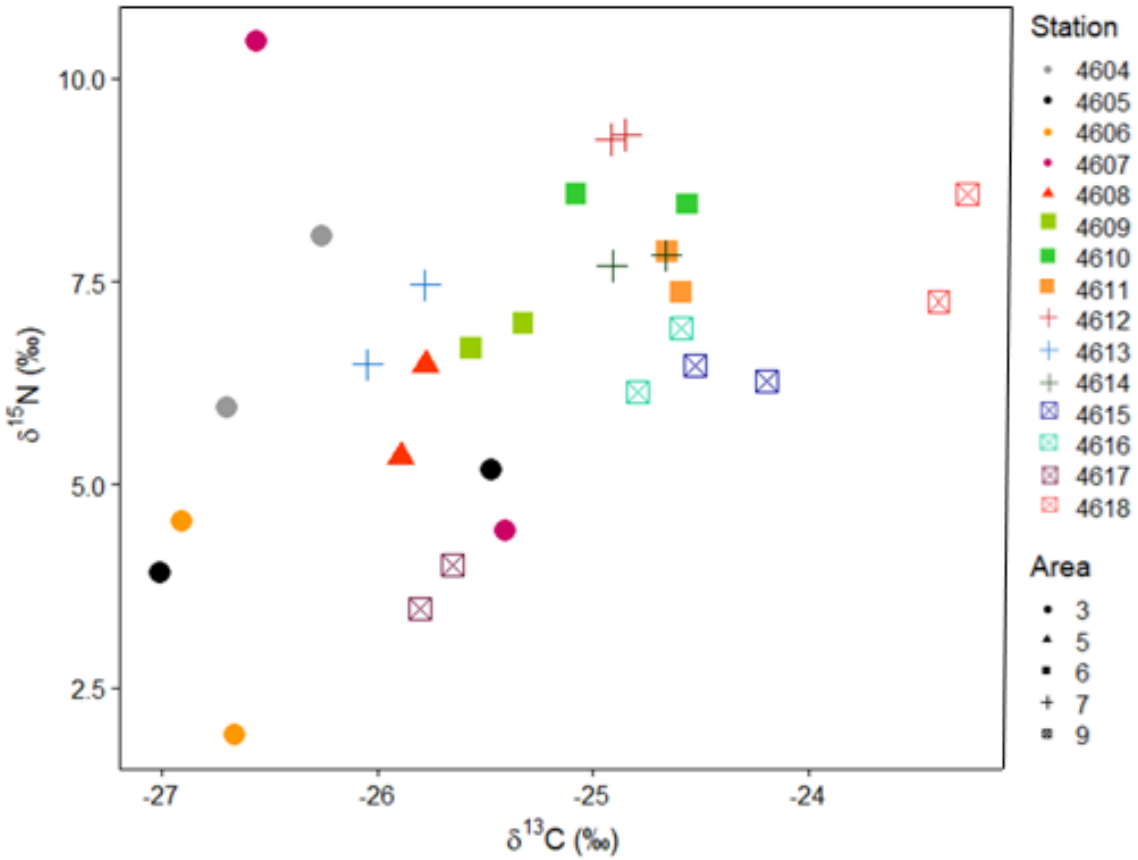

Figure 4. Stable carbon and nitrogen isotope values of the seston. Colors denote different water samples and shape the different areas.

3.2 Trophic level estimates

Species are purposely sorted from low to high mean trophic level (instead of taxonomic order) to exhibit the trophic structure (Fig 5). The estimated trophic levels (TL) ranged from TL 1.5 to TL 3.3 encompassing theoretically three trophic levels. *Searsia koefoedi* and *Cyclothone pseudopallida* were the only species that had an estimated TL 3. Most of the fish as well as some of the crustacean was found to be at TL 2, categorized as primary consumers. The crustacean species *Eucopia sculpticauda*, the fish species *Valenciennellus tripunctulatus*, *Eurypharynx pelecanoides* along with certain members of the families Gonostomatidae, Stomiidae and Myctophidae, was found the have TL closer to 3 than to 2. However, nine species, seven fish, namely *G. cocco*, *S. veranyi*, *H. benoiti*, *M. muelleri*, *M. punctatum*, *N. kroyeri* and *C. warmingii*, and two crustaceans, *G. valens* and *S. debilis*, were estimated to have a mean trophic level below 2, which was the assumed trophic level of the baseline (seston).

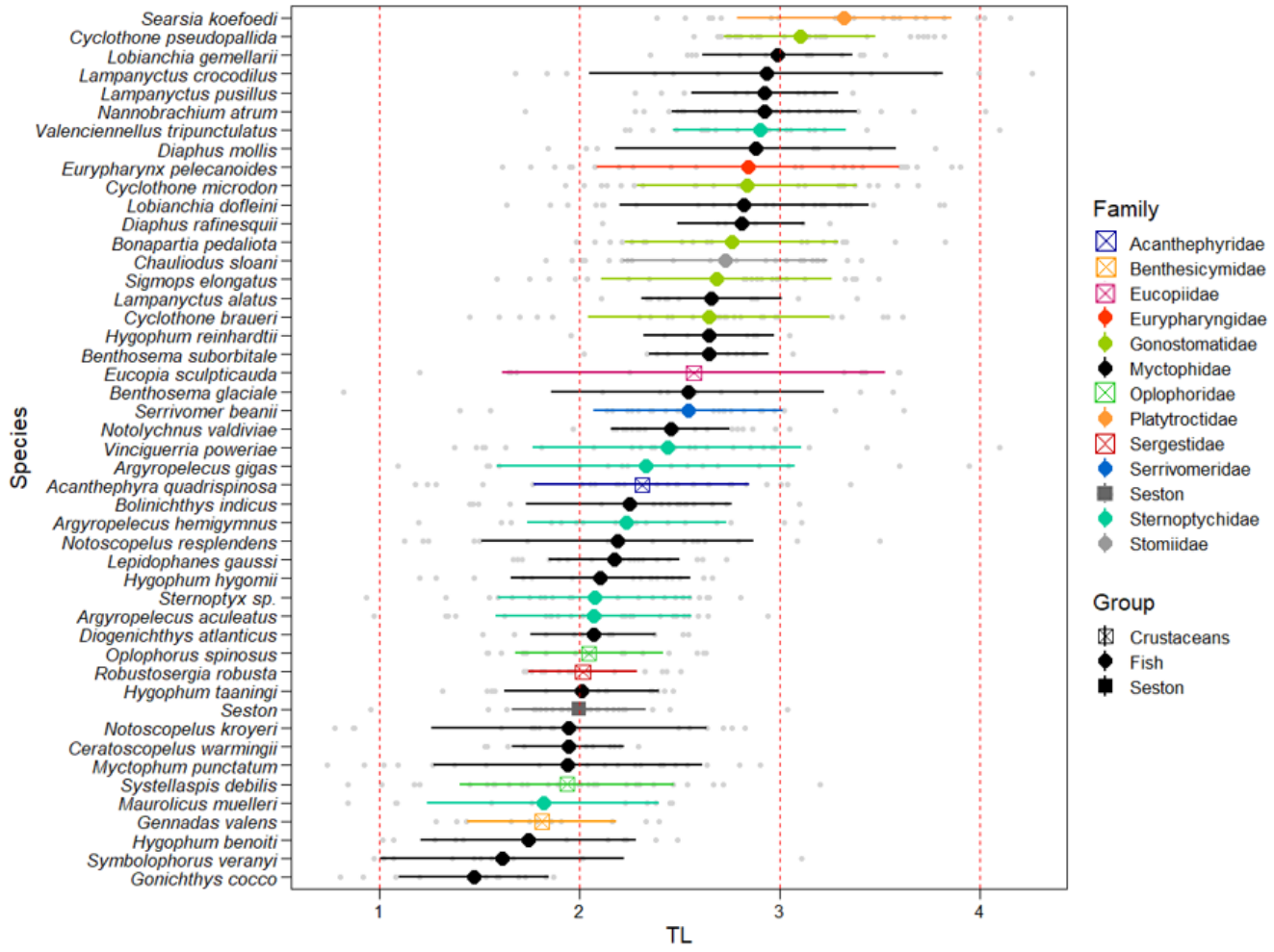

Figure 5. Colored points are the estimates of the mean trophic levels (TL) and the line is the standard derivation for each species. The colors represent the family they are belonging to. Red dashed lines represent the $\delta^{15}N$ threshold values of TL 1,2,3 and 4 when using seton as a primary consumer to set the isotopic baseline. Grey background point is the estimated TL for each individual. Species are sorted from low to high mean trophic level.

3.3 Isotopic niche

We find a distinct difference in the isotopic niche between the seston, fish and crustaceans (Fig. 6). However, the isotopic niche of some families of fish appear to have a slight overlap with the isotopic niche of some of the crustaceans. Specifically, the isotopic niches of the families Myctophidae and Sternoptychidae are overlapping with those of *R. robusta* (family Sergestidae), the largest of the crustaceans; and *E. sculpticauda* (family Eucopiidae), which is the only crustacean in this study that is not a decapod. The seston has a distinctly lower carbon level than the crustacean and fish families, but with $\delta^{15}N$ values similar to the lowest values found in some of the crustaceans. In particular, the $\delta^{15}N$ values of the Benthesicymidae (*Gennadas valens*) and Oplophoridae (*Oplophorus spinosus* and *Systellaspis debilis*) are close to those of the seston. The family Platytroctidae, only represented by *Searsia koefoedi* in this study, visibly stands out due to its high nitrogen and carbon values (Fig. 6).

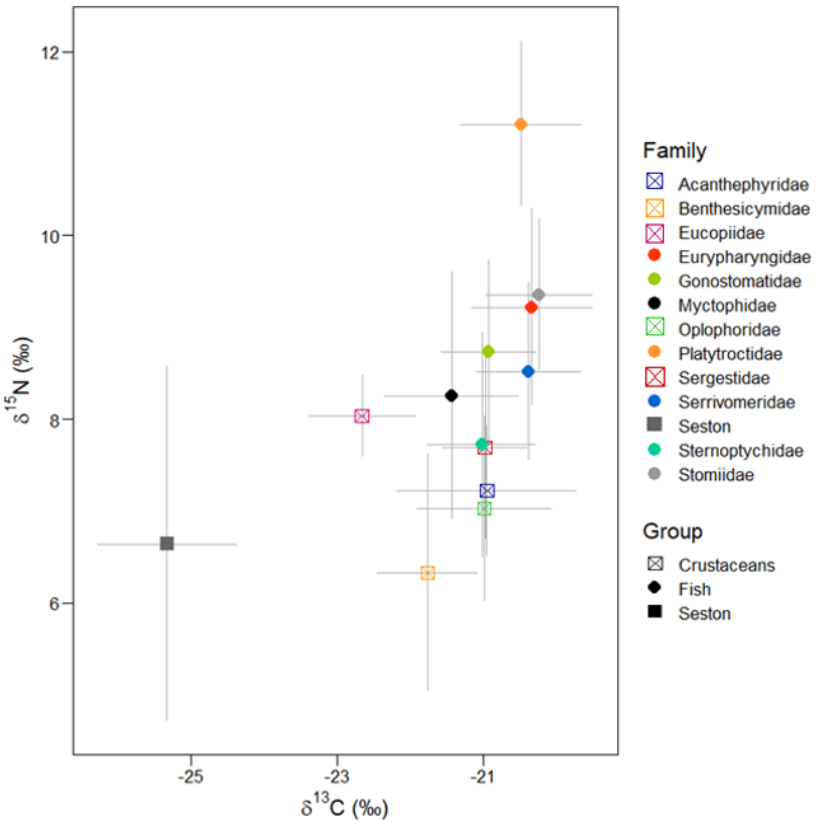

Figure 6. MeanScatterplot of the mean (±SD) of all stable carbon and nitrogen isotope values of seston and the different crustaceans and fish families.

There are also some differences in the isotopic niches of species between areas. In area nine we find a distinct niche distribution where the crustaceans in the families Acanthephyridae, Benthesicymidae and Eucopiidae stand out as a separate group with low $\delta^{13}C$ values (Fig 7. A).

Furthermore, in area nine, the isotopic niche of members of the family Sternoptychidae overlaps with that of some of the crustaceans in the families Sergestidae and Oplophoridae. Platytroctidae, Stomiidae, Serrivomeridae and Gonostomatidae stands out with higher $\delta 15^{N}$ values in area 9 (Fig. 7. A) and as a general trend in the rest of the areas. According to their isotopic values, myctophids occupy central place in the middle between two niches of the high trophic level fish families and crustacean families, suggesting that the different species in the myctophid family might feed in one or both niches (Fig 7. A).). In area 7, there is a tendency of two distinct groups. One group including Platytroctidae, Stomiidae, Serrivomeridae, Eurypharyngidae and Gonostomatidae, and a second group grouping the crustaceans and the members of the Myctophidae and Sternoptychidae (Fig. 7, A). In areas 6, however, the families of most fish and crustaceans are overlapping, which make it hard to determine any distinct niche segregation (Fig 7. C). Some positions are however noteworthy. Myctophidae stands out by having low $\delta^{13}C$ and *S. koefoedi* (Platytroctidae) stands out due to its high $\delta^{15}N$ values. There is also a slight distinction between crustaceans and fish even though it is hard to separate them into distinct groups.

Furthermore, area 5 is also characterized by close values of $\delta^{15}N$ and $\delta^{13}C$ between the families of fish and crustaceans (Fig 7.D). Four different groups can be determined. Eurypharyngidae with a high $\delta^{15}N$ and $\delta^{13}C$. Myctophidae and Sternoptychidae with lower $\delta^{13}C\delta 1^{3}C$ than the rest of the families. The two crustacean families Oplophoridae and Acanthephyridae with low $\delta^{15}N$ compared to the other families. Stomiidae, Serrivomeridae, and Gonostomatidae have similar $\delta^{15}N$ and $\delta^{13}C$ occupy a central place in the middle between the other groups. In area 3, we observed the same tendencies as in area 7, except for Gonostomatidae which has a lower $\delta^{15}N$ and $\delta^{14}C$ in this area than in the other areas (Fig 7. E). Across all areas *S. koefoedi* (Platytroctidae) stands out due to its high $\delta^{15}N$ values, making it possible that it is not sharing the same prey preference as the other families. The Myctophidae separate themselves from the rest of the fish families because of their low $\delta^{13}C$ values in all areas, the exception being area 9. Overall is also the $\delta^{13}C$ values decreases with the increasing latitude (Fig. 7)

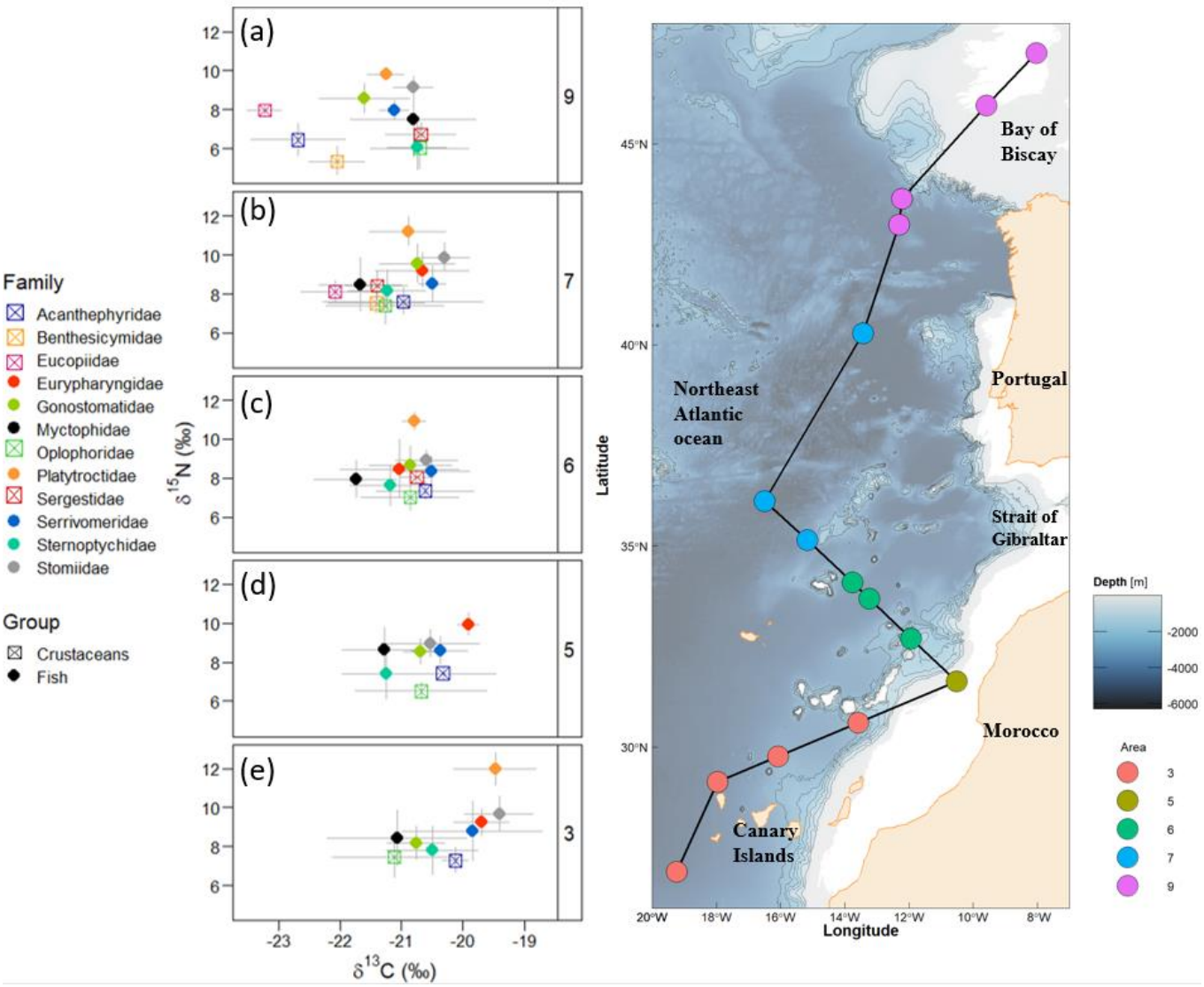

Figure 7. To the left is the mean (±SD) stable carbon and nitrogen isotope values at each area 3 (at the bottom) ,5,6,7 and 9(at the top) of the different crustaceans and fish families. To the right is the map with the areas represents in different colors.

3.4 Intraspecific patterns

A regression analysis between length (mm) and the $\delta^{15}N$ or $\delta^{13}C$ values shows that most of the species have a positive relationship between length and the $\delta^{15}N$ and $\delta^{13}C$. Appendix D have an overview of all the spices that had significant relationship between size and $\delta^{15}N$ and/or $\delta^{13}C$. Eighteen of the 48 species included in this study showed a significant increase in $\delta^{13}C$ with increasing length ($P \leq 0.05$). For $\delta^{15}N$, a total of 35 species showed a significant increase with length ($P \leq 0.05$). 25 species had a weak or no pattern between length and $\delta^{13}C$ values, and 13 species in $\delta^{15}N$ ($P > 0.05$). Five species, however, showed a significant $\delta^{13}C$ decrease when the length increased. The relationship between length and $\delta^{15}N$ and $\delta^{13}C$ are shown in Figure 8, 9 and 10 for a number of selected species.

In our data, *S. koefoedi, S.beanii* and *A. gigas* all had a significant increase ($P \leq 0.001$) in δ15N and δ13C with increasing size (Fig. 8). However, in the cases of *S. koefoedi* (Fig 8.A) and *A. gigas* (Fig 8.B) there was a clear size distribution between areas where the largest individuals were found in area 3 in both instances and smallest ones in area 6 and 7 as well as area 9 for S. *koefoedi.*

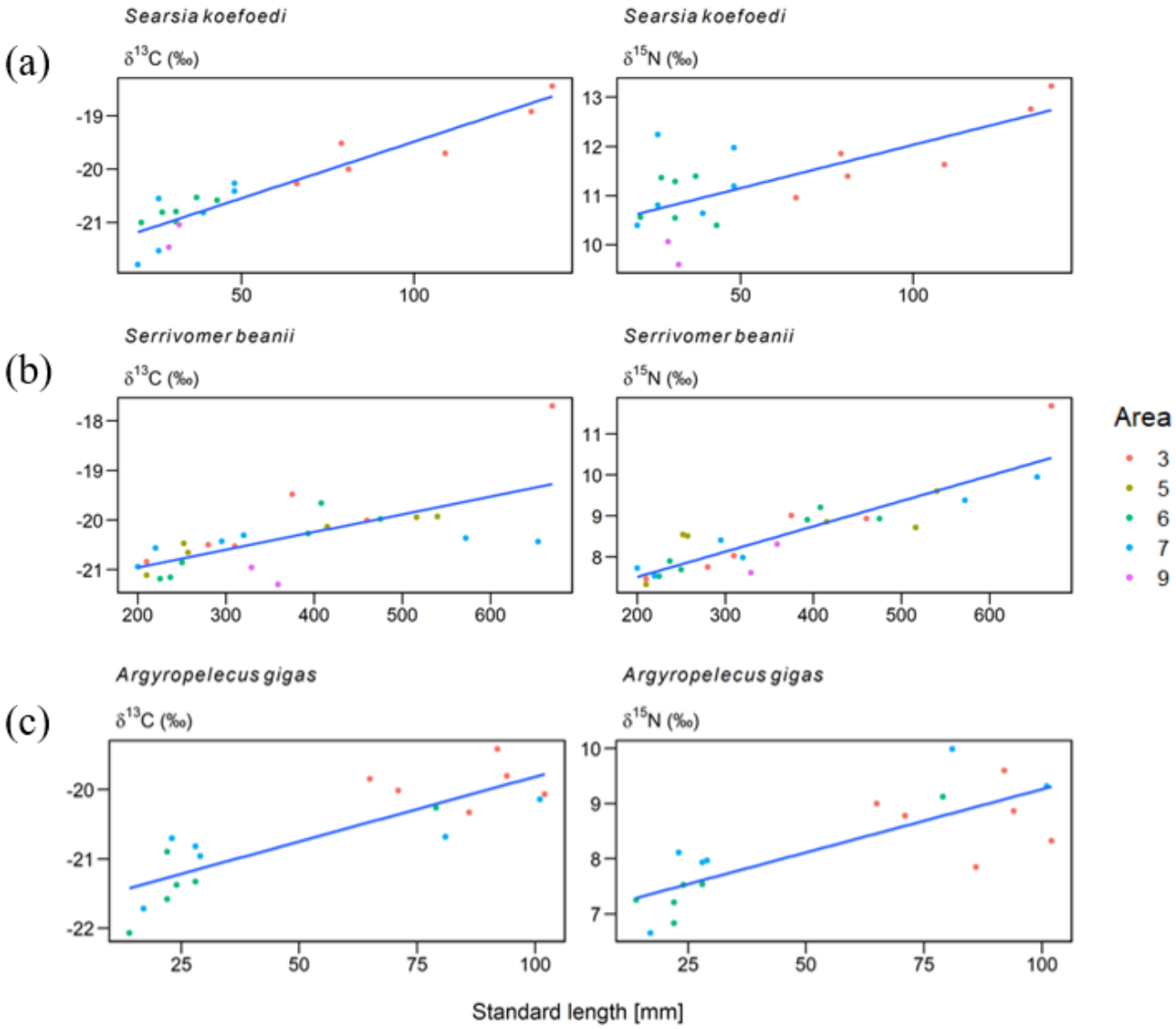

Figure 8. A selection of species showing a positive relationship between standard length and $\delta^{15}N$ and $\delta^{13}C$. (a) *S. koefoedi*, (b) *S.beanii* and (c) *A. gigas*

The absence of a clear relationship between length $\delta^{15}N$ and $\delta^{13}C$, can for instance be seen in the shrimp *G. valens* (Fig 9. A). Other examples for patterns are a clear positive relation between length and $\delta^{15}N$, but no or only a very weak correlation with $\delta^{13}C$ (as seen shown for *N. atrum*

Fig 6B). We also find the opposite; a clear negative relationship with $\delta^{13}C$, but no clear effect on $\delta^{15}N$ values (as shown for *L. gaussi* in Fig. 9 C)

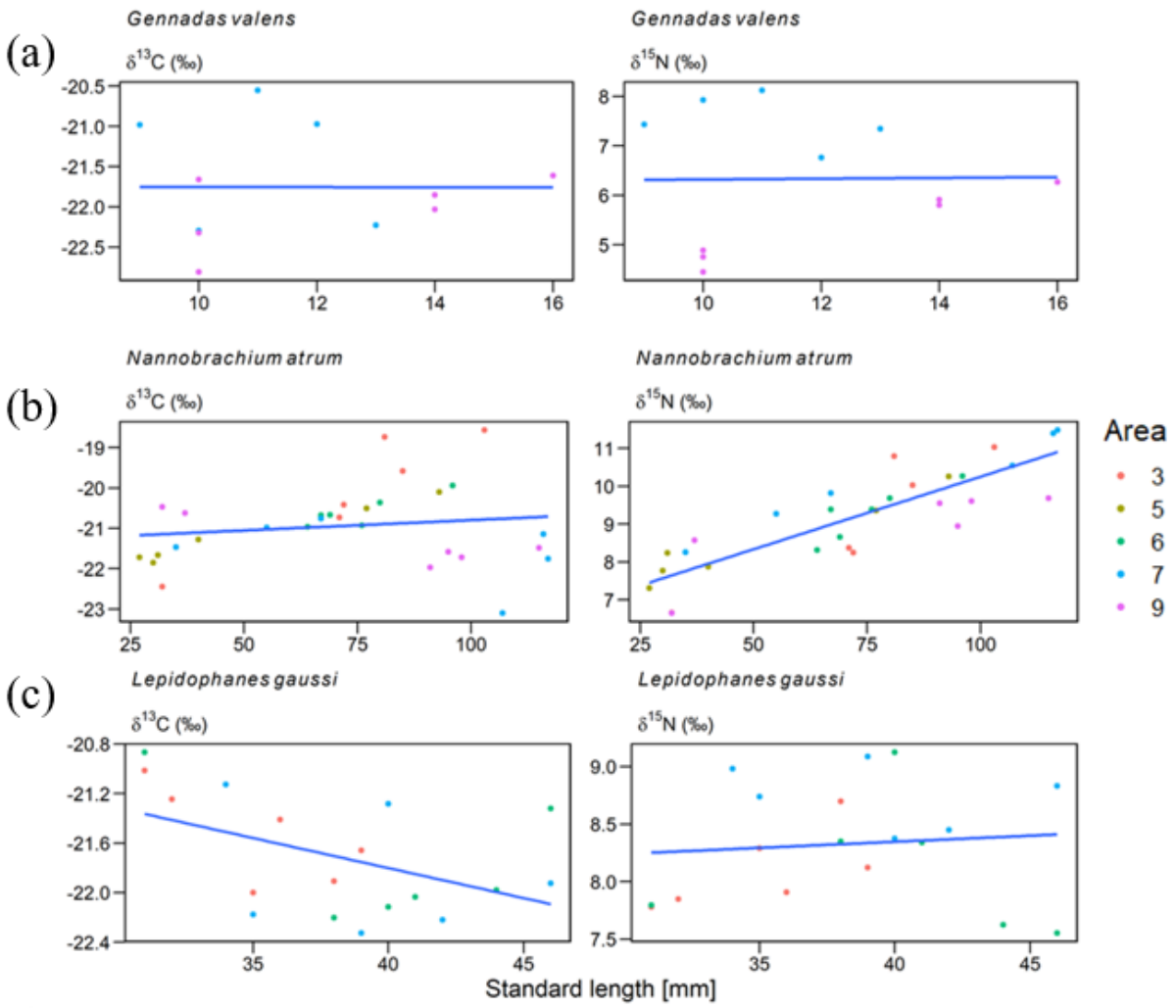

Figure 9. A selection of species showing a weak or no relationship between standard length and $\delta^{15}N$ and/or $\delta^{13}C$.

Overall, *Eucopia sculpticauda*, *Lepidophanes gaussi*, *Maurolicus muelleri*, *Notoscopelus kroyeri*, *Systellaspis debilis* shows a negative relationship between size and $\delta^{14}C$. An illustration depicting this trend is shown in for two example species in figure 10. While this is true as a general trend it is not prevalent for all species in all areas. Specific species in certain areas such as *Notoscopelus kroyeri* in area 5 (Fig.10 B) and *Maurolicus muelleri* in area 7 (Fig.10 A) show a positive relation between size and $\delta^{14}C$ in contrast to the negative relation observed overall analysing the specimen from all areas togheter.

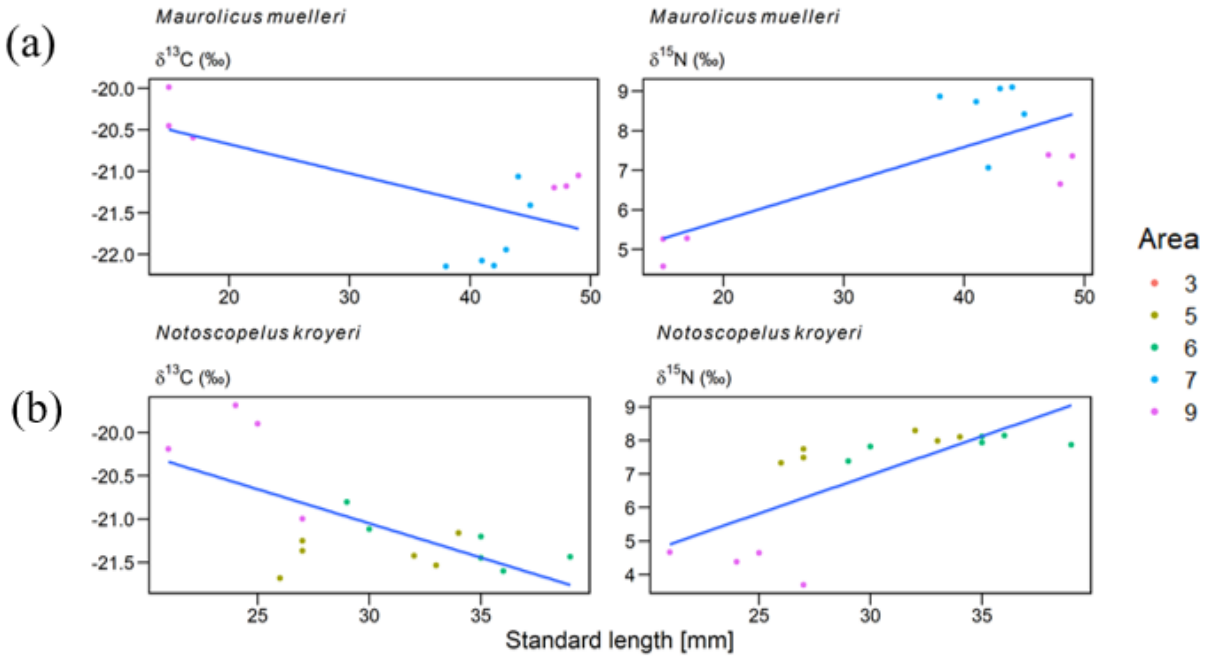

Figure 10. Two of the species with a negative relationship between size and δ^{13}C

3.5 Geographical differences and the effect of environmental features on δ^{13}C and δ^{15}N

The stations along the study transect had different latitude ranging from 26.8 degrees north to 47.2 degrees north and different proximity to mainland and islands. Stations varied also in terms of their environmental conditions. The result in Garcia et al., (2021) paper state that temperature (°C), salinity and oxygen (ml/L) varied along the transect. The southmost areas had a higher temperature and salinity in the 0-200m depth than at the same depth in the northern areas. At 300-700meter depth the temperature and salinity was almost equal along the transect, but lower in the southern areas than in the northern areas at 700-1200m depth. There was less oxygen in the water in the southern areas than the northern at all depths. This difference in environment along the transect is found to have a possible effect on the δ^{13}C and δ^{15}N values. The linear mixed model showed that the effect of distance to shore (km) on δ^{13}C is statistically significant and negative ($p< 0.01$). The same is true for the effect of salinity maximum ($p < 0.001$) and oxygen minimum ($p<0.001$). The effect of temperature maximum was statistically significant and positive ($p<0.001$). Values of δ^{15}N shows a significant positive correlation between distance to shore and temperature maximum ($p<0.001$), and a significant negative correlation between salinity maximum and oxygen minimum ($p<0.001$). A summary of the two linear mixed model is found in Appendix H.

The values of $\delta^{13}C$ and $\delta^{15}N$ vary across the different locations (Fig. 11) vary. The effect of latitude on $\delta^{13}C$ is statistically significant and negative ($p < .001$). The largest variations for $\delta^{13}C$ was found in area 3 (Fig. 11 a). The mean of $\delta^{13}C$ was lowest around 30.6°N (location for station 4607), and highest around 26.8°N (location for station 4604). Area 5, which is the area closest to the Moroccan shore, had a higher (although not significantly higher) mean $\delta^{13}C$ value than the other areas further offshore.In comparison to $\delta^{13}C$, $\delta^{15}N$ was more varied along the transect. There was a statistically significant and negative trend ($p < 0.05$) in $\delta^{15}N$ from south to north. Figure 11 B shows that there was slightly higher mean in area 7 than the other areas.

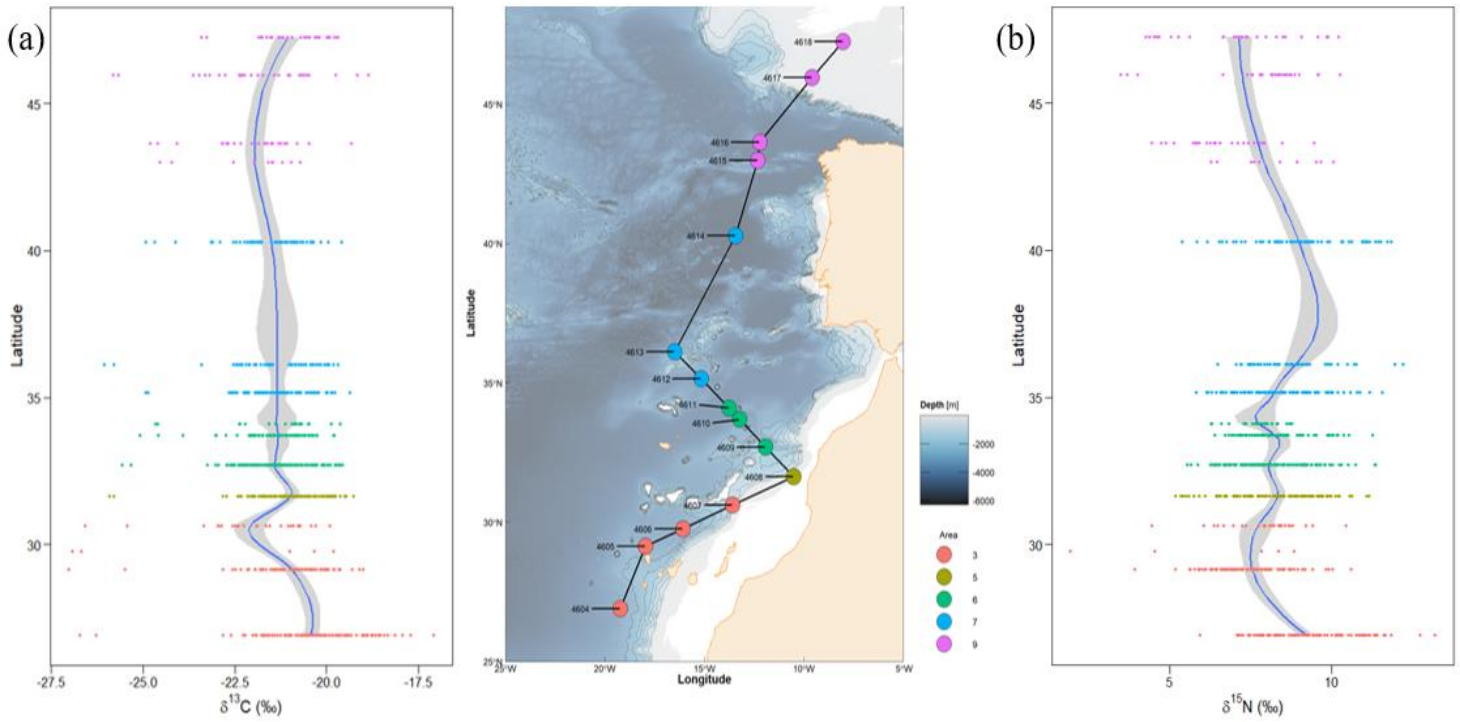

Figure 11. The mean values of $\delta^{13}C$ and $\delta^{15}N$ across the different locations (station 4604 to 4618) (a) shows all the values of $\delta^{13}C$, (b) all the values of $\delta^{15}N$ in each location. The line goes through the mean of the $\delta^{13}C$ (a) and $\delta^{15}N$ (b) in each location. The color of the points represents the area the locations is a part of. A map is included to better visualize the areas. The stations marked in the map has the same order as the location in a) and b) The same colures used for area in the map are used in figure (a) and (b).

4. DISCUSSION

This study reveals new useful information about several of the mesopelagic species that live in the northeast Atlantic Ocean. Although there is abundant literature on the trophic ecology and feeding regimes for many mesopelagic taxa, especially for myctophids (Gordon et al., 1985; T. L. Hopkins, 1996; T. L. Hopkins et al., 1994; T. L. Hopkins & Baird, 1981; Olivar et al., 2019), few studies have focused on the northeast Atlantic Ocean, encompass as many species or cover as much area as this study does. North East Atlantic is one of the most abundant fishing areas in the world (NEAFC, 2014). Information about the food web is used to make fishery management decisions to be sure of sustainable fishing (Foley, 2013). Since several of the species in the mesopelagic zone likely is part of a larger food web will information in this study be useful combined with other trophic structure in the area. Also, A good understanding of a study system is essential for stable isotope mixing models (Phillips et al., 2014) and the information found in this study can be useful for future trophic structure studies in the northeast Atlantic ocean.

4.1 Trophic structure of mesopelagic species

A total of 48 taxa from 12 different families were studied. Many mesopelagic species appear similar in shape, or lifestyle (e.g., a large fraction of them performs diel vertical migration into surface waters at night), yet their trophic ecology differs. Because of their diel vertical migration strategy, understanding their role in the food web is crucial to understand productivity and energy flows between the epipelagic and mesopelagic zone.

4.1.1 Tropic level

Estimates based on stable isotopes show that some of the trophic level estimates in this study corresponds well with those estimated in other studies. The $\delta^{15}N$ and trophic level estimates for myctophids form this study are for instance similar to those described in Olivar et al., (2019) from the equatorial Atlantic just south of the transect from this study. Twelve species (*Benthosema glaciale, Benthosema suborbitale, Ceratoscopelus warmingii, Diaphus mollis, Diaphus rafinesquii, Diogenichthys atlanticus, Lampanyctus alatus, Lampanyctus pusillus, Lobianchia dofleini, Myctophum punctatum, Notolychnus valdiviae* and *Notoscopelus resplendens*) were considered in both studies. Thereof, only *Ceratoscopelus warmingii, Myctophum punctatum* and *Notolychnus valdiviae* differed with more than 0.5 in the trophic level estimates (comparing with the scaled model (TPS) trophic levels estimates, calculated using a decreasing isotopic enrichment with the increase in trophic level). The trophic level

(TL) estimates for *Ceratoscopelus warmingii* differed by 0.7, with an estimated TL of 2 this study compared to TL 2.7 in Olivar et al., (2019). Similary, *Myctophum punctatum* was estimated to have a lower TL, here 1.9, compared to TL 2.81 in Olivar et al., (2019). In contrast, the TL of *Notolychnus valdiviae* was estimated to be higher in our study than those reported from the equatorial Atlantic further to the south, with a TL of 2.4and 1.76, respectively.

Size differences between the two studies may explain some of the deviation in TL. In Olivar et al., (2019) the mean standard length was higher, both for *C. warmingii* (44 mm vs. 58 mm) and *M. punctatum* (33 mm,vs. 66 mm). For *N. valdiviae*, however, the mean standard length was identical (21mm) and hence cannot explain the differences in TL estimates. Several other factors, like methodological differences or errors, as well as geographical and environmental variation could be the cause of the observed differences, however, it is beyond the scope of this study to disentangle those drivers.

Surprisingly, several species that had an estimated trophic level lower than baseline. Since the $\delta^{15}N$ values are correlated with the TL estimates a closer look at the $\delta^{15}N$ value of the samples is necessary to detect why some of the species had an unexpectedly low TL. Seven fish (*G. cocco, S veranyi, H. benoiti, M. muelleri, M. punctatum, N. kroyeri and C. warmingii*) and two crustaceans (*G. valens and S. debilis*) were estimated to have a mean trophic level below the baseline (here the trophic level of the baseline, the seston, was set to TL 2). However, *G. valens*, *S. debilis* and *D. atlanticus* were the only species that had a mean $\delta^{15}N$ lower than the mean $\delta^{15}N$ value of the seston. Although *D. atlanticus* had a lower mean $\delta^{15}N$, the estimated trophic level (2.1 ± 0.28) was higher than the seston. It should be noted that the trophic level estimates in this study use the sampling site specific $\delta^{15}N$ values of the seston as a baseline, not an overall mean. Consequently, the trophic level was estimated for each sample and for each location before averaging the trophic level. There was a lot of variation in the seston $\delta^{15}N$ between the different stations, i.e., water samples, that have affected the result. For example, Station 4612 had the highest $\delta^{15}N$ value measured in the seston (9.2‰) and 12.5 % of all the samples in this study were taken at this station. Here, A total of 25 species had a lower mean $\delta^{15}N$ value than the seston in this location. This means that the $\delta^{15}N$ value of the seston in this location affected the total average trophic level to a greater extent than a location with a small number of species, like station 4606 which had the lowest $\delta^{15}N$ value measured in the seston (3.2 ‰), but also only accounted for 0.5% of all the samples in this study. The reason for the large variation in $\delta^{15}N$ in the seston is unknown.

A different approach that could have been done instead is to use the $\delta^{15}N$ mean for the all the seston sample (6.6‰) as the baseline for all TL calculations regardless of which station the individuals were sampled at. The outcome would then have changes. Not only would it change from seven species to two species with a TL under seston, but also several species would have changed the estimates mean TL (Appendix F). For instance, *Gonichthys cocco* who had the lowest TL (1.5) in the original approach did with the overall mean seston baseline get a TL of 2.1. Even though the TL estimates looks better when the baseline is the mean of all seston samples does however this approach rule out station differences which is also not optimal. Disregarding the total TL average for the species is also an idea, but as mentioned some stations like 4612 had a surprisingly high $\delta^{15}N$ value which again would lead to strange TL estimates in those location. By surprisingly I mean that they are found to have almost the same $\delta^{15}N$ as for instance *Chauliodus sloani* which in the literature is found to feed mostly on fish (Battaglia et al., 2018) while we assumed seston to consist by mostly primary consumers like zooplankton.

4.1.2. Isotopic niche

Based on a visually analysis of the mean $\delta^{15}N$ and $\delta^{13}C$ values of the families they, are segregated into four isotopic niches. An isotopic niche can be defined as an area (in δ-space) with isotopic values (δ-values) as coordinates (Newsome et al., 2007). In this case, $\delta^{15}N$ on the y-axis and $\delta^{13}C$ on the x-axis. The isotopic niche should not be confused with the ecological niche, where environmental and geographical variables are used as axes instead (Hutchinson, 1957). However, these two terms are connected to a certain extent, since stable isotopes provide information on the resource (bionomic) and habitat (scenopoetic) use, which are the two fundamental axes of the ecological niche (Newsome et al., 2007). The $\delta^{15}N$ values provide information about the tropical level of a species (the bionomic dimension), while in marine environments, $\delta^{13}C$-values give an indication about the habitat (the scenopoetic dimension), for examples low vs high latitudes, or in shore vs. offshore feeding grounds (Newsome et al., 2007).

Searsia koefoedi (Platytroctidae) had distinctly higher $\delta^{15}N$ values than the rest of the species, and an estimated tropic level of 3.2 ± 0.48. This suggests that *S. koefoedi* is a piscivore, at least partially, and feeds higher in the food chain than the rest of the mesopelagic assemblage in the area. Hence, here it was considered to occupy its own isotopic niche. Little is known about *Searsia koefoedi* apart from that it inhabits the lower mesopelagics and upper bathypelagic zone and its diet has not previously been described. Other closely related species in the family Platytroctidae have been found to feed on gelatinous zooplankton, as well as chaetognaths, copepods, and ostracods (Novotny, 2018). Since *Searsia koefoedi* is considered a partial

migrator and usually does not occur occurs shallower than 500 m (Matsui & Rosenblatt, n.d.). Considering a trophic enrichment factor of 2.9 ‰ for nitrogen (Sweeting et al., 2007) and 1‰ for carbon (Caut et al., 2009) a suggestion is that *Searsia koefoedi* (11.2‰ and -20.4‰) in this study may feed on prey with a $\delta^{15}N$ value around 8‰ and a $\delta^{13}C$ value around -21.4‰. These values match the isotopic niche of several species in the families Myctophidae (e.g. Benthosema suborbital and Bolinichthys indicus), Sternoptychidae (e.g., Vinciguerria poweriae) as well as some crustaceans (the crustacean *Eucopia sculpticauda* and *Sergia robusta*). The high δ15N may also indicate that sinking, dead organic matter could play an important role in its diet. Without further stomach content analysis, it is difficult to conclude with certainty.

The families Stomiidae, Eurypharyngidae, Serrivomeridae and Gonostomatidae were characterized by generally high $\delta^{15}N$ and $\delta^{13}C$ values but were overall similar and hence grouped based on their overlapping isotopic niches. According to the literature, most stomiids are piscivores. For example, Sloane's viperfish *Chauliodus sloani* (Stomiidae) is described to feed exclusively on mesopelagic fish belonging to the families Gonostomatidae, Myctophidae, Paralepididae, Phosichthyidae, Sternoptychidae, and in some cases its own conspecifics (Battaglia et al., 2018). Loosejaws *Photostomias guernei* (Stomiidae), however, have a characteristic diet of decapod shrimps which in some studies accounted for 89% of the diet items and 97% of the prey biomass (T. L. Hopkins, 1996). In contrast, Bean's sawtooth eel *Serrivomer beanii* (Serrivomeridae) and pelican eel *Eurypharynx pelecanoides* (Eurypharyngidae), both characteristic members of their families, are described as generalists that feed mainly on crustaceans, cephalopods and other fish. (Eschmeyer & Herald, 1983; Geidner, 2008). The isotopic values of these species found here match the prey organism in the literature relatively well.

Gonostomatids, also commonly known as bristlemouths, are typically planktivores (Gordon et al., 1985). It is therefore surprising that here we estimate trophic levels of 2.6 and 3.1, which generally would suggest a piscivorous diet. Although some species like the Elongated bristlemouth *Sigmops elongatus* are known feed on macrozooplankton and small fish fishes(Badcock, 1984), we find that they have very similar $\delta^{15}N$ and $\delta^{13}C$ values as for example the longray fangjaw *Bonapartia pedaliota* and the bristlemouths *Cyclothone* sp. which are predominantly planktivorous (Badcock, 1984). Here, again marine snow, i.e., sinking dead organic material from higher trophic levels species may be part of the explanation.

Here, crustaceans were found to have on average lower $\delta^{15}N$ and $\delta^{13}C$ values than the fishes and their overlapping isotopic values suggest that *A. quadrispinosa* (Acanthephyridae), *G. valens* (Benthesicymidae), *O. spinosus* (Oplophoridae) *S. debilis* (Oplophoridae), *R. robusta* (Sergestidae) and *E. sculpticauda* (Eucopiidae) share an isotopic niche and hence may compete for the same resources. Despite overlapping isotopic values, the spread in this isotopic niche is much wider than compared to the previous niches described in this study for fishes. Especially *E. sculpticauda* has relatively lower $\delta^{13}C$ values than the other crustacean as well as the fishes. It can therefore not be excluded, that individuals of this taxa feed in another scenopoetic dimension and occupy another ecological niche. In the literature, *Eucopia sculpticauda* is described to mainly prey on decapods, while the other crustacean species are more generalist that feed on different types of zooplankton, crustaceans, as well as small fishes like *Cyclothone* sp. (T. L. Hopkins et al., 1994).

According to their isotopic values, species in the families Sternoptychidae and Myctophidae occupy a central position in the mesopelagic assemblage in the Northeast Atlantic. Some overlap with the isotopic niche of more piscivorous fish above and shrimp-like crustaceans below, suggest that the different species in these families the family might feed in one or both niches. There is a large isotopic variation among the different species within both families. Most Sternoptychidae appear to share an isotopic niche with the crustaceans, the exception being the constellation fish *Valenciennellus tripunctulatus* that had higher $\delta^{15}N$ values than the rest of the Sternoptychidae species. Sternoptychids mostly feed on copepods and/or /and amphipods (Carmo et al., 2015). The same is found for *V. tripunctulatus*. Hopkins & Baird, (1981) reported that in the Gulf of Mexico and Caribbean, copepods accounted for 95% of all food items in their diet.

Isotopic values for the different myctophid species varied even more than those of the sternoptychids. The estimated TL for myctophids in this study ranges range from TL 1.5 for *G. cocco* to TL 2.9 for *Lobianchia gemellarii*. The other 25 myctophid species fall in between those two extremes. Myctophids are opportunistic predators, meaning they have a widely variation of prey and can adapt to whatever food becomes available. They are found to prey on copepods, ostracods, euphausiids, amphipods, chaetognaths, pteropods, fish eggs and fish larvae (Catul et al., 2011). This feeding strategy makes it difficult to define one isotopic niche for myctophids.

For some species, the isotopic values varied across areas and environmental conditions. For example, in northernmost area the crustacean *A. quadrispinosa* (Acanthephyridae), *E. sculpticauda* (Eucopiidae) and *G. valens* (Benthesicymidae) are distinctly different from other species due to their low $\delta^{13}C$. In the areas more south, the same species seems to share an isotopic niche with the rest of the crustaceans, sternoptychids and myctophids. One explanation is that their prey may differ under different environmental settings. But there are several other reasons why the trophic ecology of a species may vary across areas. Disentangling the different drivers is beyond the scope of this study.

To further improve and verify improve knowledge on isotopic niches in this study, a stable isotope mixing model could have been used. These mathematical mixing models can be used to convert isotopic data into statistical estimates of source proportions (J. B. Hopkins & Ferguson, 2012; Phillips, 2012; Phillips et al., 2014).

4.2. Intraspecific patterns: Size-based diet shifts

Marine food webs are often described as highly size-structured i.e., large individuals eat smaller ones. There is an advantage for a predator to be bigger than its prey as it make it easier to eat the prey in the absence of body parts to hold the prey (Galván et al., 2010). However, investigations on the food web structure in the mesopelagic zone are underrepresented in the literature and even fewer studies addresses size-based diet shifts.

Out of the 48 species included in this study, Eighteen had a significant increase in $\delta^{13}C$ with increasing length and Five species showed a significant $\delta^{13}C$ decrease. This significant relationships can indicate that species with this trends change the location they are feeding in. For $\delta^{15}N$, a total of 35 species showed a significant increase with length indicating that these species feed higher in the food chain as they grow. 25 species had a weak or no pattern between length and $\delta^{13}C$ values, and 13 species in $\delta^{15}N$ indicating that they don't have an significant changes in diet as they grows.

Clear relationships between tropic level and size could not be established for all species. For some species the size distribution was very uniform suggesting that not all size classes where representatively sampled, while in other cases several small but only one large individual was.analyzed for isotopes. Here, the data basis is deficient and does not allow to make conclusive predictions about the relationship between size and trophic level. Another confounding factor are area differences e.g., were all large individuals were caught in one area and all small individuals in another. The prey preferences and/or prey composition might be

different to the different areas it becomes impossible to attribute the differences with certainty to either to size or area.

4.3 Limitations

4.3.1. Baseline

A correct isotopic baseline is key to provide accurate trophic level estimates and determine trophic niches, or in other words "*if your baseline is crap, your model is going to be crap and your result will be crap*" (Hayden, 2016). However, obtaining an correct baseline is described as one of the most difficult methodological issues in stable isotope studies (Post, 2002).

In this study there were large differences in the baseline (seston) across the different station. Although environmental features have showed to have a significant effect on the isotope values it is not enough explanation alone since the same large variation in seston across the stations is not found for the study species in the study. One of the sources of errors using the seston as a baseline is the uncertainty to what the seston samples contain. The large differences in the isotope values across the different seston samples make it likely that the organic contents on the filters differed. For instance, the two replicates collected at station 4607 had distinctly different $\delta^{15}N$ values (4.4‰ and 10.4‰). One possible explanation is that the seston sample with high $\delta^{15}N$ contain parts of dead organic material from high trophic level organism or more zooplankton than the replicate with lower $\delta^{15}N$. Because of the uncertainty of the containment on the filter it is hard to determine what the trophic level of the reference baseline should be, which then consequently scales all other trophic level estimates.

4.3.2 The Trouble with trophic enrichment factor

Because the trophic enrichment factor is a multiplicator when calculating the trophic level (see Equation 2) it is hugely important. However, the values for trophic enrichment are highly uncertain and may vary among consumers, and across different tissue, prey, habitat, or body sizes etc.(McCutchan et al., 2003; McMahon et al., 2015; MILL et al., 2007; Sweeting et al., 2007; Zanden & Rasmussen, 2001). For this study, a trophic enrichment factor of 2.9 was chosen for all calculation based on suggestions from (Sweeting et al., 2007). One key factor not considered here was the source of tissue used for the stable isotope analysis. In this study muscle tissue was taken from fish with a standard length of ≥60 mm, while smaller individuals were kept whole. This may have biased the results as studies suggest that the mean $\Delta\delta^{15}N$ for muscle tissues is higher than that of whole fish (Sweeting et al., 2007). In this study, 194 of 885 individuals had a length of ≥60mm and therefore muscle tissues were used, meaning that around

21% of the samples would have more correct trophic level estimates if the trophic enrichment factor was adjusted higher to 3.2 as Sweeting et al., (2007) suggests. A higher trophic enrichment factor will lead to a lower estimated trophic level for these species.

4.5. Future studies

An interesting avenue for future studies would be to confirm the trophic level estimates and isotopic niches with stomach content analysis. This should easily be While isotopes can give a more long-term pictures can stomach content provide a snapshot of the current conditions. Stable isotopes cannot however determine specific prey types a species in feeding on but only on which trophic level they mostly feed and were. Preforming a stomach content analysis for the myctophids in this study is easily possible, as all the myctophid specimen in this study had their stomach removed and are preserved in ethanol. The myctophids in this study had a great range of $\delta^{15}N$ and $\delta^{13}C$ values and it should be interesting to see if the stomach contents confirm this large variation inside the Myctophidae family. Stomach content analysis for the other study species would also be welcomed especially for the species that had a $\delta^{15}N$ and $\delta^{13}C$ values that was unexpected compared on their diets described in the literature like *Cyclothone sp*. which was typically described as planktivorous (Gordon et al., 1985) but in this study had the same $\delta^{15}N$ has species described as piscivores and for *Searsia koefoedi* were no diet description was found. Combining stomach content and isotope analyses to identify the trophic structure of mesopelagic fishes has showed to be usefulness not only for improving result in stable isotope analysis, but also the strength the studies with stomach content analysis (McClain-Counts et al., 2017; Olivar et al., 2019).

Another aspect that should be consider doing more research on is the trophic enrichment factor. As mentioned above, we lack data on the specific trophic enrichment factor for many mesopelagic fishes. To make better estimates in the future we will therefore need more research on trophic enrichment factors in the mesopelagic zone.

5. CONCLUSION

This study shows that the mesopelagic species along a transect roughly from the Canary Islands to the Bay of Biscay spans three trophic levels from TL 1.5 for *G. cocco* to TL 3.2 for *S. koefoedi*. It is also suggested that the families in this study can be coupled into three isotopic groups. Most species had a positive relationship between size and $\delta^{15}N$ and $\delta^{13}C$, except for a

few species that showed no relationship or a negative relationship. Additionally, local environmental conditions were found to be a significant predictor of $\delta^{15}N$ and $\delta^{13}C$ values in mesopelagic species in the Northeast Atlantic Ocean.

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Appendix A. Distribution of samples used in this study.

Distribution of samples in groups

Group	**Crustaceans**	**Filter**	**Fish**
Number of samples	116	30	769
Proportion	0.127	0.033	0.840

Distribution of samples in areas

Area	**3**	**5**	**6**	**7**	**9**
Number of samples	229	122	234	234	96
Proportion	0.250	0.133	0.256	0.256	0.105

4. Distribution of samples that was used in this study in each station

Stations	**4604**	**4605**	**4606**	**4607**	**4608**	**4609**	**4610**	**4611**	**4612**	**4613**	**4614**	**4615**	**4616**	**4617**	**4618**
Number of samples	103	94	5	27	122	147	67	20	114	54	66	10	24	30	32
Proportion	0.113	0.103	0.005	0.030	0.133	0.161	0.073	0.022	0.125	0.059	0.072	0.011	0.026	0.033	0.034

Table 4. Overview of the number of individuals of each species from each area

species	area_3	area_5	area_6	area_7	area_9
Acanthephyra quadrispinosa	6	6	6	6	6
Argyropelecus aculeatus	6	6	6	6	
Argyropelecus gigas	6		6	6	
Argyropelecus hemigymnus	6		6	6	
Benthosema suborbitale	6		6		
Bolinichthys indicus	6		6	6	
Bonapartia pedaliota	6	3	6	6	
Ceratoscopelus warmingii	6		2	3	
Chauliodus sloani	6	6	6	6	
Cyclothone braueri	6	6	6	6	6
Cyclothone microdon	6		6	6	6
Cyclothone pseudopallida	6	6	6	6	6
Diaphus mollis	6			3	
Diaphus rafinesquii	6			3	
Diogenichthys atlanticus	6		6		
Eurypharynx pelecanoides	6	6	6	6	
seston	8	2	6	6	8
Hygophum hygomii	6		6	6	
Hygophum reinhardtii	6		3		
Hygophum taaningi	6		5	3	
Lampanyctus alatus	6	6	2		
Lampanyctus crocodilus	6				6
Lepidophanes gaussi	6		6	6	
Lobianchia dofleini	6	6	6	6	
Lobianchia gemellarii	6		4	4	
Nannobrachium atrum	6	6	6	6	6
Nannobrachium cuprarium	6	4	6	6	
Notolychnus valdiviae	6	3	6	6	3
Notoscopelus resplendens	6		6	6	
Oplophorus spinosus	6		6	6	
Photostomias guerneiguernei	6	3	6	6	3
Searsia koefoedi	6		6	6	2
Serrivomer beanii	6	6	6	6	2
Sigmops elongatus	6	6	5	6	
Sternoptyx sp.	6	5	6	6	
Systellaspis debilis	5	6	6	6	6
Valenciennellus tripunctulatus	6	6	5	4	
Vinciguerria poweriae	6	6	6	6	
Lampanyctus pusillus		6	6		
Myctophum punctatum		6	6	6	2
Notoscopelus kroyeri		6	6		4
Gonichthys cocco			6	6	
Hygophum benoiti			6	6	
Robustosergia robusta			4	6	6

Symbolophorus veranyi			6	5	
Benthosema glaciale				6	6
Eucopia sculpticauda				6	6
Gennadas valens				5	6
Maurolicus muelleri				6	6

Table 5. Overview of the number of individuals of each species from each station. Orange color are the station that was trawl with the Multpelt and blue color was the station trawls at night at a shallower depth.

species	4604	4605	4606	4607	4608	4609	4610	4611	4612	4613	4614	4615	4616	4617	4618
Acanthephyra quadrispinosa		6			6	6			3	2	1		5	1	
Argyropelecus aculeatus		1		5	6	5	1		3	2	1				
Argyropelecus gigas	4		2			4	2		4	1	1				
Argyropelecus hemigymnus	3	3				6			3		3				
Benthosema glaciale											6	2	1	2	1
Benthosema suborbitale		6				6									
Bolinichthys indicus	4	2				6			6						
Bonapartia pedaliota	2	4			3	3	3		6						
Ceratoscopelus warmingii	6						2		1		2				
Chauliodus sloani	6				6		6		3	1	2				
Cyclothone braueri		6			6		6			3	3			6	
Cyclothone microdon		6				6				3	3				6
Cyclothone pseudopallida		6			6	6				6				6	
Diaphus mollis		5	1						3						
Diaphus rafinesquii	6								1	2					
Diogenichthys atlanticus		3		3		6									
Eucopia sculpticauda									5		1			6	
Eurypharynx pelecanoides		6			6	4	2		6						
Gennadas valens										3	2		6		
Gonichthys cocco						4	2		6						
Hygophum benoiti							4	2	3		3				
Hygophum hygomii	6					6			6						
Hygophum reinhardtii		6				3									
Hygophum taaningi	6					2	3		3						
Lampanyctus alatus				6	6	1		1							
Lampanyctus crocodilus	4			2									2	2	2
Lampanyctus pusillus					6	6									
Lepidophanes gaussi	6						4	2	3	2	1				
Lobianchia dofleini	6				6	6			6						
Lobianchia gemellarii	6					1	3		1		3				
Maurolicus muelleri											6				6
Myctophum punctatum					6	1	3	2	4		2				2
Nannobrachium atrum	3			3	6	6				2	4	3		3	
Nannobrachium cuprarium		6			4	6			3	3					
Notolychnus valdiviae		2		4	3	6				6		1	2		
Notoscopelus kroyeri					6		6							1	3
Notoscopelus resplendens	6					6			6						
Oplophorus spinosus	6						3	3	3	3					
Photostomias guernei	3	3			3	2	2	2	2	2	2		3		
Robustosergia robusta						1	3				6		3		3
Searsia koefoedi	6					3	3		3	2	1	1		1	
Serrivomer beanii	6				6	6			2	2	2	1			1
Seston	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Sigmops elongatus	5	1			6	1	2	2	4	1	1				
Sternoptyx sp.		6			5	6			3		3				

Symbolophorus veranyi							5	1	4		1				
Systellaspis debilis		5			6	3		3		2	4				6
Valenciennellus tripunctulatus		4		2	6	5				4					
Vinciguerria poweriae	1	5			6	6			6						

Appendix B. R packages

R package	Reference
here	Kirill Müller (2020). here: A Simpler Way to Find Your Files. R package version 1.0.1. https://CRAN.R-project.org/package=here
tidyverse	Wickham et al., (2019). Welcome to the tidyverse. Journal of Open Source Software, 4(43), 1686, https://doi.org/10.21105/joss.01686
dplyr	Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.6. https://CRAN.R-project.org/package=dplyr
janitor	Sam Firke (2021). janitor: Simple Tools for Examining and Cleaning Dirty Data. R package version 2.1.0. https://CRAN.R-project.org/package=janitor
cowplot	Claus O. Wilke (2020). cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.1. https://CRAN.R-project.org/package=cowplot
lme4	Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.
lmerTest	Kuznetsova A, Brockhoff PB, Christensen RHB (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." _Journal of Statistical Software_, *82*(13), 1-26. doi: 10.18637/jss.v082.i13 (URL: https://doi.org/10.18637/jss.v082.i13).
report	Makowski, D., Ben-Shachar, M.S., Patil, I. & Lüdecke, D. (2020). Automated Results Reporting as a Practical Tool to Improve Reproducibility and Methodological Best Practices Adoption. CRAN. Available from https://github.com/easystats/report. doi: .
writexl	Jeroen Ooms (2021). writexl: Export Data Frames to Excel 'xlsx' Format. R package version 1.4.0. https://CRAN.R-project.org/package=writexl
ggthemes	Jeffrey B. Arnold (2021). ggthemes: Extra Themes, Scales and Geoms for 'ggplot2'. R package version 4.2.4. https://CRAN.R-project.org/package=ggthemes
patchwork	Thomas Lin Pedersen (2020). patchwork: The Composer of Plots. R package version 1.1.1. https://CRAN.R-project.org/package=patchwork

Hmisc	Frank E Harrell Jr, with contributions from Charles Dupont and many others. (2021). Hmisc: Harrell Miscellaneous. R package version 4.5-0. https://CRAN.R-project.org/package=Hmisc

Appendix C. Illustration and short diet description of species or families

Fish

Serrivomeridae (Sawtooth eels)

Serrivomer beanii

A generalist that feeding on crustacean, cephalopods and teleost (Geidner, 2008).

Eurypharyngidae

Eurypharynx pelecanoidid

Adults feed mainly on crustaceans and fish, but also take cephalopods, and other invertebrates. (Eschmeyer & Herald, 1983)

Platytroctidae

Searsia koefoedi

No specific diet data for *S. koefoedi* is found, but other species in the family Platytroctidae is found to consumed gelatinous zooplankton as well as chaetognaths, copepods, and ostracods (Novotny, 2018)

Gonostomatidae

Species in this family is found to preyed predominantly on copepods (Gordon et al., 1985)

Bonapartia pedaliota	
Cyclothone braueri	
Cyclothone microdon	

	
Cyclothone pseudopallida	
Sigmops elongatus Feed on crustaceans and small fishes(Badcock, 1984)	

Sternoptychidae

Species do mostly feed on zooplankton and are found to be Copepod-eaters or /and Amphipod-eaters.(Carmo et al., 2015)

Argyropelecus aculeatus	
Argyropelecus gigas	
Argyropelecus hemigymnus	

	
Maurolicus muelleri	
Sternoptyx sp.	

Valenciennellus tripunctulatus

Vinciguerria poweriae

Stomiidae

Chauliodus sloani

A specialist predator which feeds exclusively on mesopelagic fish belonging to Gonostomatidae, Myctophidae, Paralepididae, Phosichthyidae, Sternoptychidae and Stomiidae. (Battaglia et al., 2018)

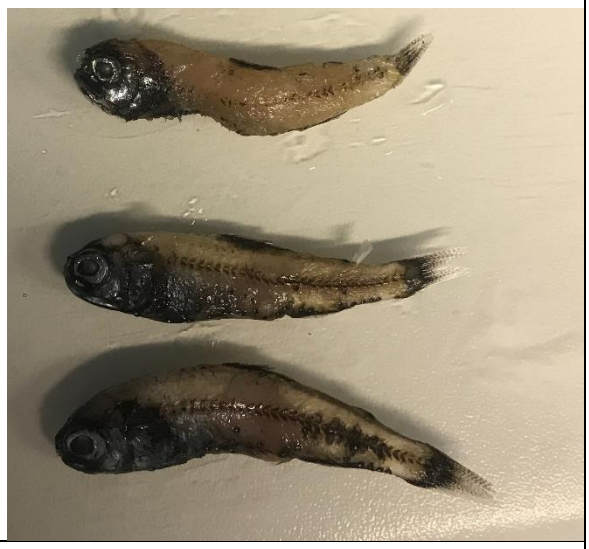
Photostomias guernei

Found characteristic diet of penaeidean shrimp. Decapod shrimps accounted for 89% of the diet items and 97% of the prey biomass of this species. The chief prey species was *Sergestes pectinatus*. Euphausiids were a significant dietary component of smaller fish (<40mm SL). (T. L. Hopkins, 1996)

Myctophidae

Opportunistic predators on copepods, ostracods, euphausiids, amphipods, chaetognaths, pteropods, fish eggs and fish larvae.(Catul et al., 2011)

Bolinichthys indicus

Ceratoscopelus warmingii 
Diaphus mollis 
Diaphus rafinesquii
Diogenichthys atlanticus 
Hygophum reinhardtii

Crustacean

2.3 **Oplophoridae**

Systellaspis debilis

Appendix D. Size and $\delta^{13}C$ and $\delta^{15}N$

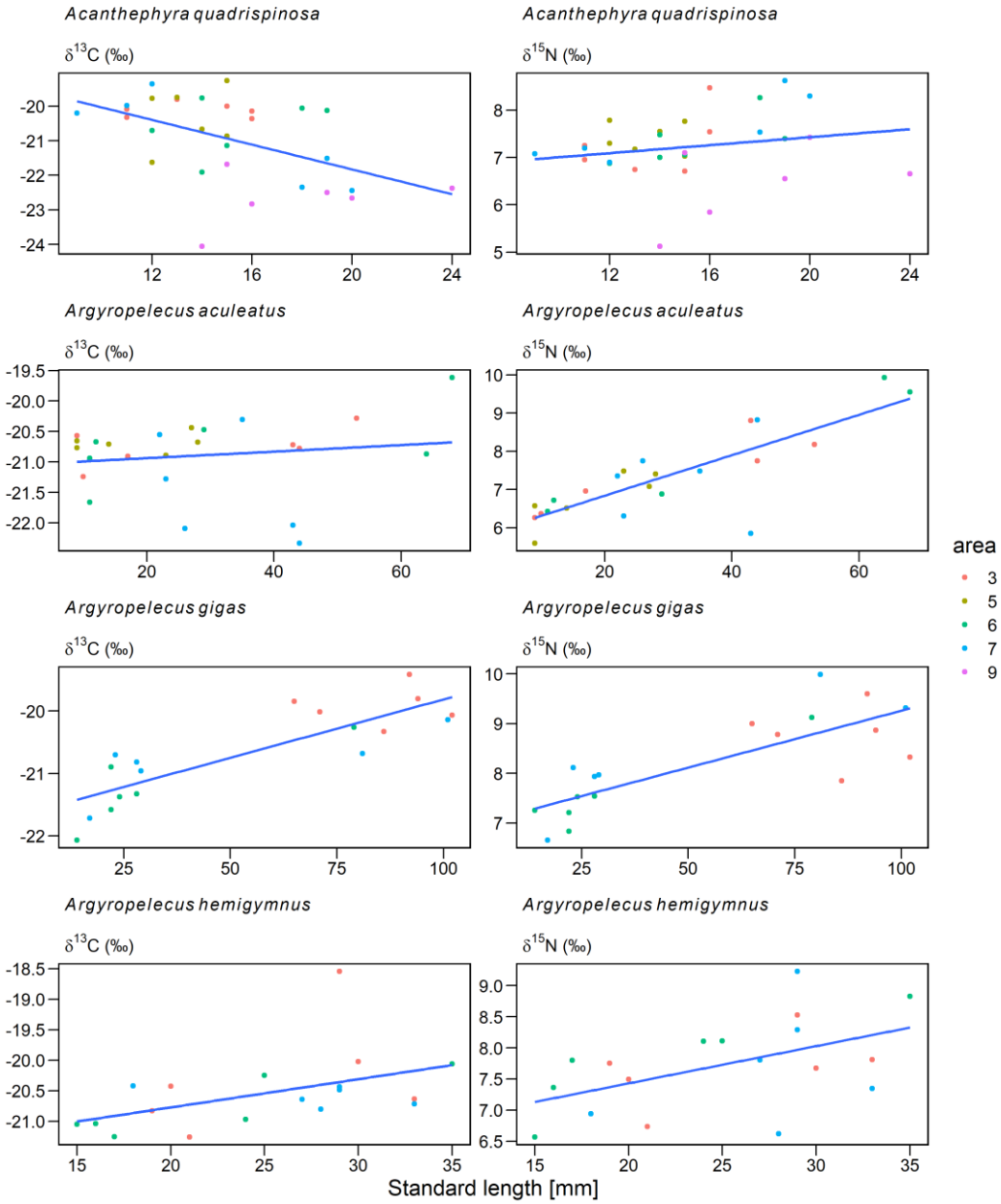
Table 6. Linear model test checking relationship between length of the species and $\delta^{13}C$ and $\delta^{15}N$ values. Trend are p=positive trend and N= negative trend. Significant stars symbolize ns = non-significant,(P > 0.05). * = P ≤ 0.05; ** = P ≤ 0.01, *** = P ≤ 0.001

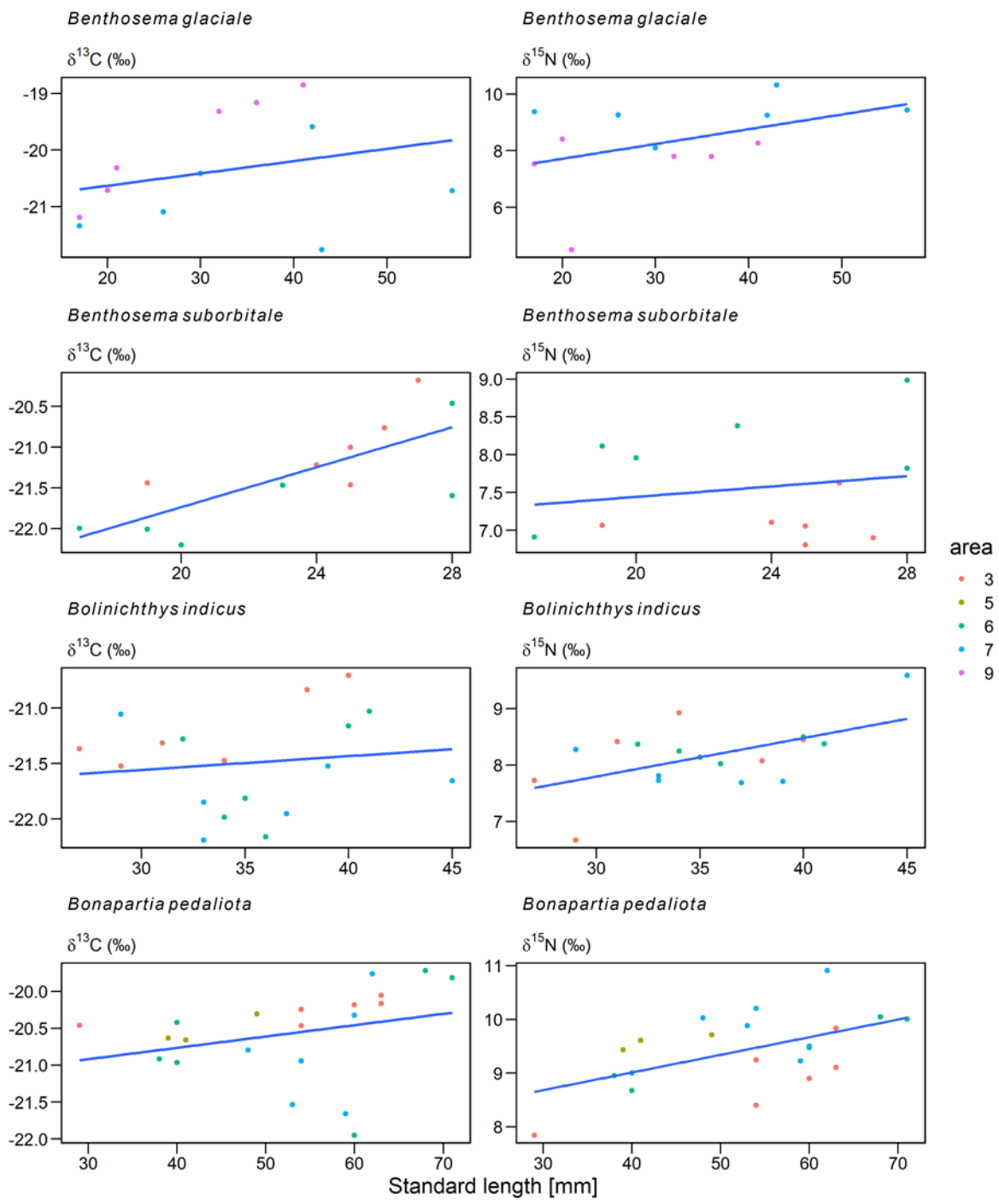
species	var	P value	adjusted R squared	Significance	Trend
Acanthephyra quadrispinosa	δ13C	0,005774309	0,214799953	**	P
Acanthephyra quadrispinosa	δ15N	0,291286331	0,005381938	ns	
Argyropelecus aculeatus	δ13C	0,476337336	-0,021086019	ns	
Argyropelecus aculeatus	δ15N	5,0336E-07	0,67601978	***	P
Argyropelecus gigas	δ13C	8,87107E-06	0,701218179	***	P
Argyropelecus gigas	δ15N	8,07301E-05	0,608926678	***	P
Argyropelecus hemigymnus	δ13C	0,046938418	0,176139023	*	P
Argyropelecus hemigymnus	δ15N	0,028626998	0,219629345	*	P
Benthosema glaciale	δ13C	0,366450954	-0,009647079	ns	
Benthosema glaciale	δ15N	0,152960007	0,112362644	ns	
Benthosema suborbitale	δ13C	0,005334854	0,512173728	**	P
Benthosema suborbitale	δ15N	0,559403505	-0,061303544	ns	
Bolinichthys indicus	δ13C	0,596302031	-0,043448461	ns	
Bolinichthys indicus	δ15N	0,020783319	0,247014724	*	P
Bonapartia pedaliota	δ13C	0,209056051	0,033383975	ns	
Bonapartia pedaliota	δ15N	0,011325635	0,255458449	*	P
Ceratoscopelus warmingii	δ13C	0,245774544	0,051369817	ns	
Ceratoscopelus warmingii	δ15N	0,885543254	-0,108410524	ns	
Chauliodus sloani	δ13C	4,76176E-06	0,604262051	***	P
Chauliodus sloani	δ15N	0,003273592	0,300490764	**	P
Cyclothone braueri	δ13C	0,746113195	-0,031774476	ns	
Cyclothone braueri	δ15N	0,002974222	0,248395078	**	P
Cyclothone microdon	δ13C	0,153037359	0,049216117	ns	
Cyclothone microdon	δ15N	0,000341927	0,423764184	***	P
Cyclothone pseudopallida	δ13C	0,055365451	0,093670401	ns	
Cyclothone pseudopallida	δ15N	4,56169E-05	0,433738007	***	P
Diaphus mollis	δ13C	0,162397445	0,152361753	ns	
Diaphus mollis	δ15N	0,529670298	-0,075686078	ns	
Diaphus rafinesquii	δ13C	0,335675892	0,008485929	ns	
Diaphus rafinesquii	δ15N	0,015593737	0,531674014	*	P
Diogenichthys atlanticus	δ13C	0,307494258	0,014009791	ns	
Diogenichthys atlanticus	δ15N	0,022777531	0,361407272	*	P
Eucopia sculpticauda	δ13C	0,019008843	0,382127858	*	N
Eucopia sculpticauda	δ15N	0,276888625	0,028490251	ns	
Eurypharynx pelecanoides	δ13C	2,37753E-05	0,543746345	***	P
Eurypharynx pelecanoides	δ15N	0,000178549	0,45544088	***	P

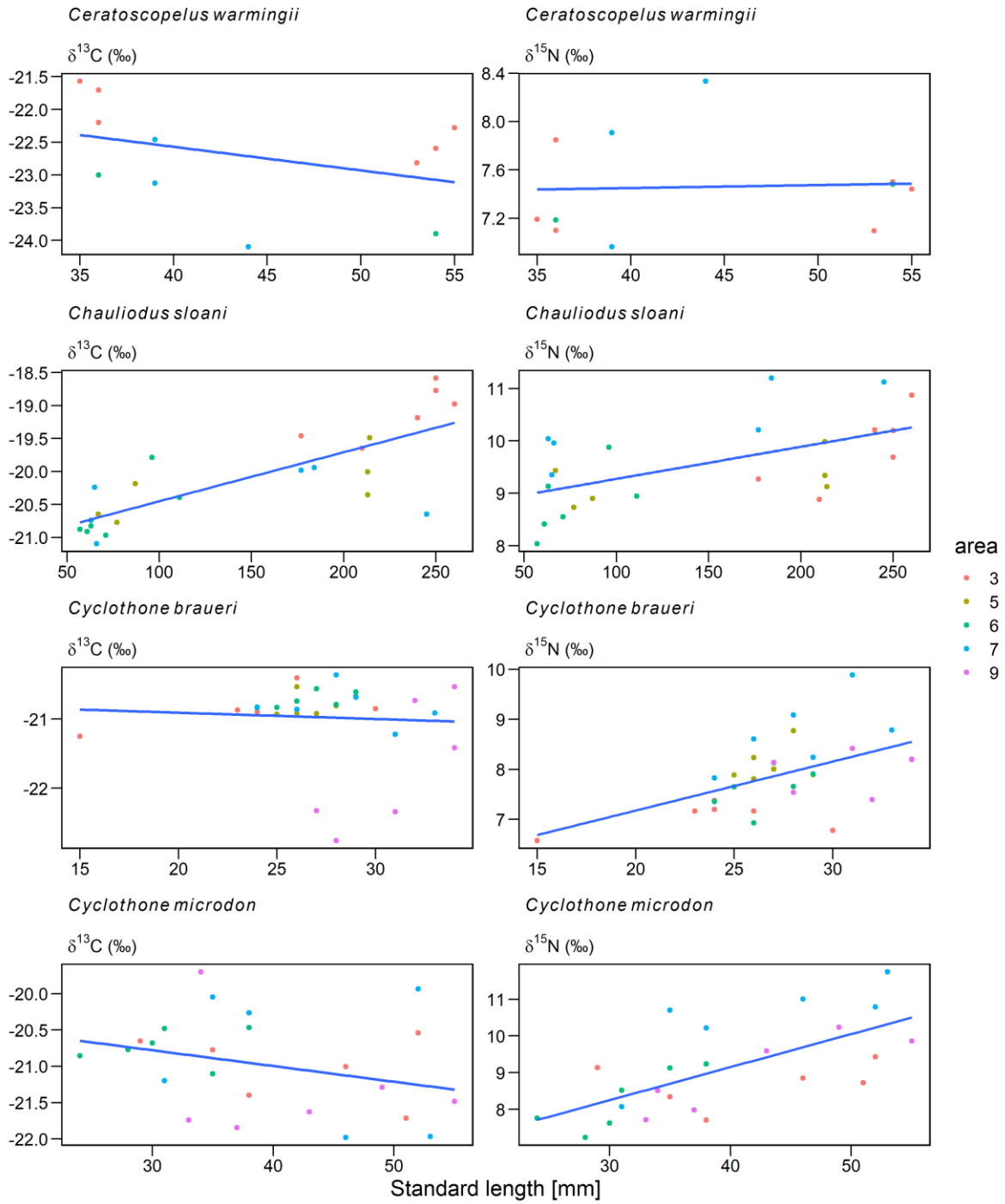
Gennadas valens	δ13C	0,992247106	-0,11109879	ns	
Gennadas valens	δ15N	0,968460757	-0,110907124	ns	
Gonichthys cocco	δ13C	0,161750338	0,104439238	ns	
Gonichthys cocco	δ15N	0,066522676	0,227451458	ns	
Hygophum benoiti	δ13C	0,074517702	0,212226226	ns	
Hygophum benoiti	δ15N	1,45739E-05	0,844959425	***	P
Hygophum hygomii	δ13C	0,193400209	0,047264025	ns	
Hygophum hygomii	δ15N	2,16418E-05	0,666805935	***	P
Hygophum reinhardtii	δ13C	0,000257042	0,849100757	***	P
Hygophum reinhardtii	δ15N	0,001025376	0,777735035	**	P
Hygophum taaningi	δ13C	0,012482024	0,369409443	*	P
Hygophum taaningi	δ15N	0,041780079	0,243845136	*	P
Lampanyctus alatus	δ13C	0,030288521	0,278952897	*	P
Lampanyctus alatus	δ15N	0,003159172	0,49077936	**	P
Lampanyctus crocodilus	δ13C	3,65448E-05	0,814166689	***	P
Lampanyctus crocodilus	δ15N	2,57909E-06	0,88992798	***	P
Lampanyctus pusillus	δ13C	0,579831791	-0,065128232	ns	
Lampanyctus pusillus	δ15N	0,005750814	0,505223488	**	P
Lepidophanes gaussi	δ13C	0,043841256	0,182221122	*	N
Lepidophanes gaussi	δ15N	0,700004016	-0,052376854	ns	
Lobianchia dofleini	δ13C	0,023622275	0,176024149	*	P
Lobianchia dofleini	δ15N	3,74721E-06	0,612591683	***	P
Lobianchia gemellarii	δ13C	0,153650342	0,092162358	ns	
Lobianchia gemellarii	δ15N	0,03604873	0,260093465	*	P
Maurolicus muelleri	δ13C	0,020675653	0,372568258	*	N
Maurolicus muelleri	δ15N	0,003909668	0,540012583	**	P
Myctophum punctatum	δ13C	0,039570827	0,171189304	*	P
Myctophum punctatum	δ15N	2,0734E-05	0,624115633	***	P
Nannobrachium atrum	δ13C	0,432445313	-0,012767613	ns	
Nannobrachium atrum	δ15N	1,41328E-09	0,72604709	***	P
Nannobrachium cuprarium	δ13C	0,378333691	-0,009046799	ns	
Nannobrachium cuprarium	δ15N	0,494846258	-0,025215916	ns	
Notolychnus valdiviae	δ13C	0,931704398	-0,045097566	ns	
Notolychnus valdiviae	δ15N	0,032404967	0,154886494	*	P
Notoscopelus kroyeri	δ13C	0,005885149	0,388306082	**	N
Notoscopelus kroyeri	δ15N	0,001513476	0,490413151	**	P
Notoscopelus resplendens	δ13C	0,003234555	0,392024717	**	P
Notoscopelus resplendens	δ15N	1,46569E-06	0,760469301	***	P
Oplophorus spinosus	δ13C	0,849655033	-0,060041031	ns	
Oplophorus spinosus	δ15N	0,004672379	0,365337058	**	P
Photostomias guernei	δ13C	0,000268392	0,435761283	***	P
Photostomias guernei	δ15N	0,000254261	0,43840972	***	P

Robustosergia robusta	δ13C	0,925480243	-0,070734955	ns	
Robustosergia robusta	δ15N	0,000681086	0,542984236	***	P
Searsia koefoedi	δ13C	3,33505E-09	0,855545341	***	P
Searsia koefoedi	δ15N	0,000355399	0,489896927	***	P
Serrivomer beanii	δ13C	0,000104782	0,450460973	***	P
Serrivomer beanii	δ15N	1,11817E-09	0,78423354	***	P
Sigmops elongatus	δ13C	0,046128957	0,136999717	*	P
Sigmops elongatus	δ15N	1,62913E-07	0,724058719	***	P
Sternoptyx sp.	δ13C	7,75776E-06	0,60415575	***	P
Sternoptyx sp.	δ15N	2,99409E-07	0,707854718	***	P
Symbolophorus veranyi	δ13C	0,763276373	-0,099337347	ns	
Symbolophorus veranyi	δ15N	0,001185603	0,674410332	**	P
Systellaspis debilis	δ13C	0,049995191	0,10285735	*	N
Systellaspis debilis	δ15N	0,067591228	0,085743592	ns	
Valenciennellus tripunctulatus	δ13C	0,590040231	-0,03625026	ns	
Valenciennellus tripunctulatus	δ15N	0,457297015	-0,021672703	ns	
Vinciguerria poweriae	δ13C	0,588043081	-0,031288298	ns	
Vinciguerria poweriae	δ15N	0,058163699	0,115186986	ns	

Linear models for all species. δ^{13}c on the left side and δ^{15} on the right side. Figures for each species are in alphabetic order. The color of the point represent the different areas.




Benthosema glaciale
δ13C (‰)
Benthosema glaciale
δ15N (‰)
Benthosema suborbitale
δ13C (‰)
Benthosema suborbitale
δ15N (‰)
Bolinichthys indicus
δ13C (‰)
Bolinichthys indicus
δ15N (‰)
Bonapartia pedaliota
δ13C (‰)
Bonapartia pedaliota
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Ceratoscopelus warmingii
δ13C (‰)
Ceratoscopelus warmingii
δ15N (‰)
Chauliodus sloani
δ13C (‰)
Chauliodus sloani
δ15N (‰)
Cyclothone braueri
δ13C (‰)
Cyclothone braueri
δ15N (‰)
Cyclothone microdon
δ13C (‰)
Cyclothone microdon
δ15N (‰)
Standard length [mm]
area
3
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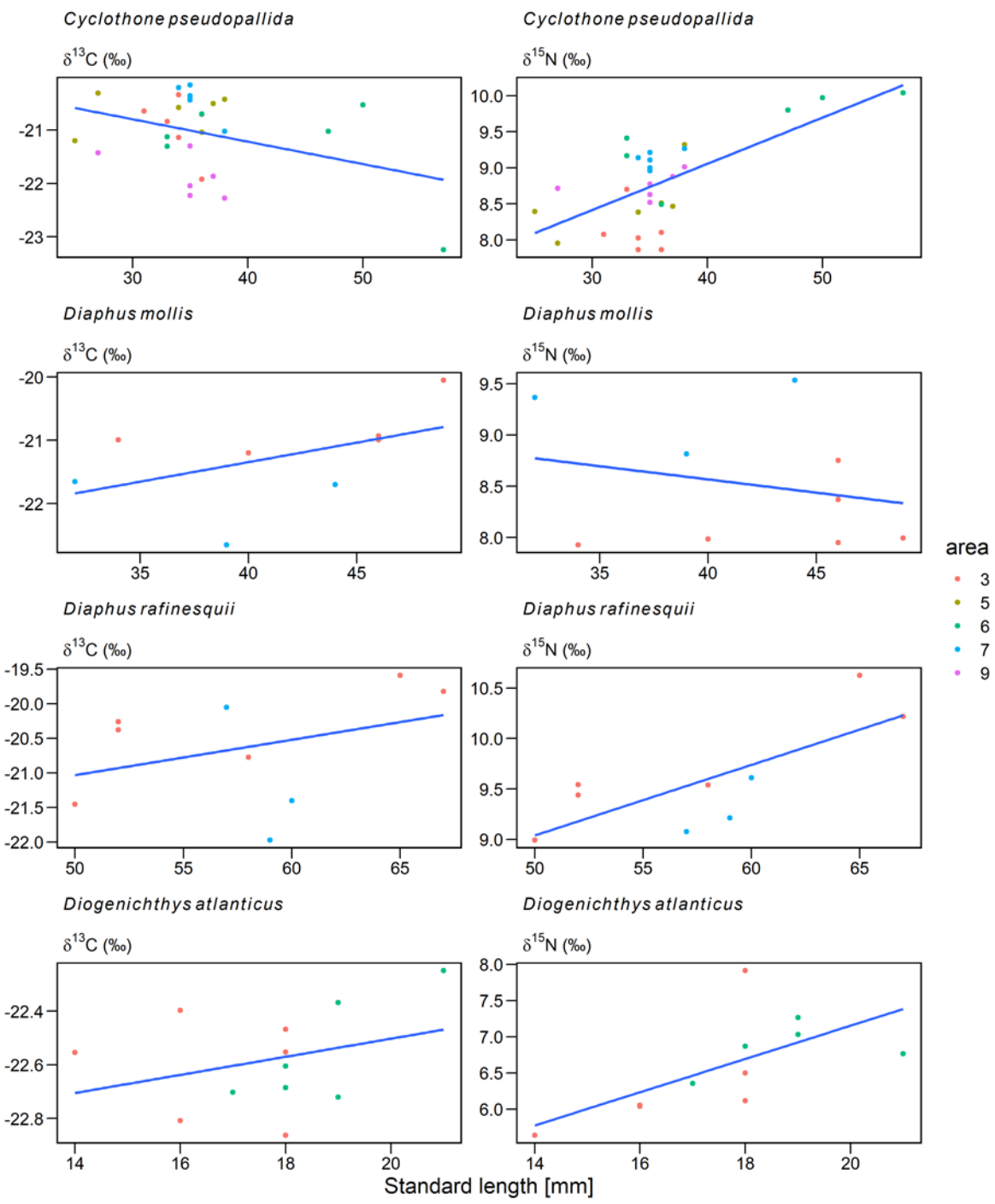
7
9


Cyclothone pseudopallida
δ13C (‰)
Cyclothone pseudopallida
δ15N (‰)
Diaphus mollis
δ13C (‰)
Diaphus mollis
δ15N (‰)
Diaphus rafinesquii
δ13C (‰)
Diaphus rafinesquii
δ15N (‰)
Diogenichthys atlanticus
δ13C (‰)
Diogenichthys atlanticus
δ15N (‰)
Standard length [mm]
area
3
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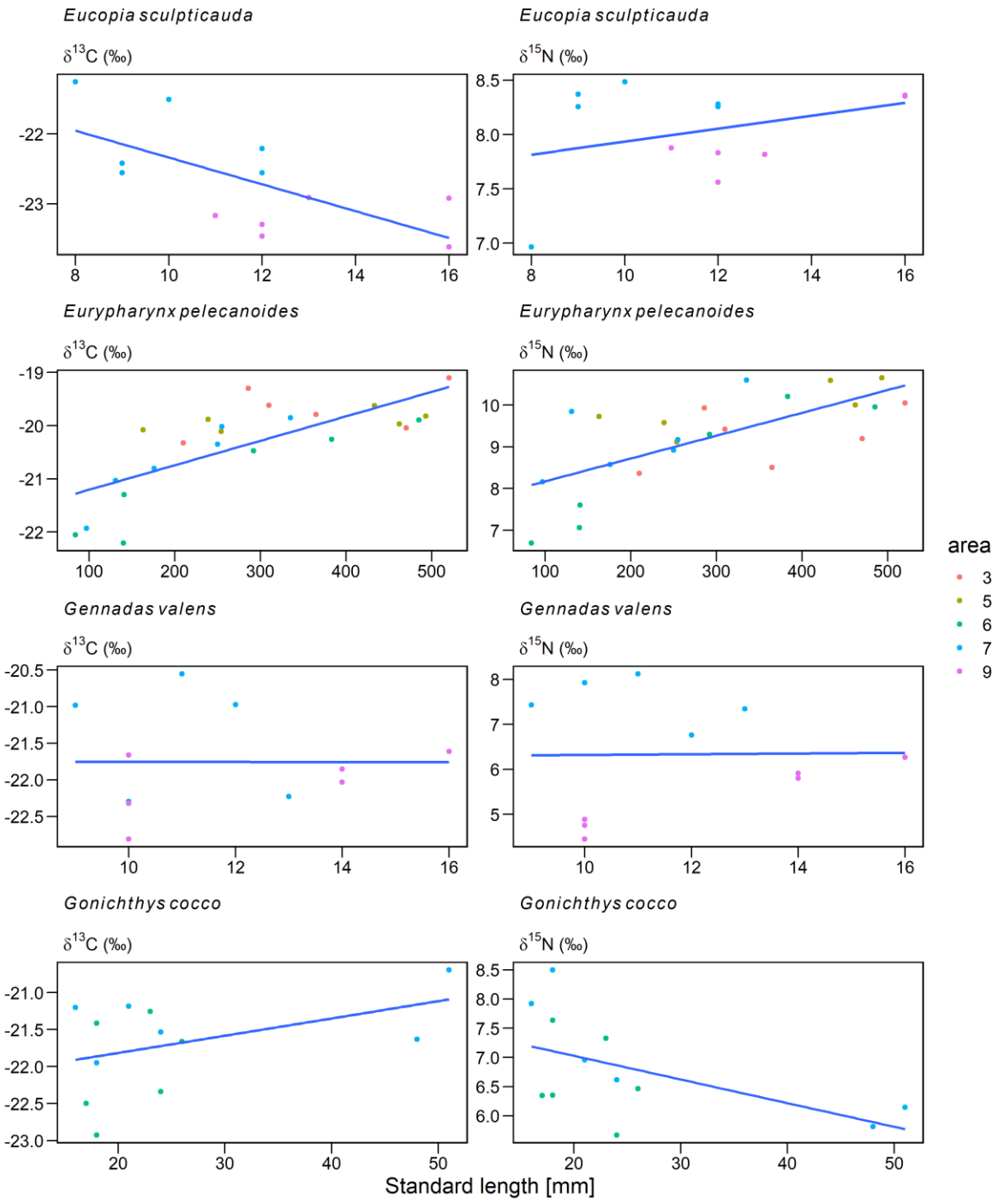
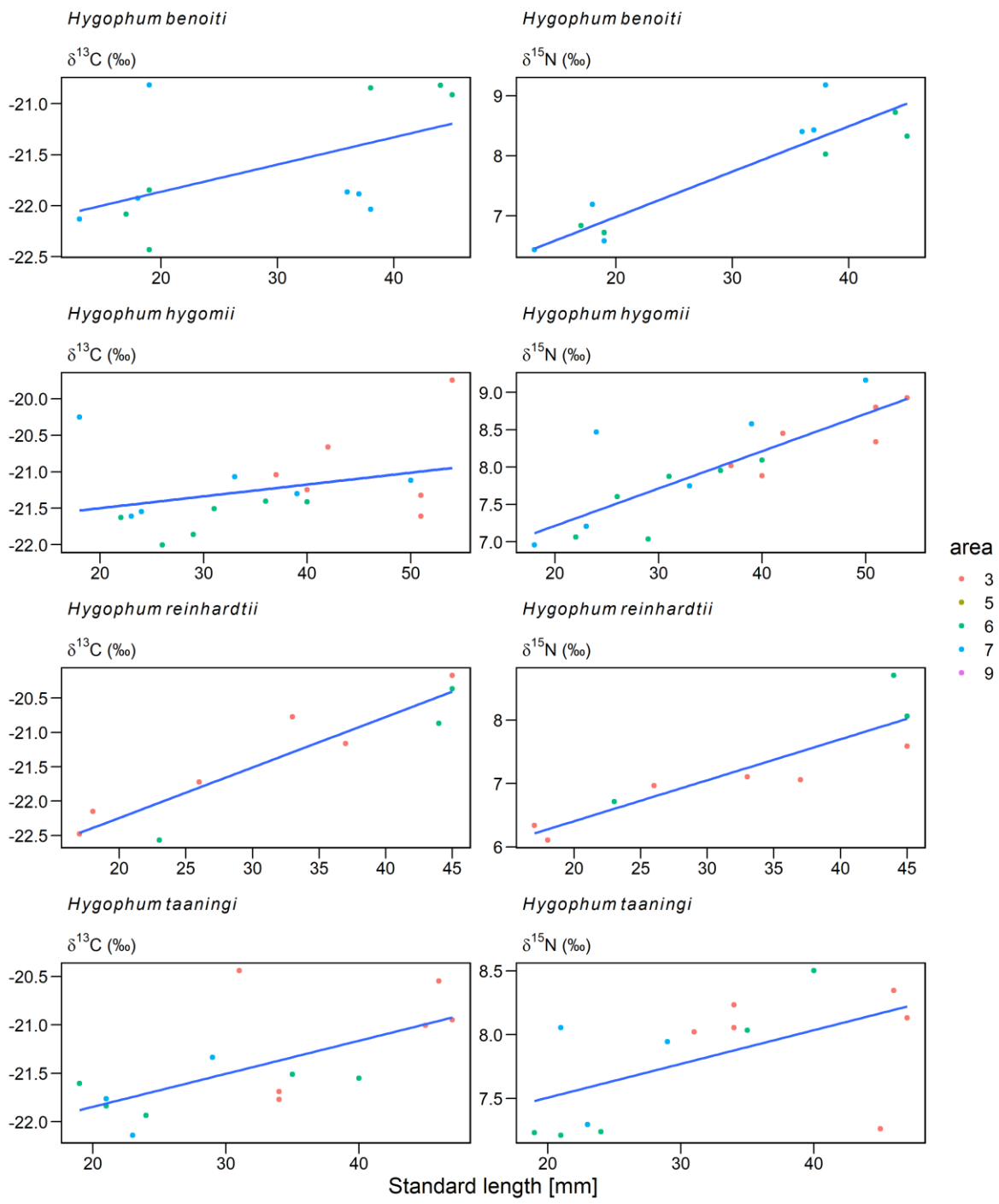
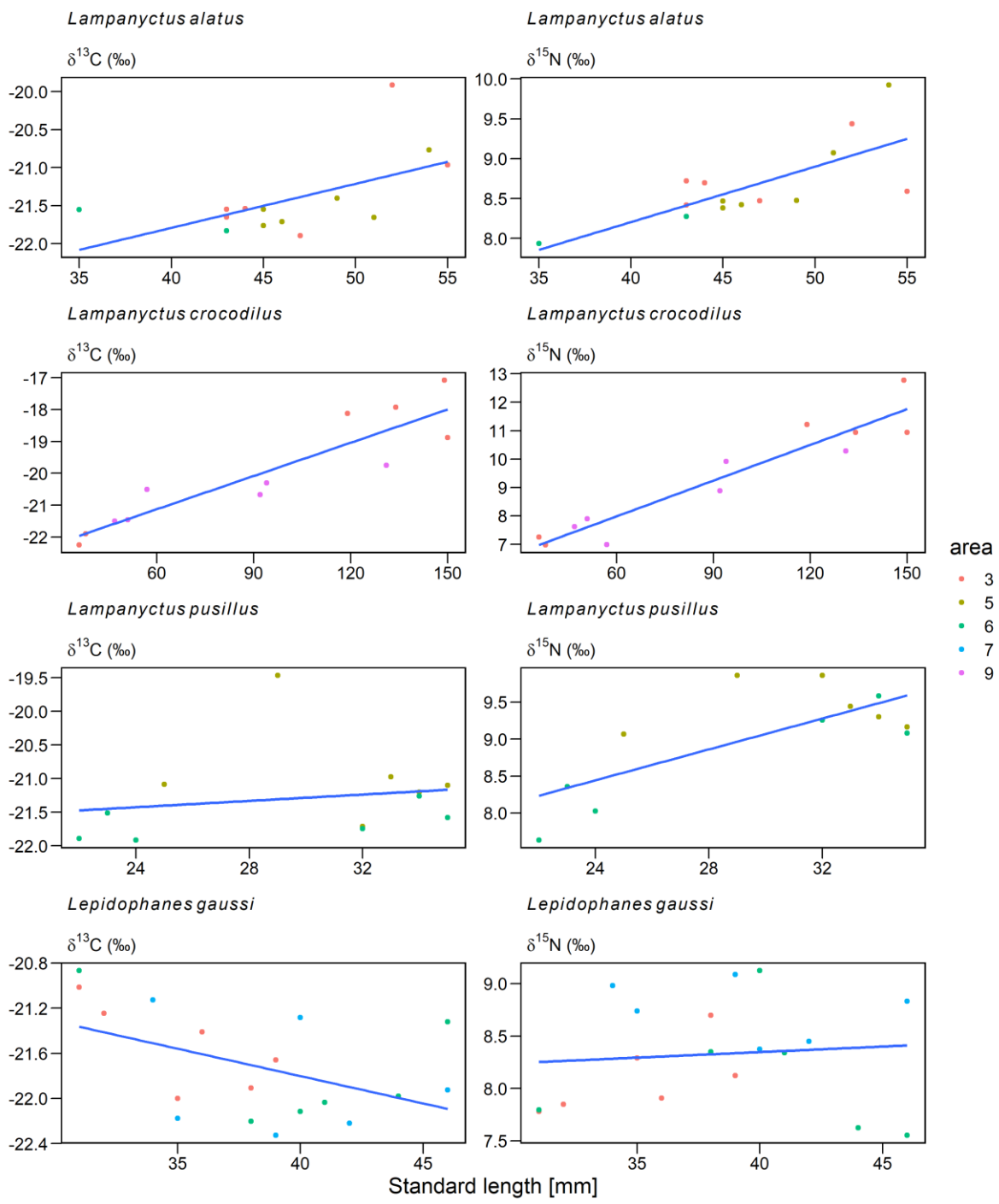
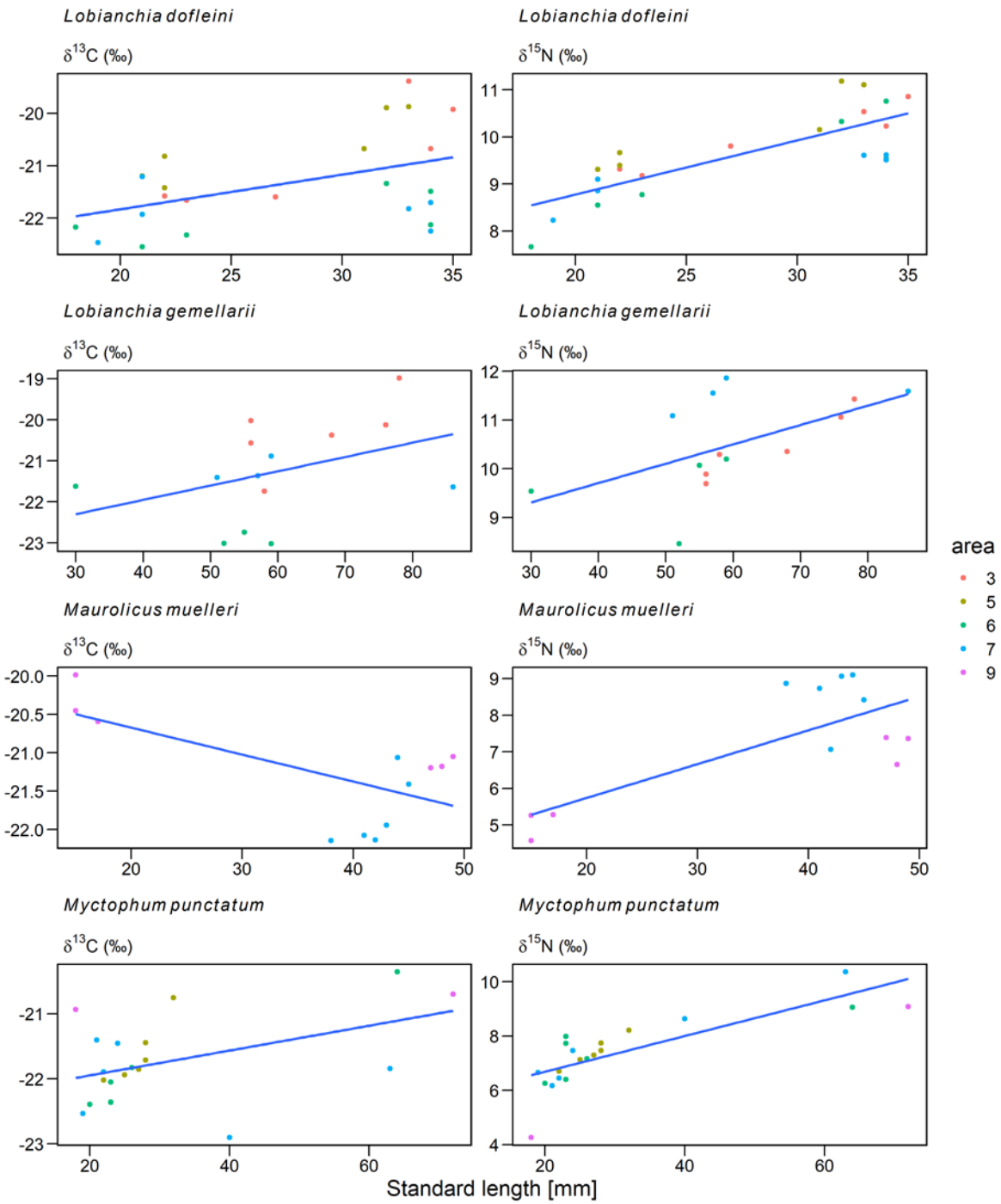
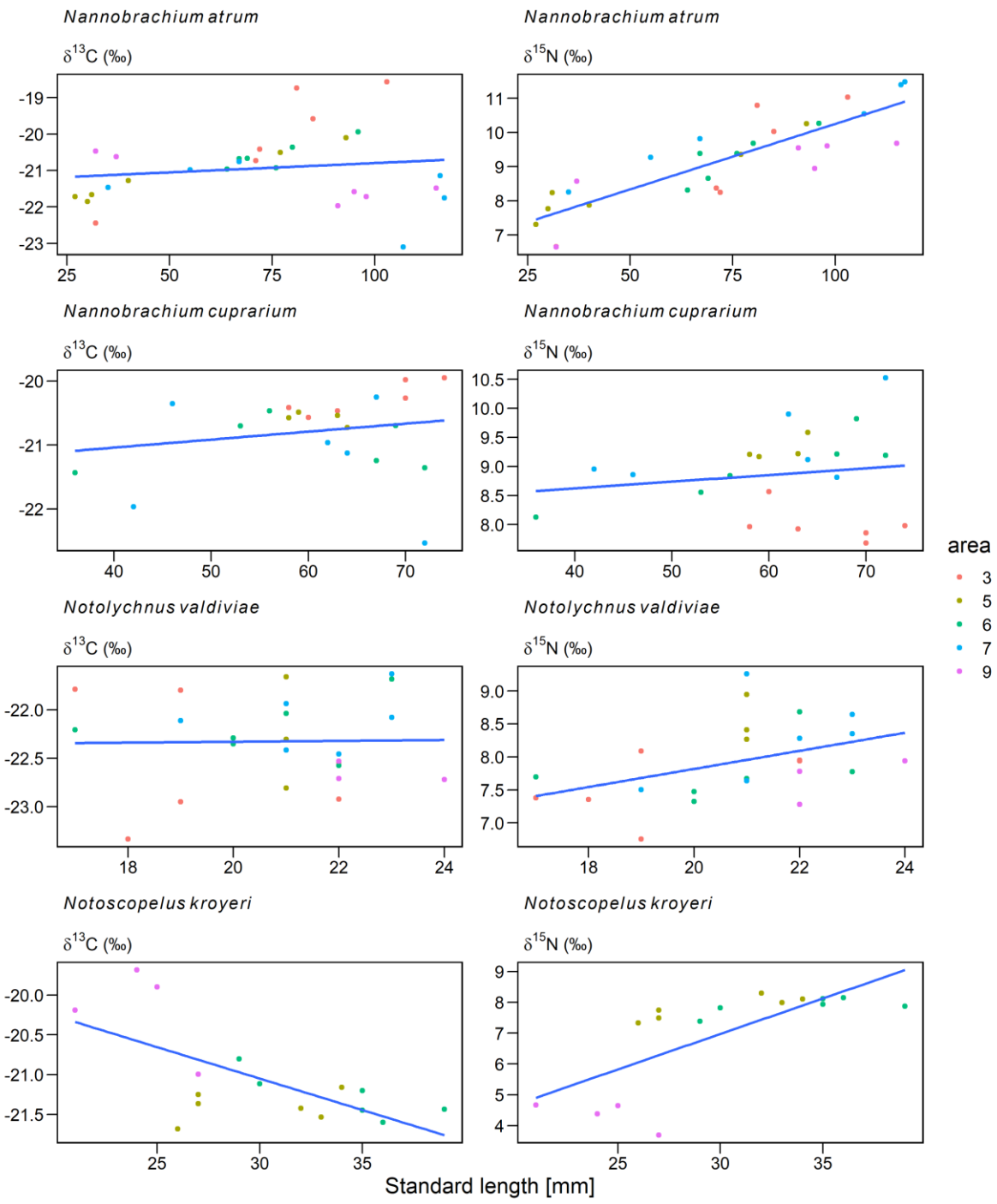
6
7
9

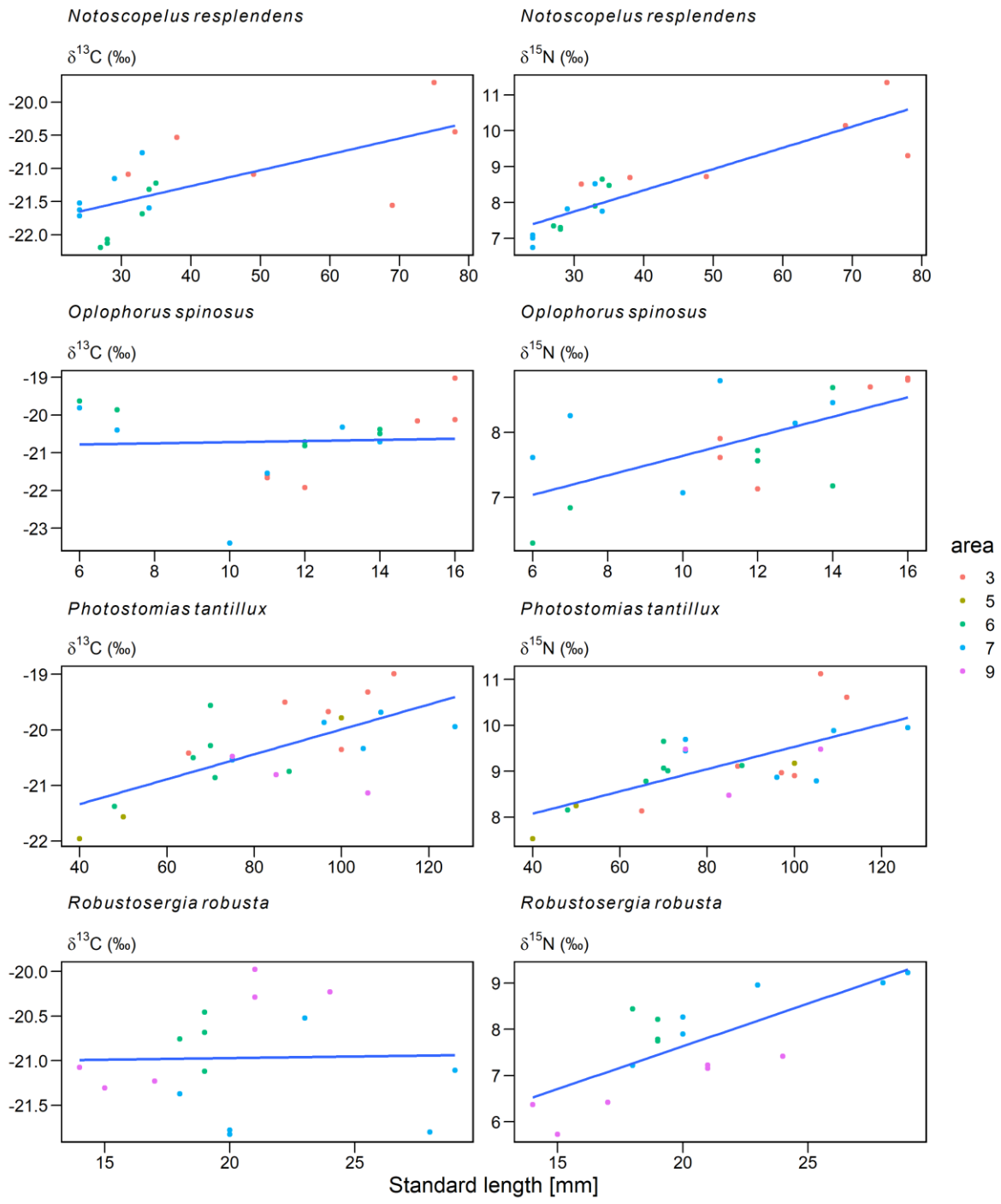

Eucopia sculpticauda
δ13C (‰)
Eucopia sculpticauda
δ15N (‰)
Eurypharynx pelecanoides
δ13C (‰)
Eurypharynx pelecanoides
δ15N (‰)
Gennadas valens
δ13C (‰)
Gennadas valens
δ15N (‰)
Gonichthys cocco
δ13C (‰)
Gonichthys cocco
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9

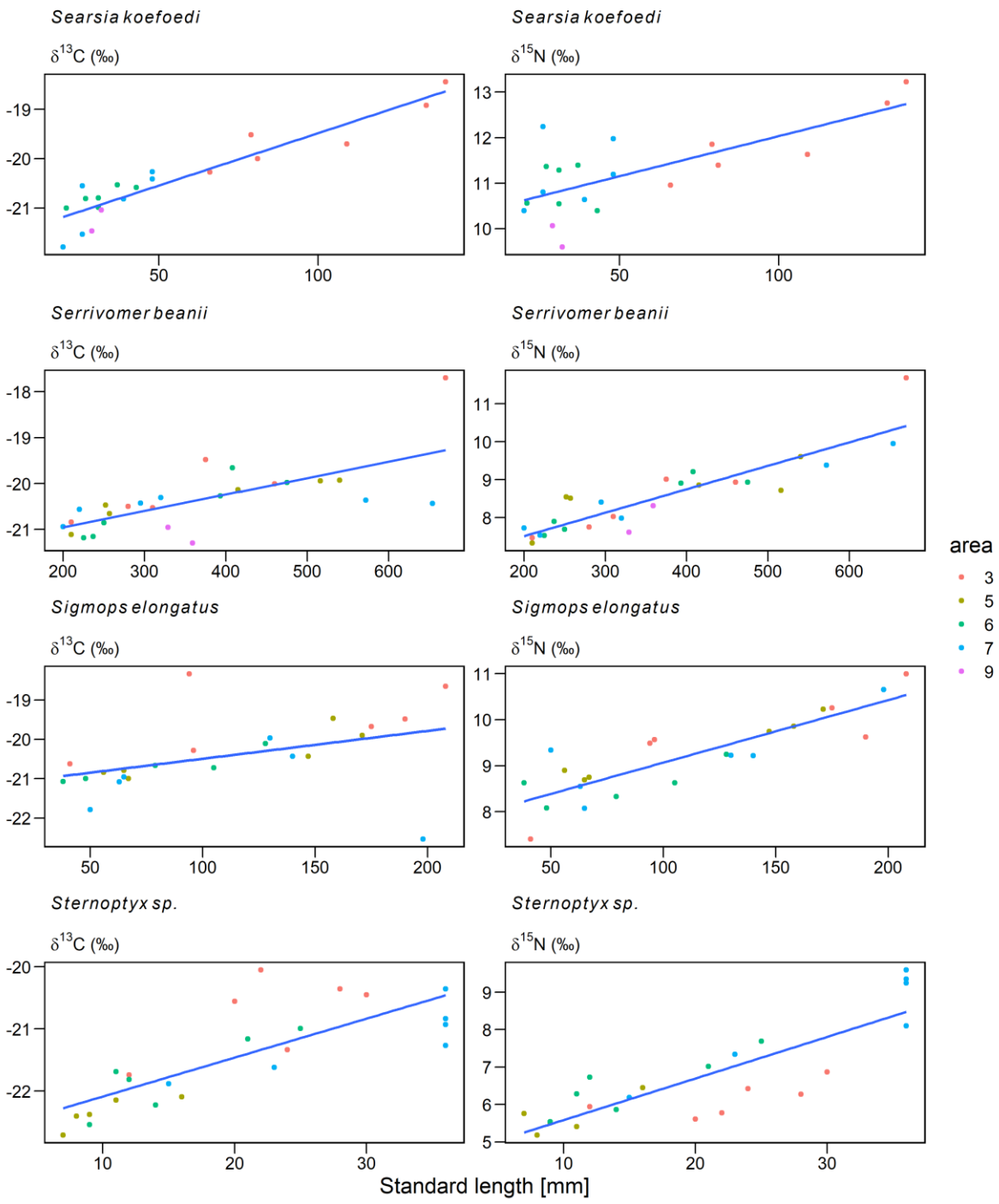

Hygophum benoiti
δ13C (‰)
Hygophum benoiti
δ15N (‰)
Hygophum hygomii
δ13C (‰)
Hygophum hygomii
δ15N (‰)
Hygophum reinhardtii
δ13C (‰)
Hygophum reinhardtii
δ15N (‰)
Hygophum taaningi
δ13C (‰)
Hygophum taaningi
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9

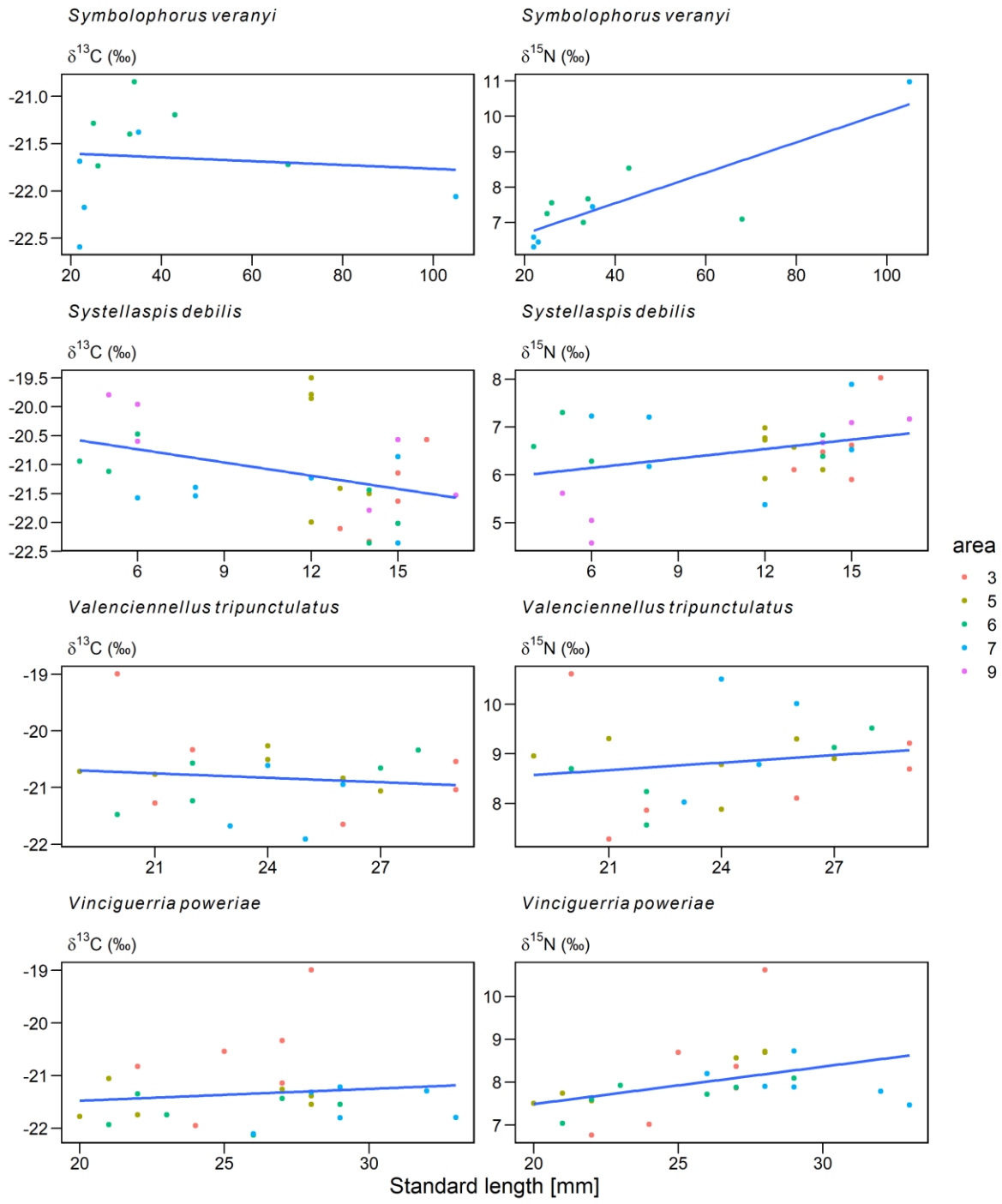

Lampanyctus alatus
δ13C (‰)
Lampanyctus alatus
δ15N (‰)
Lampanyctus crocodilus
δ13C (‰)
Lampanyctus crocodilus
δ15N (‰)
Lampanyctus pusillus
δ13C (‰)
Lampanyctus pusillus
δ15N (‰)
Lepidophanes gaussi
δ13C (‰)
Lepidophanes gaussi
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Lobianchia dofleini
δ13C (‰)
Lobianchia dofleini
δ15N (‰)
Lobianchia gemellarii
δ13C (‰)
Lobianchia gemellarii
δ15N (‰)
Maurolicus muelleri
δ13C (‰)
Maurolicus muelleri
δ15N (‰)
Myctophum punctatum
δ13C (‰)
Myctophum punctatum
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Nannobrachium atrum
δ13C (‰)
Nannobrachium atrum
δ15N (‰)
Nannobrachium cuprarium
δ13C (‰)
Nannobrachium cuprarium
δ15N (‰)
Notolychnus valdiviae
δ13C (‰)
Notolychnus valdiviae
δ15N (‰)
Notoscopelus kroyeri
δ13C (‰)
Notoscopelus kroyeri
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Notoscopelus resplendens
δ13C (‰)
Notoscopelus resplendens
δ15N (‰)
Oplophorus spinosus
δ13C (‰)
Oplophorus spinosus
δ15N (‰)
Photostomias tantillux
δ13C (‰)
Photostomias tantillux
δ15N (‰)
Robustosergia robusta
δ13C (‰)
Robustosergia robusta
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Searsia koefoedi
δ13C (‰)
Searsia koefoedi
δ15N (‰)
Serrivomer beanii
δ13C (‰)
Serrivomer beanii
δ15N (‰)
Sigmops elongatus
δ13C (‰)
Sigmops elongatus
δ15N (‰)
Sternoptyx sp.
δ13C (‰)
Sternoptyx sp.
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9


Symbolophorus veranyi
δ13C (‰)
Symbolophorus veranyi
δ15N (‰)
Systellaspis debilis
δ13C (‰)
Systellaspis debilis
δ15N (‰)
Valenciennellus tripunctulatus
δ13C (‰)
Valenciennellus tripunctulatus
δ15N (‰)
Vinciguerria poweriae
δ13C (‰)
Vinciguerria poweriae
δ15N (‰)
Standard length [mm]
area
3
5
6
7
9

APPENDIX E. Summary on linear mixed model

Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: **d13c_av_std** ~ dist_shore_km + temp_max + sal_max + oxy_min + (1 | species)
Data: master_df

REML criterion at convergence: 2177.2

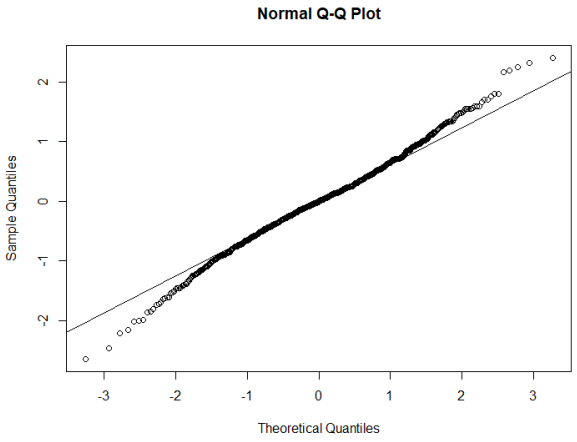
Scaled residuals:
Min 1Q Median 3Q Max
-3.6678 -0.5835 0.0000 0.5763 3.3383

Random effects:
Groups Name Variance Std.Dev.
species (Intercept) 0.7024 0.8381
Residual 0.5196 0.7209
Number of obs: 915, groups: species, 49

Fixed effects:
Estimate Std. Error df t value Pr(>|t|)
(Intercept) 1.933e+01 1.180e+01 8.744e+02 1.638 0.101788
dist_shore_km -9.695e-04 3.692e-04 8.814e+02 -2.626 0.008782 **
temp_max 2.517e-01 5.543e-02 8.748e+02 4.542 6.35e-06 ***
sal_max -1.200e+00 3.494e-01 8.740e+02 -3.433 0.000625 ***
oxy_min -3.051e-01 6.278e-02 8.847e+02 -4.861 1.38e-06 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
(Intr) dst_s_ tmp_mx sal_mx
dist_shr_km -0.414
temp_max 0.931 -0.281
sal_max -0.999 0.409 -0.943
oxy_min 0.153 -0.515 0.215 -0.175



Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: **d15n_av_std** ~ dist_shore_km + temp_max + sal_max + oxy_min + (1 | species)
Data: master_df

REML criterion at convergence: 2624.7

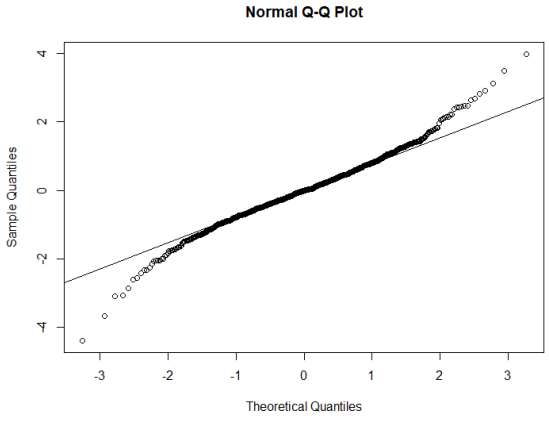
Scaled residuals:
Min 1Q Median 3Q Max
-4.7384 -0.5596 -0.0168 0.5574 4.2957

Random effects:
Groups Name Variance Std.Dev.
species (Intercept) 0.9715 0.9857
Residual 0.8573 0.9259
Number of obs: 915, groups: species, 49

Fixed effects:
Estimate Std. Error df t value Pr(>|t|)
(Intercept) 1.152e+02 1.515e+01 8.758e+02 7.605 7.32e-14 ***
dist_shore_km 2.654e-03 4.736e-04 8.839e+02 5.602 2.82e-08 ***
temp_max 5.718e-01 7.114e-02 8.763e+02 8.038 2.95e-15 ***
sal_max -3.188e+00 4.485e-01 8.754e+02 -7.108 2.44e-12 ***
oxy_min -4.148e-01 8.053e-02 8.876e+02 -5.151 3.19e-07 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
(Intr) dst_s_ tmp_mx sal_mx
dist_shr_km -0.414
temp_max 0.931 -0.281
sal_max -0.999 0.409 -0.943
oxy_min 0.154 -0.514 0.215 -0.175



APPENDIX F. Alternatively trophic level result

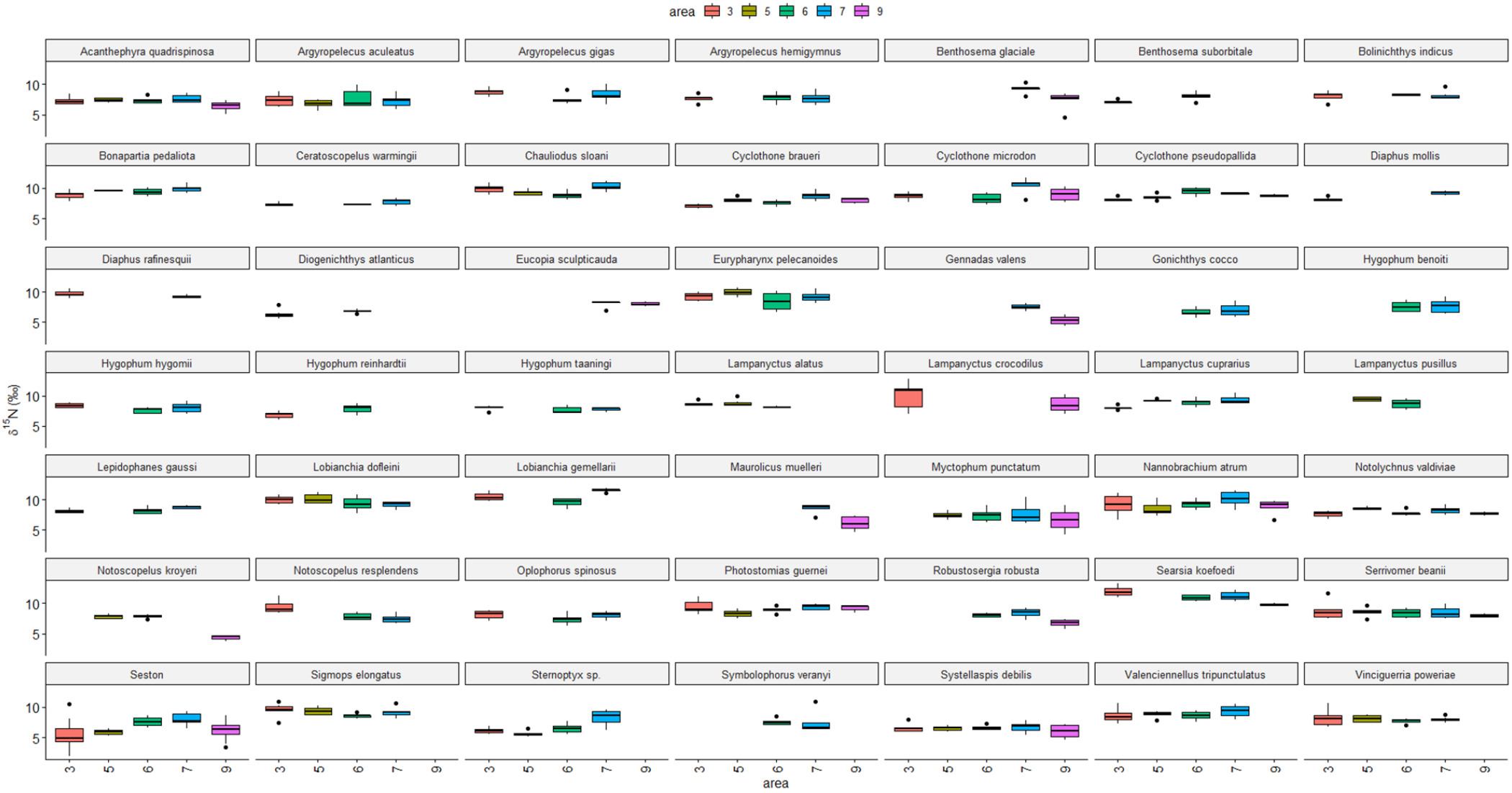
species	**TL (original)** Baseline = mean of both seston samples from each station	**TL (mean seston baseline)** Baseline= mean of all seston sample (6.6‰)
Searsia koefoedi	3.3 ± 0.53	3,6
Lobianchia gemellarii	3 ± 0.37	3,3
Lobianchia dofleini	2.8 ± 0.62	3,0
Bonapartia pedaliota	2.8 ± 0.53	3,0
Chauliodus sloani	2.7 ± 0.51	3,0
Diaphus rafinesquii	2.8 ± 0.32	3,0
Lampanyctus crocodilus	2.9 ± 0.88	2,9
Eurypharynx pelecanoides	2.8 ± 0.75	2,9
Sigmops elongatus	2.7 ± 0.58	2,9
Photostomias guernei	2.8 ± 0.53	2,9
Nannobrachium atrum	2.9 ± 0.46	2,9
Cyclothone microdon	2.8 ± 0.55	2,8
Lampanyctus cuprarius	2.9 ± 0.46	2,8
Valenciennellus tripunctulatus	2.9 ± 0.43	2,8
Lampanyctus pusillus	2.9 ± 0.37	2,8
Lampanyctus alatus	2.7 ± 0.35	2,7
Cyclothone pseudopallida	3.1 ± 0.38	2,7
Benthosema glaciale	2.5 ± 0.68	2,6
Notoscopelus resplendens	2.2 ± 0.68	2,6
Serrivomer beanii	2.5 ± 0.47	2,6
Lepidophanes gaussi	2.2 ± 0.32	2,6
Diaphus mollis	2.9 ± 0.7	2,6
Eucopia sculpticauda	2.6 ± 0.96	2,5
Argyropelecus gigas	2.3 ± 0.74	2,5
Vinciguerria poweriae	2.4 ± 0.67	2,5
Bolinichthys indicus	2.2 ± 0.51	2,5
Hygophum hygomii	2.1 ± 0.45	2,5
Hygophum taaningi	2 ± 0.38	2,4
Oplophorus spinosus	2 ± 0.37	2,4
Notolychnus valdiviae	2.5 ± 0.29	2,4
Robustosergia robusta	2 ± 0.27	2,4
Cyclothone braueri	2.6 ± 0.6	2,4
Argyropelecus hemigymnus	2.2 ± 0.5	2,4
Myctophum punctatum	1.9 ± 0.67	2,3
Symbolophorus veranyi	1.6 ± 0.61	2,3
Hygophum benoiti	1.7 ± 0.54	2,3
Ceratoscopelus warmingii	1.9 ± 0.28	2,3
Benthosema suborbitale	2.6 ± 0.3	2,3
Maurolicus muelleri	1.8 ± 0.58	2,2
Acanthephyra quadrispinosa	2.3 ± 0.54	2,2
Argyropelecus aculeatus	2.1 ± 0.49	2,2
Hygophum reinhardtii	2.6 ± 0.32	2,2
Notoscopelus kroyeri	1.9 ± 0.68	2,1
Gonichthys cocco	1.5 ± 0.37	2,1
Systellaspis debilis	1.9 ± 0.53	2,0
Sternoptyx sp.	2.1 ± 0.48	2,0
Seston	2 ± 0.33	2,0
Diogenichthys atlanticus	2.1 ± 0.31	2,0
Gennadas valens	1.8 ± 0.37	1,9

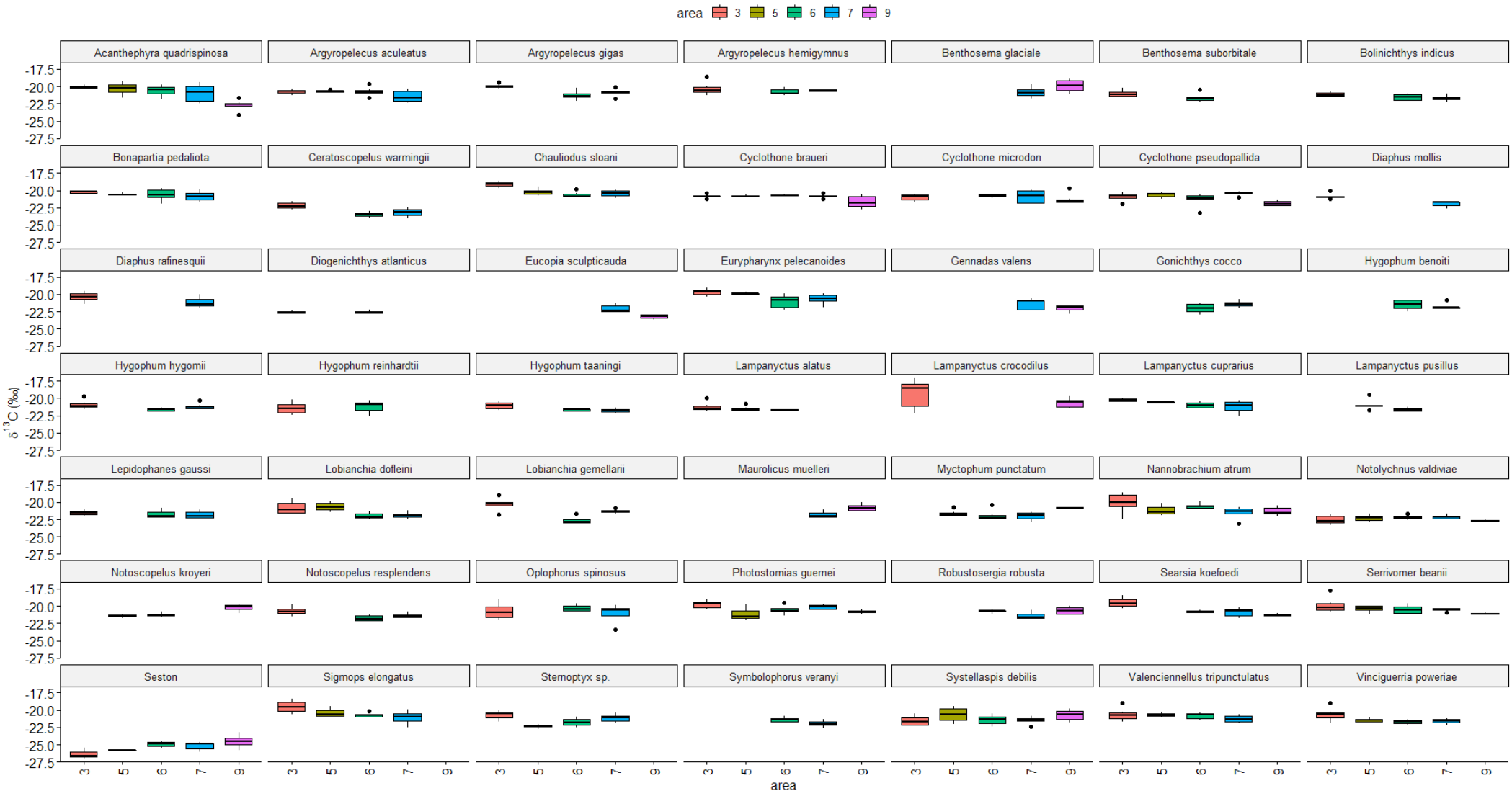
APPENDIX G. Length measurements compared to max length

The table is comparing mean length of the species used in this study to the max length of the species described in the "Identification guide to the mesopelagic fishes of the central and south east Atlantic ocean" (Tracey T Sutton et al., 2020). The last two columns showed the how close the mean length of this study is to the max length. For instance, the mean length of *Ceratoscopelus warmingii* in this study is just the half of what the documented max length of the species is.

Species	**Mean Length(mm)** (This study)	**Length range (mm)** (This study)	**Max length (mm)** (Tracey T Sutton et al., 2020)	How much smaller mean length is than max length	Mean length/ max length
Nannobrachium atrum	72	27-117			
Lobianchia gemellarii	60	30-86	60	0 %	1
Notolychnus valdiviae	21	17-24	25	16 %	5/6
Bolinichthys indicus	35	27-45	45	22 %	7/9
Valenciennellus tripunctulatus	24	19-29	31	23 %	7/9
Lampanyctus alatus	47	35-55	61	23 %	7/9
Lepidophanes gaussi	38	31-46	50	24 %	3/4
Bonapartia pedaliota	53	29-71	72	26 %	3/4
Cyclothone braueri	27	15-34	38	29 %	5/7
Lampanyctus pusillus	30	22-35	43	30 %	2/3
Diogenichthys atlanticus	18	14-21	27	33 %	2/3
Diaphus rafinesquii	58	50-67	90	36 %	2/3
Diaphus mollis	42	32-49	66	36 %	2/3
Cyclothone pseudopallida	36	25-57	58	38 %	5/8
Vinciguerria poweriae	26	20-33	43	40 %	3/5
Benthosema suborbitale	23	17-28	39	41 %	3/5
Lampanyctus cuprarius	61	36-74	110	45 %	5/9
Ceratoscopelus warmingii	44	35-55	81	46 %	1/2
Lobianchia dofleini	27	18-35	50	46 %	1/2
Lampanyctus crocodilus	92	36-150	172	47 %	1/2
Hygophum hygomii	36	18-54	68	47 %	1/2
Hygophum benoiti	29	13-45	55	47 %	1/2
Photostomias guernei	84	40-126	160	48 %	1/2
Hygophum reinhardtii	32	17-45	61	48 %	1/2
Hygophum taaningi	32	19-47	61	48 %	1/2
Cyclothone microdon	39	24-55	76	49 %	1/2
Argyropelecus hemigymnus	25	15-35	51	51 %	1/2
Serrivomer beanii	360	200-670	750	52 %	1/2
Maurolicus muelleri	37	15-49	80	54 %	1/2
Argyropelecus gigas	54	14-102	120	55 %	4/9
Chauliodus sloani	150	57-260	350	57 %	3/7
Gonichthys cocco	25	16-51	60	58 %	3/7
Notoscopelus resplendens	38	24-78	95	60 %	2/5
Sigmops elongatus	110	38-208	275	60 %	2/5
Searsia koefoedi	53	20-140	150	65 %	1/3
Sternoptyx sp.	20	7-36	60	67 %	1/3
Argyropelecus aculeatus	28	9-68	84	67 %	1/3
Symbolophorus veranyi	40	22-105	120	67 %	1/3
Benthosema glaciale	32	17-57	103	69 %	1/3
Myctophum punctatum	31	18-72	110	72 %	2/7
Notoscopelus kroyeri	30	21-39	143	79 %	1/5
Eurypharynx pelecanoides	290	84-520	1 800	84 %	1/6

APPENDIX H. Boxplots with δ13c and δ^{15} N values for each species in each area




area 3 5 6 7 9
Acanthephyra quadrispinosa
Argyropelecus aculeatus
Argyropelecus gigas
Argyropelecus hemigymnus
Benthosema glaciale
Benthosema suborbitale
Bolinichthys indicus
Bonapartia pedaliota
Ceratoscopelus warmingii
Chauliodus sloani
Cyclothone braueri
Cyclothone microdon
Cyclothone pseudopallida
Diaphus mollis
Diaphus rafinesquii
Diogenichthys atlanticus
Eucopia sculpticauda
Eurypharynx pelecanoides
Gennadas valens
Gonichthys cocco
Hygophum benoiti
Hygophum hygomii
Hygophum reinhardtii
Hygophum taaningi
Lampanyctus alatus
Lampanyctus crocodilus
Lampanyctus cuprarius
Lampanyctus pusillus
Lepidophanes gaussi
Lobianchia dofleini
Lobianchia gemellarii
Maurolicus muelleri
Myctophum punctatum
Nannobrachium atrum
Notolychnus valdiviae
Notoscopelus kroyeri
Notoscopelus resplendens
Oplophorus spinosus
Photostomias guernei
Robustosergia robusta
Searsia koefoedi
Serrivomer beanii
Seston
Sigmops elongatus
Sternoptyx sp.
Symbolophorus veranyi
Systellaspis debilis
Valenciennellus tripunctulatus
Vinciguerria poweriae
-17.5
-20.0
-22.5
-25.0
-27.5
δ13C (‰)
3
5
6
7
9
area