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Unexpected feeding behaviour inferred by DNA metabarcoding of Barents Sea skates

A diet analysis of spinytail skate *Bathyraja spinicauda,* Arctic skate *Amblyraja hyperborea*, and thorny skate *Amblyraja radiata*

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Abstract

Feeding studies give an indication about the role and position of species within ecosystems and provide crucial knowledge for management. Traditional methods are based on morphological identification of prey. DNA metabarcoding is a promising tool that allow for identification of specific prey items, also when highly digested. A total of 63 stomachs from three skate species caught in the Barents Sea region were investigated by DNA metabarcoding. Differences were found between species and size classes, reflecting the distributions and respective lengths of the skates. No sexual differences were found. The smaller size classes preferred small and soft-bodied prey species. Arctic skate have been observed to perform short-term depth changes, being confirmed by a higher portion of pelagic diet compared to the other two. Spinytail skate, reaching the largest maximum size, was more piscivorous. The smaller thorny skate predominantly preyed upon soft-bodied invertebrates. A comparison with traditional methods was performed, revealing that DNA metabarcoding identified up to twenty times more species. This includes rapidly digestible prey such as Annelida, Cnidaria and Chaetognatha, although secondary predation cannot be ruled out. DNA metabarcoding is a powerful tool for mapping the range of prey diversity and is an important complementation to traditional methods.

Keywords

Feeding ecology, COI, Leray fragment, Next-Generation Sequencing, NGS, Pelagic diet, Benthic diet, Ontogenetic shifts

Own contribution to the thesis

Most of the work in this thesis was conducted by me. The Institute of Marine Research contributed with part of the materials, and the sequencing was conducted by a commercial company. The bioinformatic analysis was performed by my co-supervisor, who also assisted during some of the data analyses.

Introduction

Feeding ecology studies

Feeding ecology studies provide information about partitioning, habitat preferences, prey selection, predation, evolution, competition, and energy transfer within and between ecosystems (Braga et al., 2012). It is a cornerstone for understanding predator biology and the function of predators in ecosystems (Bergmann et al., 2021).

Predator-prey relationships are important ecological interactions, affecting biotic community compositions and energy flow through a system (Waraniak et al., 2019). Predator-prey relationships includes movements that can range from fine-scale aggregations that result in a small shift in home range or territory that is opportunistic, to large-scale movements that may occur reliably year after year (Furey et al., 2018). Migratory coupling is defined as large-scale movements both by predators and prey (predators move beyond home range to feed on migrant prey) (Furey et al., 2018). One example of migratory coupling is the North Atlantic right whale (*Eubalaena glacialis*) that migrate hundreds of kilometres to pursue calanoid copepods (*Calanus finmarchicus*) when the copepod migrate to shallower waters during spring to feed and reproduce (Baumgartner et al., 2003; Firestone et al., 2008; Pershing et al., 2009). Benthic predators are observed to have some form of migratory behaviour. Decapods (Aguzzi & Company, 2010), catsharks (*Scyliorhinus canicular*) (Sims et al., 2006) and skates (Humphries et al., 2017) are observed to perform nektobenthic diel vertical migration (DVM). Nektobenthic DVM, or nektobenthic displacement, is a movement that occur in a rhythmic fashion along depth gradients close to the bottom, but not within the water column (Aguzzi & Company, 2010). This movements observed in skates, between inshore and offshore, are thought to be foraging excursions (Humphries et al., 2017). Some skates are also observed to perform large depth changes over a short time period (Peklova et al., 2014)

Quantifying the dietary composition of predator fishes in the content of relative prey availability in the environment is necessary to investigate the ecological relationship between predators and prey and to determine predator-prey preference (Waraniak et al., 2019). The prey's relative abundance is a driving factor for predator preferences. A high abundance of prey leads to more encounters and in some cases, this leads an abundant taxon to be targeted by predators (Waraniak et al., 2019). Contrary, with low prey abundance, some predators spend more time foraging than when the prey abundance is high. For example will the female Antarctic fur seal (*Arctocephalus gazella*) spend more time foraging when prey abundance is

low (Boyd et al., 1994). High prey abundance can also reduce interspecific competition, which allows predators to coexist (Waraniak et al., 2019). Intraspecific competition is also reduced in high resource environments, and opposite in low resource environments (Svanfeldt et al., 2017). Understanding how the variation of the prey community affects these relationships is important to conservation, because predator preference can indicate what members of the community function as important energetic links between trophic levels. Estimates of dietary overlap between different predators may indicate the degree of interspecific competition (Waraniak et al., 2019). Feeding ecology is therefore linked to the species' population dynamics. The feeding ecology and quantitative assessment of food habits/animal dietary information (portion of protein, fat, and carbohydrates) provides the foundation for understanding trophic relationships. Understanding trophic relationships is essential for developing conservation strategies for prey and predator to have proper ecosystem management (Bergmann et al., 2021; Braga et al., 2012). Proper ecosystem management will in turn support ecosystem services such as primary and secondary production, resource use, nutrient cycling, and ecosystem stability (Worm et al., 2006). The reconstruction of trophic links between fishes allows including predator-prey interactions into assessments for setting a balanced exploitation across trophic levels, preventing fishing-induced trophic level decline (Riccioni et al., 2018).

There are several methods to analyse the diet of animals; morphological analysis (Braley et al., 2010), lipid and fatty acid analysis (Navarro & Villanueva, 2000; Phillips, Jackson, et al., 2003), stable isotope analysis (Cherel & Hobson, 2005), serological analysis (Grisley & Boyle, 1985; Kear, 1992), and molecular methods (Braley et al., 2010). Morphological studies of the diet of animals require visual identification of partially digested prey items (Waraniak et al., 2019). Often the use of microscope is needed to identify finely masticated food (such as the diet composition of granivorous small rodents) (Calhoun, 1941; Hamilton, 1941). Presence of prey taxa are recorded, and percentage estimates of prey species composition is made. This is used to infer relative abundance/mass (Braley et al., 2010). Hard remains such as fish otoliths, scales, vertebrae, cephalopod beaks and suckers, and crustacean exoskeletons are used for identification. Left and right fish otoliths and upper and lower cephalopod beaks can be counted, and the greatest value are used to estimate the minimum number of individuals (MNI) (Braley et al., 2010). For other prey taxa, the enumeration of eyes (e.g. krill) and other appendages can be used to estimate MNI (Braley et al., 2010). Morphological analysis allows broad trophic relationships to be inferred (Braley et al., 2010).

There are mainly two methods used to analyse the prey composition, the presence-absence method, and the relative-fullness method. The presence-absence method (also referred to as the frequency of occurrence method) simply relies on the positive identification of a prey or an identifiable part of the prey to provide an accurate recording of which prey taxa are present in the diet, without taking the amount or relative contribution of each prey into account (Amundsen & Sánchez-Hernández, 2019). The relative-fullness method takes the relative prey contribution in the stomach scored in percentage. With this method, the total fullness of all stomach contents is first visually assessed and usually expressed on a scale from empty (0 %) to full (100 %). The fullness contribution of each prey category is then assigned summing up the total stomach fullness (Amundsen & Sánchez-Hernández, 2019). Amundsen & Sánchez-Hernández (2019) recommended a combination of the presence-absence and relative-fullness methods for stomach-content analysis as the optimal approach for studies addressing research objectives and questions related to dietary composition and relative prey importance. Together the methods provide a solid reliable estimation of the diet composition in terms of both relative prey abundance (relative abundance describe how common or rare a species is compared to other species in a defined place or community) and frequency of occurrence (Amundsen & Sánchez-Hernández, 2019).

Lipid and fatty acids can give information on the diet that cannot be obtained from conventional stomach content analyses alone, e.g., fatty acids stored in the digestive gland of the squid *Moroteuthis ingens* (Phillips et al., 2001). To determine the lipid and fatty acid composition, it is extracted from the digestive glands of the predator and blended with a stick mixer to a homogenate. This homogenate is then analysed to determine the proportion of major lipid classes (Phillips et al., 2003). The advantage of fatty acids as biomarker-based measures is that due to their nature, fatty acids have the strong advantage of being available in long-term compartments such as adipose tissue, medium-term compartments such as erythrocytes and short-term plasma or serum components (Arab & Akbar, 2002).

Stable isotope analysis is a way to study the long-term diet choices of animals (Mustamäki et al., 2014) and is also used to improve the understanding of animal movements and trophic linkages in aquatic ecosystems (Fry & Sherr, 1984; Hobson, 1999; Michener, 1994). Complete nitrogen isotope turnover varies from species to species, and for stingrays, complete turnover requires more than a year (MacNeil et al., 2006). A complete turnover of e.g., nitrogen isotopes is when all nitrogen isotopes have been replaced by new nitrogen isotopes. Metabolic processing of ingested organic matter causes isotopic fractionation, or

division of different stable isotopes, such as ^{13}C:^{12}C and ^{15}N:^{14}N stable isotope pairs (Ponsard & Averbuch, 1999). On average, $\delta^{13}C$ and $\delta^{15}N$ increase by 0.5 to 1.0 and 2.8 to 3.4‰, respectively, from one tropic level to the next (Michener, 1994; Minagawa & Wada, 1984; Peterson & Fry, 1987). Trophic position is predicted to increase with body size (Cohen et al., 1993). Isotope values vary across trophic levels due to differences between diet and consumer tissues, known as discrimination factors (Del Rio & Wolf, 2005). Isotopic discrimination factors, which vary across species (Macko et al., 1982) and tissue types (Pinnegar & Polunin, 1999), are included in food web mixing models (models that simulate food web interactions) (Phillips & Gregg, 2001) and trophic position estimates (Post, 2002).

Serological analysis utilizes passive immunodiffusion to test the specificity of antisera (Grisley & Boyle, 1985). Predator-prey interactions can be uncovered by use of crossed immunoelectrophoretic (a method for identification of proteins in serum or other fluid by electrophoresis and subsequent immunodiffusion) (Grisley & Boyle, 1985). Serological methods of prey identification have been applied to the complex food webs involving organism within freshwater (Young, 1973, 1980) and marine (Feller et al., 1979; Feller & Gallagher, 1982) benthic communities.

Weaknesses with morphological methods are the low resolution of data when it comes to species level of prey items and that it only gives a snapshot picture of the food choices (Mustamäki et al., 2014). Morphological methods utilize visual identification of prey items from the gastrointestinal tract (GI-tract) which is labour-intensive, requires extensive taxonomic knowledge (Baker et al., 2014; Gosselin et al., 2017) by the taxonomist identifying semi-digested fragments, time expensive (Riccioni et al., 2018) and is often inaccurate (Waraniak et al., 2019). Common issues are that organisms in the GI-tract are continuously being digested, so morphological traits might be harder, or impossible to look for. The organisms being identified are often organisms that take longer time to digest, and other organisms might only be identified to family/genus or simply overlooked (e.g., soft-bodied-animals or animals that lack diagnostic taxonomic features). This also introduces a bias towards prey that are slower digested (Baker et al., 2014; Gosselin et al., 2017; Riccioni et al., 2018). Lipid and fatty acid analysis and stable isotope analysis have posed methodological difficulties when applied to field-based studies, and used alone, are restrictive in their taxonomic and numerical resolution of diet (Ivanovic & Brunetti, 1994; Phillips et al., 2002; Stowasser et al., 2006). Serological methods are labour-intensive, expensive, require

specialized facilities and length development times, and cannot identify specific prey items (Chen et al., 2000).

DNA metabarcoding

DNA-based methods are useful for analysing the diet of animals with greater accuracy and resolution than traditional morphological methods (Waraniak et al., 2019). DNA barcoding is a method that can provide precise and semi-automatable species identification through the design of forward-reverse primer sets for highly conserved regions of mitochondrial DNA (mtDNA) (Hebert et al., 2003). The combination of DNA barcoding and high-throughput sequencing is termed metabarcoding. Metabarcoding utilizes conserved short regions of DNA to amplify sequences in samples that are unique in different taxa (Waraniak et al., 2019) and is a method used to detect biodiversity down to species level (Taberlet et al., 2012). Metabarcoding can be used for identifying species fast and objectively, separate between similar species, develop new stem trees, detect illegal use of threatened species, food and medicine control, study biodiversity, surveillance of ecosystems and study the diet of animals (Taberlet et al., 2012). Such molecular methods have advantages over morphological analysis of diets that require visual identification of partially digested prey items, because metabarcoding can identify prey items to a greater taxonomic resolution and for longer periods after consumption (Waraniak et al., 2019). Metabarcoding can also contribute to better identification of trophic links than traditional morphological methods (Riccioni et al., 2018). Metabarcoding clearly outperforms the morphological method in the taxonomic identification of prey describing more complex trophic relationships (Riccioni et al., 2018).

DNA extracted from multiple organisms sampled from stomach-contents (Berry et al., 2015) faecal matter (Berry et al., 2017), sediments (Drummond et al., 2015), water (Stat et al., 2017), or air (Kraaijeveld et al., 2015) can be analysed simultaneously using metabarcoding. To identify the species in the samples that is metabarcoded, a reference database with the DNA sequences previously barcoded is needed. The sequences from the samples are then matched with the sequences in the reference database (Taberlet et al., 2012). If the sample DNA is previously unsequenced, it is often possible to identify the species to a higher taxonomic level.

DNA metabarcoding of faecal matter and stomach contents have been developed with accurate taxonomic resolution of dietary information, in attempts to infer trophic interactions among both terrestrial (Bohmann et al., 2011; Clare et al., 2009) and aquatic organisms

(Berry et al., 2015). DNA metabarcoding have been used for analysing the diet of ticks, leeches, mosquitoes, fishes, bats, birds, cats, and whales (Johnson et al., 2021). In 72 % of field collected blacklegged tick (*Ixodes scapularis*) DNA from its host (blacklegged tich is a bloodsucker) was identified (Johnson et al., 2021). In medical leeches (*Hirudo medicinalis*) (leeches are bloodsuckers), bloodmeal have been found to contain host DNA for at least four months after its last meal, and for wild leeches 84 % were found to yield host DNA (Johnson et al., 2021). Fully digested gut content of lionfish (*Pterois volitans*) was identified to species level using next-generation sequencing (Johnson et al., 2021) and for marine-phase Arctic lamprey (*Lethenteron camtschaticum*), that feeds on fish blood, metabarcoding was useful for characterizing the intestinal content (Johnson et al., 2021). The feeding strategy of the European hake (*Merluccius merluccius*) was characterized by metabarcoding and revealed that the diet was truly diverse across sizes and sites (Riccioni et al., 2018). Atlantic cod (*Gadus morhua*) DNA was detected in spiny dogfish (*Squalus acanthias*) GI-tract using molecular techniques, where Atlantic cod have not been observed before by morphological observation (Pichford et al., 2020). Soininen et al., (2013) used metabarcoding on stomach content from Norwegian lemmings (*Lemmus lemmus*) and shed new light on the diet of the Norwegian lemming. By using chloroplast DNA, Soininen et al., (2013) were able to get a taxonomical precision and diversity of food items which were clearly higher than observed in previous studies on the Norwegian lemming (Hansson, 1969; Stoddart, 1967). Metabarcoding can be used to resolve prey taxa to species level in systems where this would be otherwise impossible, such as in fluid feeding invertebrates e.g. spiders, insects, and centipedes (Cuff et al., 2021; Eitzinger et al., 2018; Krehenwinkel et al., 2017; Pompanon et al., 2012).

A limitation of metabarcoding is its current dependency on polymerase chain reaction (PCR) (Piñol et al., 2019), because errors (errors can be overrepresentation of some taxa, or underrepresentation of other taxa) may be introduced during amplification (Taberlet et al., 2012). Other limitations are degraded template DNA and errors during sequencing (such as tag jumps from other libraries) (Taberlet et al., 2012). Other drawbacks using PCR on environmental DNA (eDNA) is its reliance finding a barcode that possesses a short variable DNA region (between different species) suitable for a primer to target (Taberlet et al., 2012). Universal primers are lacking, and different groups of organisms (archaea, bacteria, fungi, plants, arthropods, vertebrates, etc.) must be analysed separately, making it difficult to assess the relative proportions of each group (Taberlet et al., 2012). A prerequisite with metabarcoding is that you need a high-quality taxonomic reference database to compare your

data (Taberlet et al., 2012). Another issue with metabarcoding is that it is not a good quantitative method, it is a qualitative method. That means that the presence-absence of a species is a more robust approximation than the abundance of the same species. However, in some studies, it is shown that metabarcoding can be used quantitatively, but with mixed results (Lamb et al., 2019). Waraniak (et al., 2019) suggest that the number of sequencing reads is generally a good approximation of the relative biomass of organism in a sample. However, due to amplification bias of the primers, biomass and number of reads can be variable among taxa. In Waraniak's study some taxa were consistently overrepresented (e.g., the Crustacea *Cambaridae*) and some were under-represented (the insect *Perlidae*) (Waraniak et al., 2019). Taxonomic resolution could be improved by using different sets of barcoding primers targeting different regions (Waraniak et al., 2019). In some cases, it is impossible to distinguish different species. By using a barcode region in the cytochrome c oxidase I (COI) gene it is not possible to distinguish *Sebastes norvegicus* from *Sebastes mentella* and/or *Sebastes viviparus (Barcode of Life Data System, 2022)*. The universal approach COI to identify redfish species is inadequate (Shum et al., 2017). To correctly identify the different species of redfish a more variable mtDNA fragment (d-loop), other than COI, allows for the distinction of monophyletic groups (Shum et al., 2017). Furthermore, public databases are currently compromised by wrong reference sequence entries (Shum et al., 2017). Therefore, in some cases for the COI barcode region, the genus/family will be the identified taxa, not the species. Incidental consumption of eDNA in the water by predators and secondary predation (the prey's prey) can be mistaken as predation (Tercel et al., 2021; Waraniak et al., 2019), and could be difficult to account for. Prey that share the same DNA sequence as the predator is also a problem, so cannibalism is not possible to detect with metabarcoding (Waraniak et al., 2019).

Biology and feeding ecology of Batoidea

Batoidea (rays: skates, stingray, electric rays, and shovelnose rays) are dorsoventrally flattened cartilaginous fishes within Chondrichthyes (sharks, rays, and chimera). Rays around the world are caught in high numbers as by-catch in many fisheries targeting teleost species (ICES, 2021; Oliver et al., 2015). They are also in some cases commercially important (Frisk, 2000). Rays are the most species-rich group of Chondrichthyes, and many are threatened with extinction (Flowers et al., 2021). They are widely distributed all over the globe, living in freshwater, brackish water, saltwater, coastal, and deep-sea environments. Despite being widespread, bycaught, and to some extent commercially important, skates have received less

scientific attention than other vertebrates (Flowers et al., 2021). Trophic interactions and skates importance to ecosystem structure and function are examples of areas that needs further investigation to amongst others understand potential consequences of environmental changes on population and ecosystems (Flowers et al., 2021).

Skates are K-selected species that are oviparous (Walker, 1998), have internal fertilization (Luer et al., 2007), low fecundities (compared to teleost fishes) (Zorzi et al., 2001), late maturity, high juvenile survivorship, slow somatic growth, and long life spans (Camhi, 1998; Hoenig, 1990; King & McFarlane, 2003; Winemiller & Rose, 1992).

In the Barents Sea region five species of skates are found but little is known about their ecology (ICES, 2021). Among those are spinytail skate (*Bathyraja spinicauda*), Arctic skate (*Amblyraja hyperborea*), and thorny skate (*Amblyraja radiata*) which are all considered as *Least Concern* in the Barents Sea by the International Union of Conservation of Nature (IUCN) (Dolgov & Dulvy, 2015; Kulka et al., 2020; Stehmann et al., 2015). They all are demersal species with the Arctic skate being an Arctic species and spinytail skate and thorny skate both being Arcto-Boreal species (Mecklenburg et al., 2018).

Spinytail skates (*Bathyraja spinicauda*) reach a length of 182 cm (Dolgov, 2006). They are found at a depth of 140–2,000 m (Gibson et al., 2008) and prefer temperatures between 2–6°C (Dolgov et al., 2005). Spinytail skate is found in the northern North Sea to the Barents Sea and off Iceland and Greenland in the western Atlantic (Bigelow & Schroeder, 1953; Gibson et al., 2008; Peklova et al., 2014).

Arctic skates (*Amblyraja hyperborea*) reach a length of 95 cm (Gibson et al., 2008). They are found at depths between 260–2,500 m (Bigelow & Schroeder, 1953; Gibson et al., 2008; Kyne & Simpfendorfer, 2010) and prefer temperatures between -1°C to 4°C (Bigelow & Schroeder, 1953; Gibson et al., 2008; Peklova et al., 2014). Arctic skate might also be an active swimmer since depth changes of more than 150 m per 0.5 hour has been reported (Peklova et al., 2014). Arctic skate has the widest distribution among cartilaginous fishes across the Arctic seas and shelves and are found in Atlantic and Pacific Arctic waters (Lynghammar et al., 2013; Weigmann, 2016).

Thorny skates (*Amblyraja radiata*) reach a length of 105 cm, a weight of 11 kg (Sulikowski et al., 2005) but in the Barents sea they only reach a length of 65 cm and a weight of 3.2 kg (Bjelland et al., 2000). The Thorny skate are found at a depth of 18–1,400 m (Byrkjedal &

Høines, 2007; Gibson et al., 2008) and prefers waters with a temperature between -1°C to 8°C (Bigelow & Schroeder, 1953; Gibson et al., 2008; Peklova et al., 2014). Thorny skate belong to the North Atlantic (Chevolot et al., 2007; Gibson et al., 2008) and is the most common of all skate species occurring in the Barents Sea (Dolgov et al., 2005).

Skates in general are benthic and feed mostly on fish, Crustacea, and Polychaeta (Bizzarro et al., 2007; Mabragana & Giberto, 2007; Smale & Cowley, 1992). Skates in the Barents Sea feed mostly on fish, (herring (*Clupea harengus*), capelin (*Mallotus villosus*), redfish (*Sebastes* sp.), haddock (*Melanogrammus aeglefinus*), Atlantic cod and long rough dab (*Hippoglossoides platessoides)),* Arthropoda (Decapoda, northern shrimp (*Pandalus borealis*), Gammaridea, and Euphausiidae), and Polychaeta (Dolgov, 2005). Some skates feed on other skates as well (Dolgov, 2005). Spinytail skate mainly feed on fish (redfish, haddock, long rough dab, and *Raja* spp.), Arthropoda (Decapoda, northern shrimp, and Gammaridea), and Polychaeta (Dolgov, 2005). Arctic skate mainly feed on fish (herring, capelin, redfish), Arthropoda (Decapoda and northern shrimp), and polychaeta (Dolgov, 2005). Herring, capelin, and blue whiting (*Micromesistius poutassou*) are most likely discarded from fisheries (Byrkjedal et al., 2015). It is observed that Arctic skate can eat glacial eelpouts up to 80 % of its own total length, and such a large multi-meal are suggested to be digested over several months (Byrkjedal et al., 2015). Thorny skate mainly feed on fish (cod, capelin, long rough dab), Arthropoda (Decapoda, Euphausiidae, Gammaridea, and northern shrimp), and Polychaeta (Dolgov, 2005). A significant portion of thorny skates' diet is fisheries waste (Dolgov, 2005).

Expectations and aims

Expected outcomes are differences in diet between groups such as species, size classes, and sexes. Larger skates are expected to feed on larger prey items such as fish, while smaller skates are expected to feed on smaller prey items such as annelids, arthropods, and molluscs. Environmental variables are expected to follow differences in diet, e.g., Arctic skate is expected to prefer more cold adapted prey species compared to spinytail skate and thorny skate. Compared to previous morphology-based studies, higher resolution of prey items is expected, with more soft-bodied species.

The aims with this master thesis are to firstly identify specific prey items. Secondly, compare feeding ecology between spinytail skate, Arctic skate, and thorny skate, look for ontogenetic shifts, sexual differences, if the diet varies along environmental gradients such as depth and

temperature, and if the diet varies from location to location. Thirdly, compare the results with traditional morphology-based diet studies (Dolgov, 2005; Eriksen et al., 2020).

Materials and methods

Samples and preparation

A total of 63 individuals of spinytail skate (19), Arctic skate (16), and thorny skate (28) were collected in the Barents Sea (Figure 1). Individuals were selected from 4–6 size classes and both sexes (Table 1). Samples were collected by bottom trawling from several research cruises, both by the Institute of Marine Research (IMR) as well as personal participation on Norwegian College of Fishery Science (NCFS) cruises, and frozen on board. For each individual, metadata such as station data are found in Appendix 1, Table 1.

The skates were thawed overnight in freshwater (causing slightly increased weight than if thawed in air, however weight was not used in the analysis, so it should not matter). Each individual was measured, weighted, photographed, the sex was identified, and stomachs was dissected and collected in 96 % ethanol and stored in the freezer until homogenizing with a stick mixer. The stomachs were weighted with and without stomach content after thawing. Individual stomach fullness (Appendix 3, Figure 1) was calculated with the following formula:

1-(stomach without contents)/stomach with contents) (Formula 1)

The stomach content was homogenized using a stick mixer. Three subsamples were collected and stored in the freezer. Some blanks were also collected between every 10^{th} sample from the tray and bowl used for dissecting the stomach and making the homogenate. Visible prey items were photographed prior to homogenization and later identified by using Moen & Svensen (2014). Sampling, dissection, and homogenization of samples are described in Appendix 4.

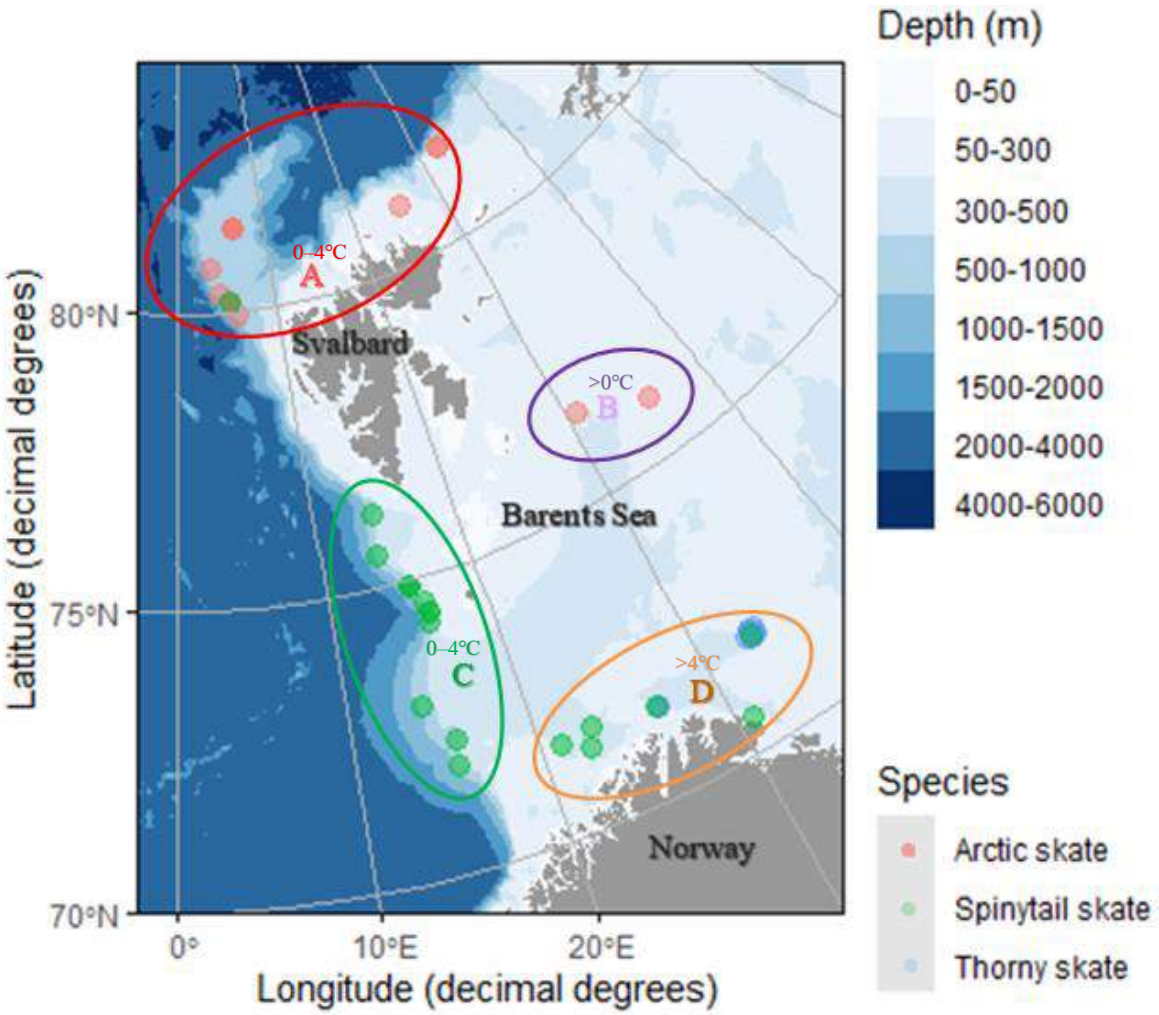

Figure 1. Collection of spinytail skate (green dots), Arctic skate (red dots) and thorny skate (blue dots) in the Barents Sea and adjacent waters. The different circles with the letters A–D indicate locations with dissimilar environmental conditions. Approximate bottom temperatures in August–September for location A–D are extracted from Christian et al., (2015).

Table 1. Different size classes divided by species, total length (TL), and sex. Skates were selected based on size and sex. Prior to sampling 6 size classes were defined, and skates were selected to fill these 6 size classes with 4 individuals (2 males and 2 females) in each size class. After sampling the size ranges were redefined to fit the number of skates and length distribution better.

Skate	Size class	TL (mm)	Female	Male
Spinytail skate	1	280–348	2	2
	2	512–580	2	2
	3	610–780	2	1
	4	800–995	0	3
	5	1220–1385	2	2
	6	1420–1420	0	1
Arctic skate	1	157–185	2	2
	2	225–270	3	2
	3	345–455	1	1
	4	604–730	2	3
Thorny skate	1	110–150	3	2
	2	210–240	2	3
	3	250–340	2	1
	4	390–440	3	3
	5	470–510	2	2
	6	560–590	2	3

DNA extraction and amplification

Samples were extracted with the Qiagen DNeasy Powersoil Kit and Qiagen DNeasy Powersoil Pro Kit, following standard protocols from the manufacturer, described in Appendix 5 and 6. DNA quantities were checked regularly (every 4th sample) with the Invitrogen Qubit 4 Fluorometer (ThermoFisher) following standard protocols.

The Leray fragment (Wangensteen et al., 2018) of cytochrome c oxidase subunit I (COI), a 313-base pair (bp) fragment were amplified using a simple 1-step PCR protocol with the Leray-XT primer set following standard protocols described in Appendix 7. All three subsamples per stomach sample were amplified. The metabarcoding primers have an 8-base sample-tag attached (each tag with at least 3 differences out of 8 bases). A variable number

(2–4) of leading Ns are added to increase sequence variability to improve Illumina sequencing. Each forward and reverse primer has the same sample-tag attached in both ends. The PCR protocol uses AmpliTaq Gold 360 master mix (ThermoFisher) and bovine serum albumin (BSA). The PCR mix consisted of 10.00 µL AmpliTaq Gold 360 master mix, 0.16 µL BSA 20 µg/µL, 5.84 µL H_2O, 1 µL forward primer 5 µM, 1 µL reverse primer 5 µM, and 2 µL DNA template. DNA qualities were checked with gel electrophoresis in 1 % agarose for 32 of the samples following standard protocols using 2µL of PCR product.

PCR clean-up and library preparation

The PCR products were pooled together in Eppendorf tubes (one tube per library). The pool was then purified using MinElute columns for removing DNA fragments below 70 bp and MinElute PCR Purification Kit (Qiagen) following standard protocols described in Appendix 8. These steps also concentrate the amplified DNA around 10 times. MinElute columns have a maximum sample volume of 130 µL per sample, so 10 or 12 columns were used. Then all elutes were pooled together and homogenized by vortexing. DNA concentrations were checked with an Invitrogen Qubit 4 Fluorometer using broad-range DNA quantification kit following standard protocols.

Library preparation is the first step of next generation sequencing. It allows DNA (or RNA) to adhere to the sequencing flowcell and allows the sample to be identified. Library preparations were done following a modified PCR-free ligation protocol, NEXTflex PCR-Free DNA Sequencing Kit (BIOO Scientific) as described in Appendix 9. A total of 3 µg of DNA (up to 40 µL of the previous pool) were used as starting material. With this kit, the amplicons were ligated to the Illumina adapter and a 6-base library tag. This protocol follows step A–F, which was End Repair, Clean-Up, 3’ Adenylation, Adapter Ligation, Clean-Up, and Quantification, respectively.

Sequencing and bioinformatic analysis

The library prepped DNA were sent in a pool for sequencing at a commercial sequencing platform (NOVOGENE) using Illumina Novasq6000, next-generation sequencing (NGS). The data with sequences and reads were ran through a bioinformatic pipeline, MJOLNIR (Metabarcoding Joining Obitools & Linkage Networks In R) where it was filtered, trimmed, and clustered. After that, numbers of reads per species present in each stomach was determined. The reads were compared with a local database, DUFA_COLR (owned by “DNA

Universal-databank for Fisheries and Aquaculture"), to see which species was present. The different steps of the pipeline are described in Appendix 10. The steps consist of RAN (Reads Allotment in N portions), FREYJA (Filtering of Reads, Enrollment, Yoke-reads, Joining and Alignment), HELA (Hierarchical Elimination of Lurking Artifacts), ODIN (OTU Delimitation Inferred by Networks), THOR (Taxonomy with Higher-than-Order Ranks), FRIGGA (Final Recount and Integration of Generated Genealogies and Abundances), LOKI (LULU Overseeing with Kinship Identification), and RAGNAROC (Replace Agnomens with Names And Recover Original Codification).

Data analysis

Molecular Operational Taxonomic Units (MOTUs) are in this thesis used for each unique prey item with the same DNA sequence. Operational Taxonomic Units (OTUs) are used in the same way as MOTUs, however, they are not separated by DNA sequences since they were identified with morphology-based methods. MOTUs and OTUs will therefore describe the lowest identifiable taxa of a prey item. In this thesis a species is a MOTU, or an OTU identified to the rank of species with at least a 98 % match with the reference database.

In cases where DNA sequences returned no match in the DUFA_COLR database, the sequences were blasted through BOLDSYSTEMS (*Barcode of Life Data System*, 2022). All sequences were processed through R-studio (R Core Team, 2013) where subsamples have been clustered. MOTUs that were removed were:

- MOTUs with low read numbers (under 100 reads)
- MOTUs with an identity below 85 % from the reference database (MOTUs with an identity between 85–98 % match was not referred to as a species)
- MOTUs which most likely have been incidentally consumed by the skate or secondary prey items
- Parasites
- Host DNA (DNA from the three skate species in this thesis)
- Suspected tag-jumps from other libraries
- Other contaminations, such as human DNA

The complete list of incidentally consumed prey, secondary prey, parasites, skate (host) DNA, tag-jumps and other contaminations are: Amoebozoa, Archaeplastida, Chromalveolata, Fungi, Nematoda, Mytiloida, Primates, Rajiformes, *Leptogorgia sarmentosa*, *Antedon mediterranea*,

Caprella scaura, Laticorophium baconi, *Cilicaea* sp. 72, *Eukrohnia bathyantarctica, Jassa slatteryi,* and *Watersipora subovoidea*.

After clustering and filtering the dataset, both the presence-absence and relative-fullness methods were used to assess % frequency of occurrence and relative abundance of prey items. The presence-absence and relative-fullness methods were based on reads rather than visual identification of prey. This study will be focusing on relative abundance and % frequency of occurrence. The difference is that relative abundance look at the importance of one prey compared to another, using the number of reads as an indication of how important the prey is. The % frequency of occurrence describes how often a prey occur, making every occurrence of a prey just as important as other occurrences of other prey items.

MOTUs/OTUs, phylum, classes and species composition have been compared for the different skate species, size classes, and sexes. Seven different parameters were tested to see if there are differences in the diet and what influences the diet of Arctic skate, spinytail skate and thorny skate. The seven different parameters were skate species diet differences, intraspecies size diet differences, interspecies size diet differences, sex diet differences, depth variation in diets, temperature variation in diets, and location variation in diets. Skate, size, depth, and temperature were illustrated in nMDS plots to show the distances in diet (Appendix 3, Figure 7–10). This was tested both for relative abundance and % frequency of occurrence of prey items. R (R Core Team, 2013) was used to make graphical representations from the filtered data. The map was made using the ggOceanMaps (Vihtakari, 2022), barplots were made using ggplot2 (Wickham, 2016), the circular plots were made using circlize (Gu et al., 2014) and rarefaction curves and accumulation curves were made using base R (R Core Team, 2013). The R-script are in Appendix 11.

SeaLifeBase (Palomares & Pauly, 2022) and FishBase (Froese & Pauly, 2022) have been used to categorise what are pelagic prey items (Appendix 2, Table 5) and what are benthic prey items (Appendix 2, Table 6). Species with unknown habitat use or uses both benthic and pelagic habitat are described in Appendix 2, Table 7.

Results

The final output from the bioinformatic pipeline was 65,295,400 reads with 897 Eukaryotic MOTUs. Prior to filtering the dataset, 275 MOTUs were assigned to species rank. After the filtration process, the dataset resulted in 17,854,361 reads containing 160 MOTUs where 112 of those were assigned to the rank of species. Rarefaction curves (Appendix 3, Figure 2) indicated that a sequencing depth of approximately 17 million reads was adequate to represent the composition of the samples. An average of 50,000 reads per sample covered the taxonomic composition of the samples. The sequencing depth were not a limiting factor. The same analysis was made for the three skate species separately, and a sufficient sequencing depth was achieved (Appendix 3, Figure 3–5). Accumulation curves indicated that not enough samples were used in this thesis to represent the full variation of prey taxa for these three skate species (Appendix 3, Figure 6).

Skate diet differences

Arctic skate did not have overlapping diet with the other two skates (Appendix 3, Figure 7–8), and the relative abundance of prey groups (phyla) varied for each skate species (p-value < 0.05) (Figure 2). List of different p-values are in Appendix 2, Table 2–4. The Relative abundance of the most important phyla was 38 %, 27 %, and 19 % for Chordata, Arthropoda, and Annelida respectively (Figure 2). For spinytail skate, the prey group that had the highest relative abundances was Chordata (74 %). For the Arctic skate, the prey groups were Chordata (36 %), Mollusca (30 %), and Arthropoda (24 %). For thorny skate, the prey group was Annelida (53%). Other important prey groups for the different skate species that had a high (>10%) relative abundance or % frequency of occurrence were Priapulida and Cnidaria.

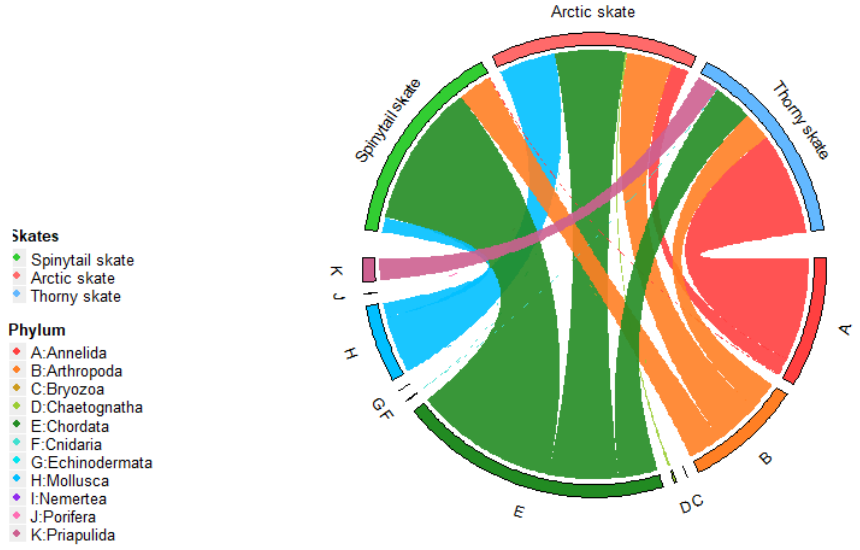

Figure 2. The relative abundance of prey groups for spinytail skate (green), Arctic skate (red), and thorny skate (blue). The skates are at the top half of the circle, and the prey are at the bottom half of the circle. The bars show the importance of the prey group, both for the individual skate species and the prey.

Ontogenetic shifts were confirmed (p-value < 0.05) for the three skate species, both intra- and interspecies. The relative abundance of Chordata became more important when skates became larger (Figure 3). Chordata was most important for spinytail skate (74 %), followed by Arctic skate (36 %) and thorny skate (20 %) (Figure 3). Smaller prey items, such as Annelida, became less important for larger skates (Figure 3). The relative abundance of Annelida was 5 %, 9 %, and 53 % respectively for spinytail skate, Arctic skate, and thorny skate.

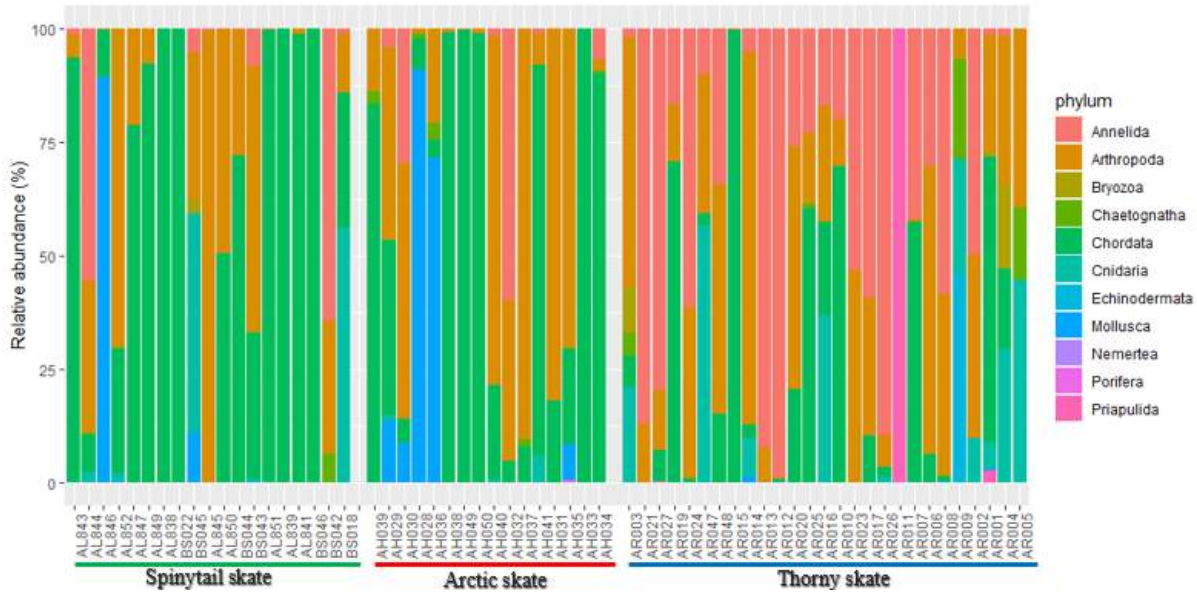

Figure 3. Relative abundance of prey items per skate sorted after skate species and skate size with the smallest individuals to the left.

The three different skate species had different preferred prey items. For spinytail skate, greater eelpout (*Lycodes esmarkii)* (40 %) and haddock (26 %) had the highest relative abundance. For Arctic skate, Atlantic gonate squid (*Gonatus steenstrupi)* (30 %) and Polar cod *(Boreogadus saida)* (26 %) were the most important prey items. For thorny skate the polychaete *Laonice cirrata* (27 %) and Greenland halibut (*Reinhardtius hippoglossoides)* (12 %) were the most important prey items. Fish's relative abundance was higher for larger skates than smaller skates (Figure 4). Norway pout, haddock, Greenland halibut, and Arctic rockling (*Gaidropsarus argentatus*) were the most important fish prey of spinytail skate. The combined relative abundance of these four fish preys were 33 %, 21 %, 85 %, 98 %, 97 %, and 30 % respectively for size class 1–6 of spinytail skate (Figure 4). Polar cod and lumpfish were the most important fish prey of Arctic skate. The combined relative abundance of these two fish preys were 29 %, 0 % 99 %, and 66 % respectively for size class 1–4 of Arctic skate (Figure 4). Greenland halibut, spotted snake (*Leptoclinus maculatus*), and haddock were the three most important fish prey for thorny skate. The combined relative abundance of these three fish preys was 34 %, 36 %, 0 %, 16 %, 0 %, and 30 % respectively for size class 1–6 of thorny skate (Figure 4).

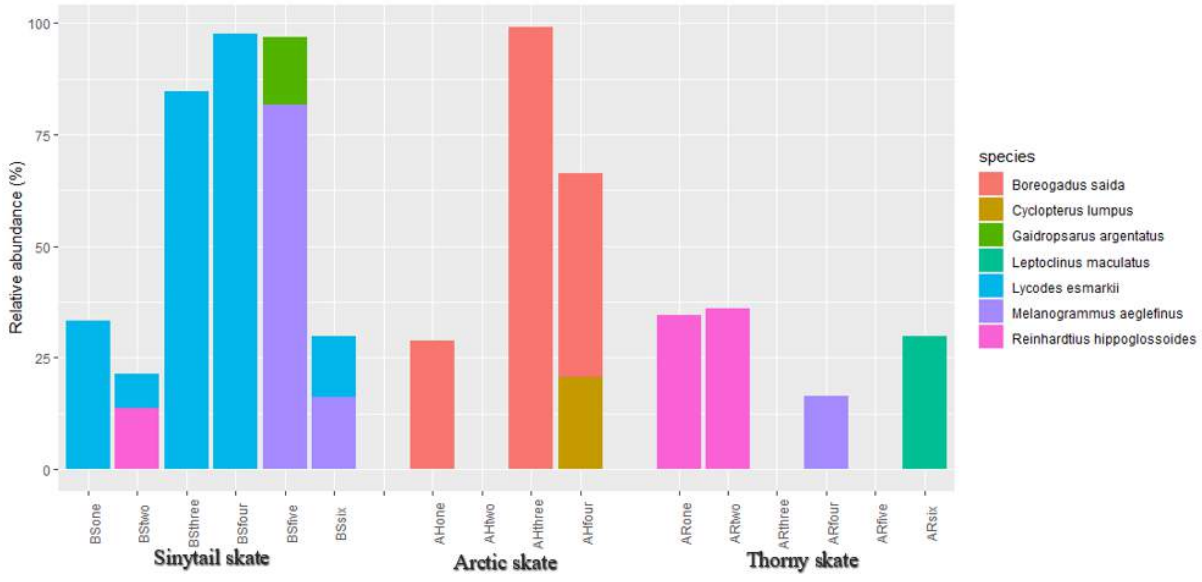

Figure 4. Relative abundance of most important (>4 %) species of Chordata for different size classes of skates. This are filtered data where prey species with a relative abundance below 4 % are filtered out.

Other prey species than fish generally had a lower relative abundance for larger skates than smaller skates (Figure 5). Examples of this were northern krill *(Meganyctiphanes norvegica)* that was important for the first size class of spinytail skate, the squid *G. steenstrupi* that was

important for the second size class of Arctic skate, and the priapulid worm *Priapulus caudatus* that was important for the third size class of thorny skate (Figure 5).

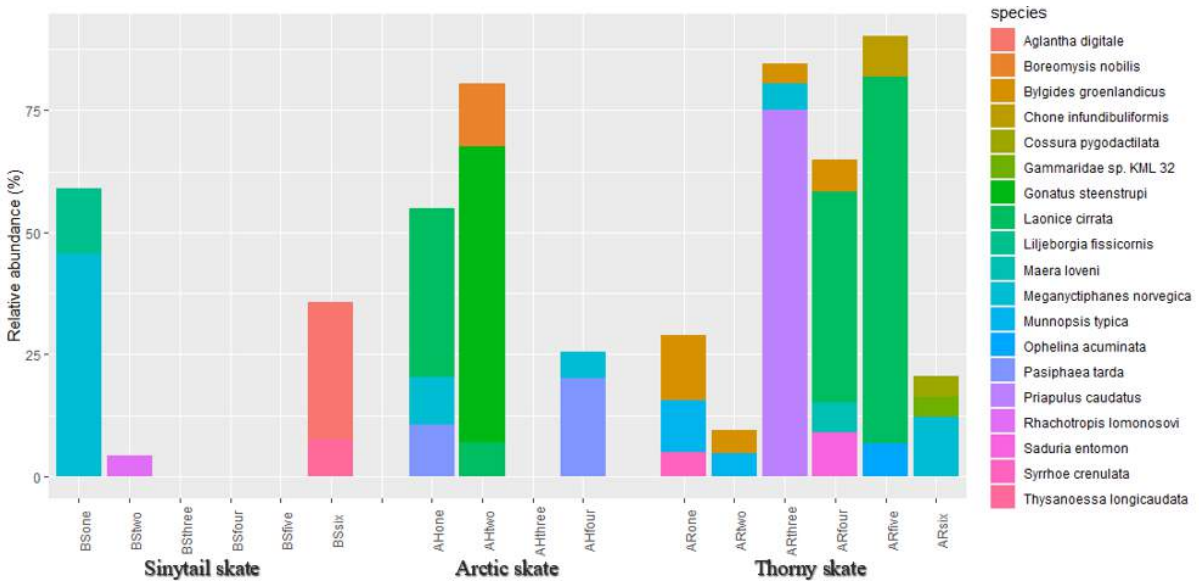

Figure 5. Relative abundance of most important (>4 %) species of other classes than Chordata for different size classes of skates. This are filtered data where prey species with a relative abundance below 4 % are filtered out.

The % frequency of occurrence of prey groups (phyla) varied for each skate species (p-value < 0.05) (Figure 6). For spinytail skate, Arctic skate, and thorny skate, the prey group that had the highest % frequency of occurrence was Arthropoda (54 %, 40 %, and 48 % respectively) followed by Annelida (12 %, 17 %, and 24 % respectively), Chordata (15 %, 18 %, and 10 % respectively), and Cnidaria (12 %, 12 %, and 11 % respectively) (Figure 6). Other differences were that Nemertea only occurred in spinytail skate, Porifera only occurred in Arctic skate, and Priapulida only occurred in thorny skate (Figure 6).

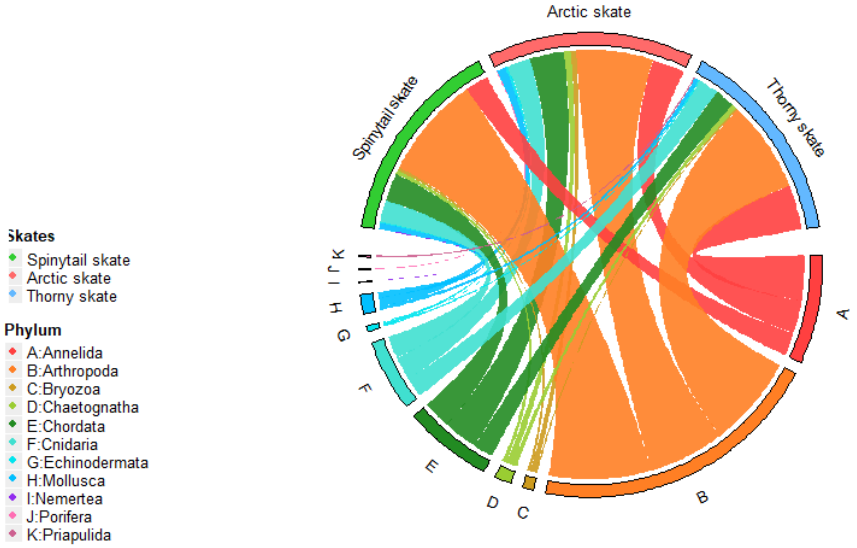

Figure 6. The % frequency of occurrence of prey groups for spinytail skate (green), Arctic skate (red), and thorny skate (blue). The skates are at the top half of the circle, and the prey are at the bottom half of the circle. The bars show the importance of the prey group, both for the individual skate species and the prey.

The phylum that had the highest % frequency of occurrence was Arthropoda (47 %), followed by Annelida (17 %), Chordata (14 %) and Cnidaria (13 %) in all three skate species combined (Figure 7). The % frequency of occurrence of Arthropoda became more frequent for the larger size classes of spinytail skate, while in Arctic skate Arthropoda was most frequent in size class three and in thorny skate Arthropoda was most frequent for the smallest size classes (Figure 7). The % frequency of occurrence of Annelida was most frequent in size group 3–6 in spinytail skate, the smallest size group of Arctic skate, while the frequency did not vary in thorny skate for the different size classes (Figure 7). The % frequency of occurrence of Chordata became more frequent for the larger size classes of spinytail skate, Arctic skate, and thorny skate (Figure 7). The % frequency of occurrence of Cnidaria was highest for the largest size class of spinytail skate, did not vary in Arctic skate, and was highest in the largest size class of thorny skate (Figure 7). So, the % frequency of occurrence varied for Chordata, Arthropoda, Annelida, and Cnidaria generally, while the other phyla had a % frequency of occurrence that was similar for all size classes of the three skate species. Fish species occurred more often in larges skates (Appendix 3, Figure 16), while other species than fish generally became less frequent in larger skates (Appendix 3, Figure 17).

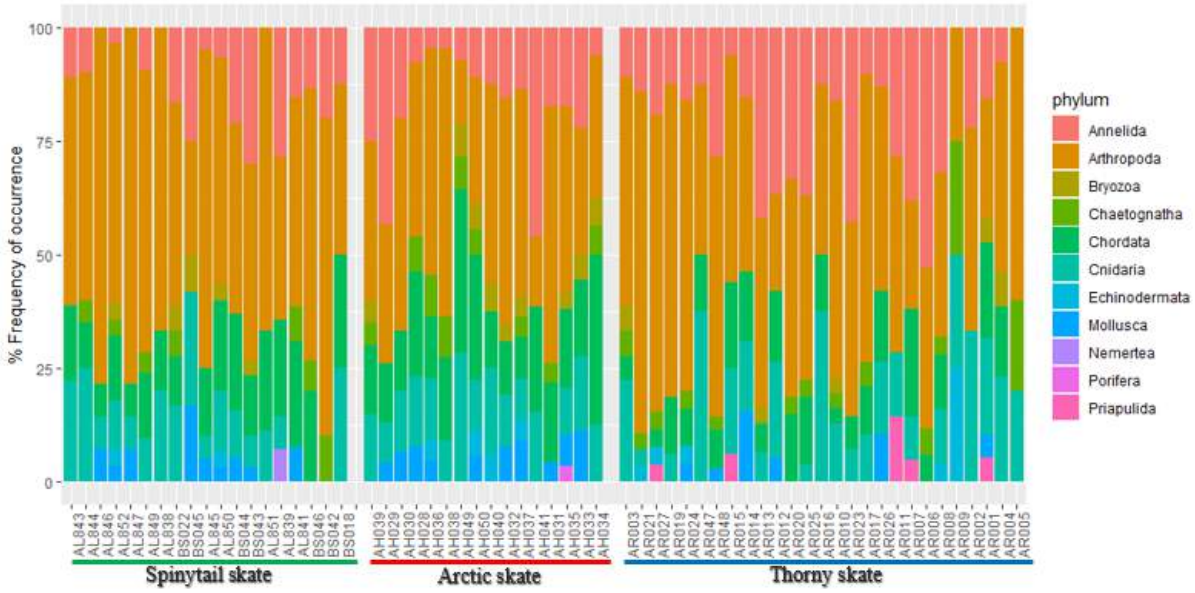

Figure 7. % Frequency of occurrence of prey items per skate sorted after skate species and skate size with the smallest individuals to the left.

When comparing the sex differences in diet (both relative abundance and % frequency of occurrence) between the three skate species, none were found (p-value > 0.05).

Pelagic vs Benthic diet

An ANOVA test revealed that there were differences (p-value < 0.05) between the three skate species when comparing the relative abundance of pelagic prey items (Appendix 2, Table 5) versus benthic prey items (Appendix 2, Table 6). Arctic skates had a larger portion of their diet that were pelagic species compared to spinytail skate and thorny skate. The relative abundance of pelagic species was 84 % in the diet of Arctic skate, and only 3–10 % for both spinytail skate and thorny skate (Figure 8). The relative abundance of benthic species was 9 % in the diet of Arctic skate, and 75–79 % for spinytail skate and thorny skate respectively (Figure 8). Polar cod and the squid *G. steenstrupi* were two prey species contributing to the high pelagic diet in Arctic skate.

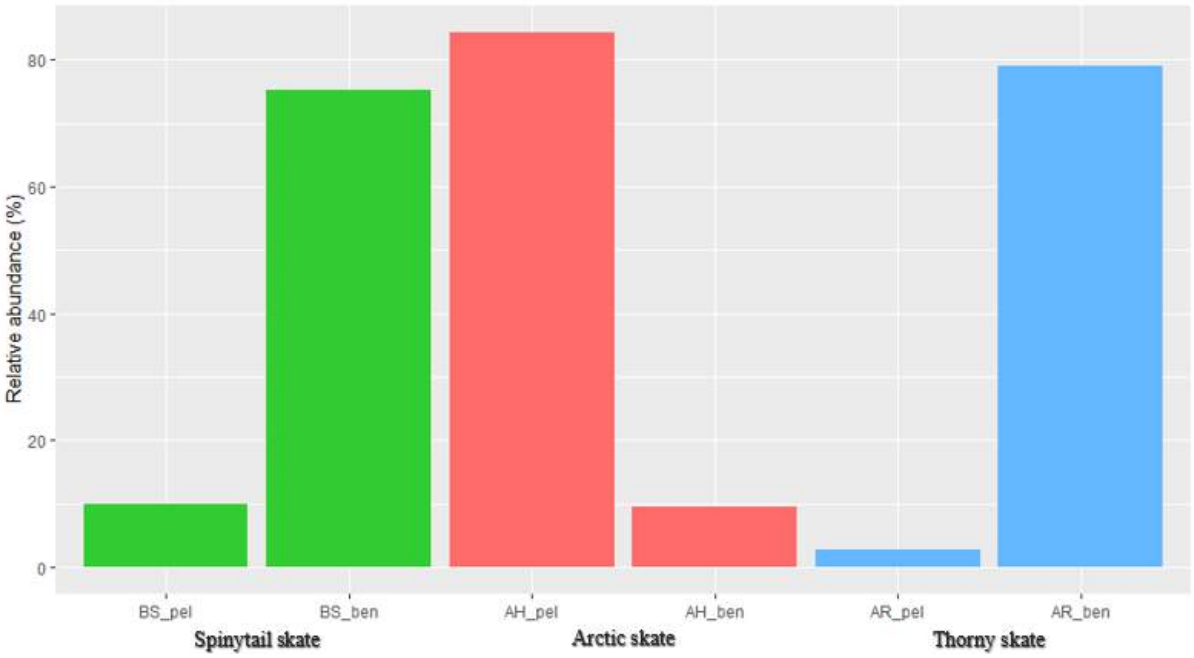

Figure 8. Relative abundance of pelagic (pel) and benthic (ben) prey items per skate species. Spinytail skate (BS), Arctic skate (AH), and thorny skate (AR).

The % frequency of occurrence of pelagic and benthic diet between the different skate species were not significantly different (p-value > 0.05). The % frequency of occurrence of pelagic prey items was still highest for Arctic skate compared to the two other skate species, and the benthic diet was still highest for spinytail skate and thorny skate (Appendix 3, Figure 11).

Pelagic species' relative abundance did not differ significantly between the different size classes of Arctic skate (p-value > 0.05). Figur 9 descibes the relative abundance of pelagic prey groups of Arctic skate. The three most important prey groups were Chordata (33 %), Mollusca (30 %), and Arthropoda (20 %), with Chordata becoming more important for the larger size classes and Arthropoda and Mollusca being more important for the smaller size classes (Figure 9).

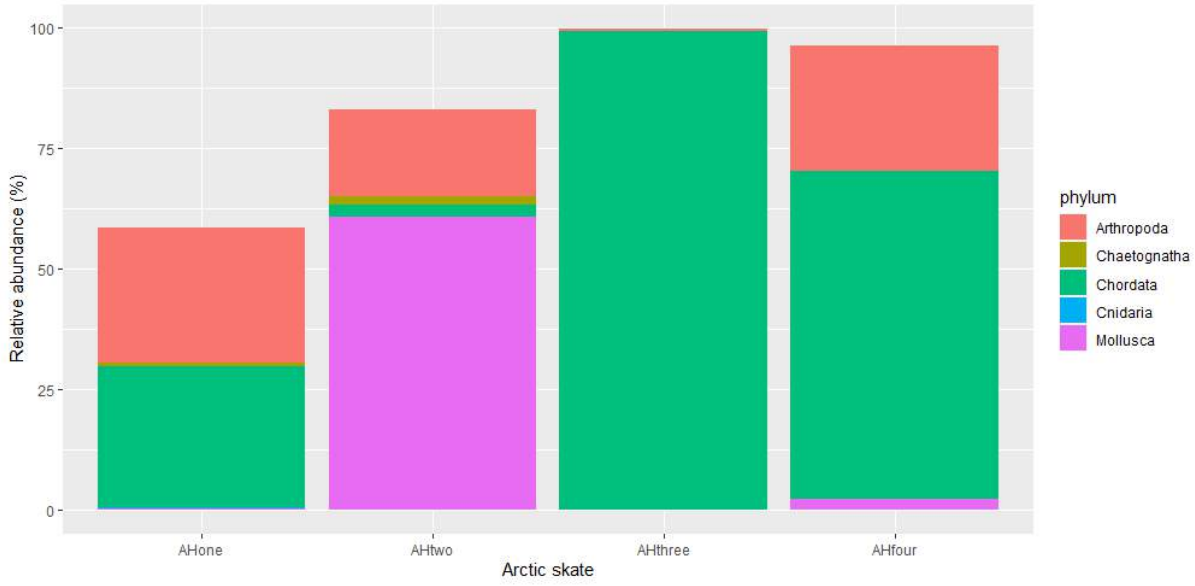

Figure 9. Relative abundance of pelagic prey groups (phyla) for the different size classes of size classes (1–4) of Arctic skate (AH). The different size classes are 1 (one), 2 (two), 3 (three), and 4 (four), with 1 being the smallest skates and 4 being the largest skates.

The squid *G. steenstrupi* (30 %), and Polar cod (26 %) (Figure 10) had the highest relative abundances and were the most important prey items at species level for Arctic skate, but the % frequency of occurrence of different prey items had a similar distribution for the different size groups with no prey items occurring more than 6 %. The relative abundance of Polar cod became more important for larger Arctic skates (Figure 10), while the squid *G. steenstrupi* was important for the second size class of Arctic skate.

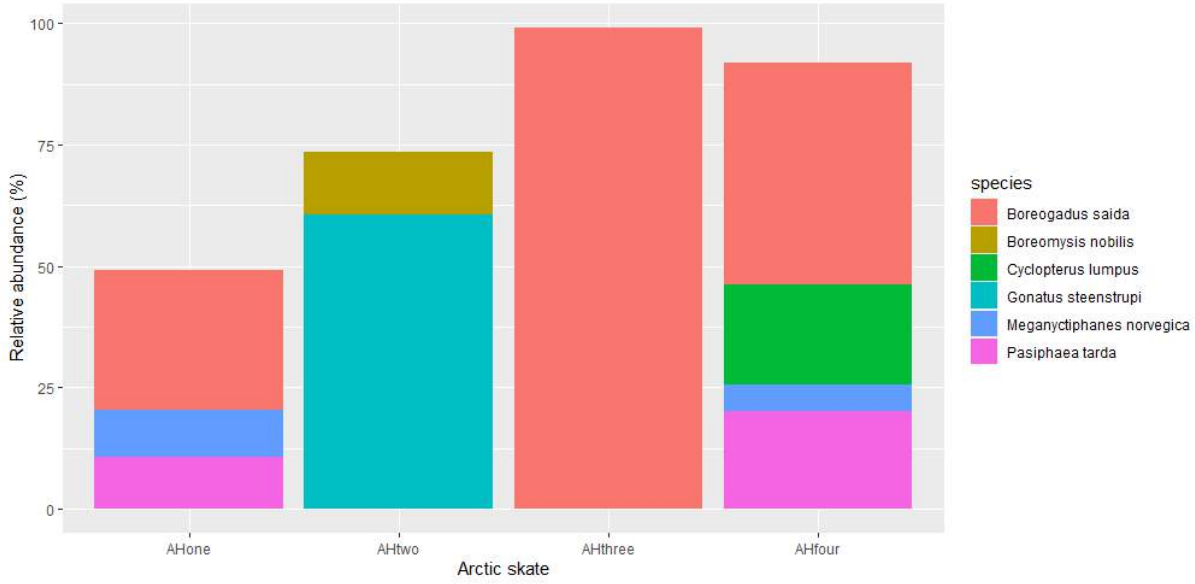

Figure 10. Relative abundance of most important pelagic prey items (>5 %) for the different size classes (1–4) of Arctic skate (AH). The different size classes are 1 (one), 2 (two), 3 (three), and 4 (four), with 1 being the smallest skates and 4 being the largest skates. This are filtered data where prey species with a relative abundance below 5 % are filtered out.

Environmental variables

There were differences in diet along depth and temperature gradients and between the different locations (A–D (Figure 1)) for the different skate species (p-value < 0.05) (Appendix 3, Figure 9-10). However, there were none intraspecies differences in diet for the different environmental gradients or locations (p-value > 0.05). The prey composition varied for the different locations (Figure 11). Mollusca was the most important prey group in location A with a relative abundance of 34 %, while Chordata were most important in location B and C (99 % and 65 % respectively)), and Annelida was the most important prey group in location D (45 %) (Figure 11).

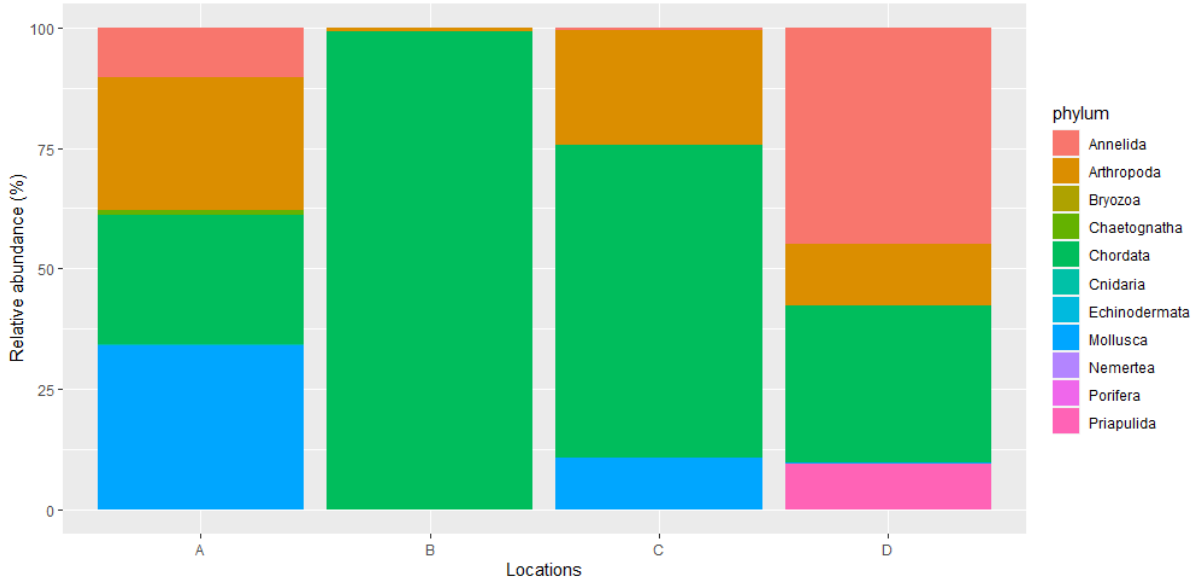

Figure 11. Relative abundance of prey groups (phyla) at various locations. The various locations are described in Figure 1.

Arthropoda was the prey group with the highest % frequency of occurrence in location A, C and D. In location B, the prey group with the highest % frequency of occurrence was Chordata (Figure 12). Other differences were Annelida that had a higher % frequency of occurrence in location D than the other locations and Cnidaria that had a higher % frequency of occurrence in location B (Figure 12).

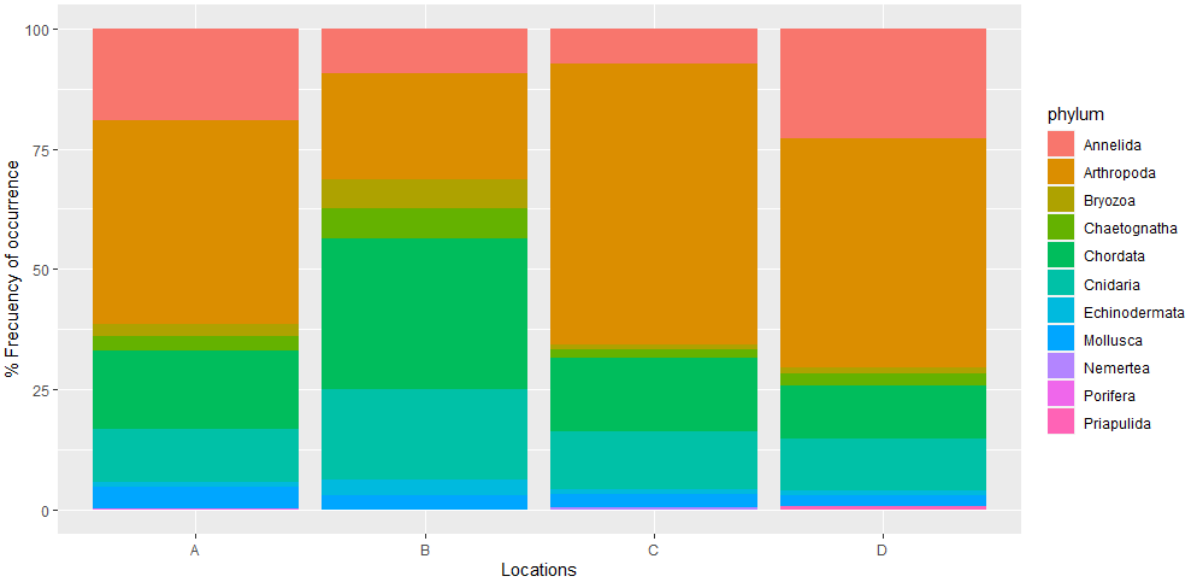

Figure 12. % Frequency of occurrence of prey groups (phyla) at various locations. The various locations are described in Figure 1.

There were also species differences in the various locations (Figure 13). In the two northern most locations (A and B), Polar cod was the dominant fish prey, while greater eelpout dominated as fish prey in location C, and haddock dominated as fish prey in location D. Lumpfish was also important in location B compared to the other locations (Figure 13). Other prey species than fish also varied from location to location (Figure 13). The squid *G. steenstrupi* was important in location A, the polychaete *L. cirrata* was important in location A and D, and the krill *M. norvegica* was important in location C (Figure 13). The % frequency of occurrence of the different prey species for the separate locations was between 0–7 %, with location B having most species occurring more than 3 % of the time (Appendix 3, Figure 18).

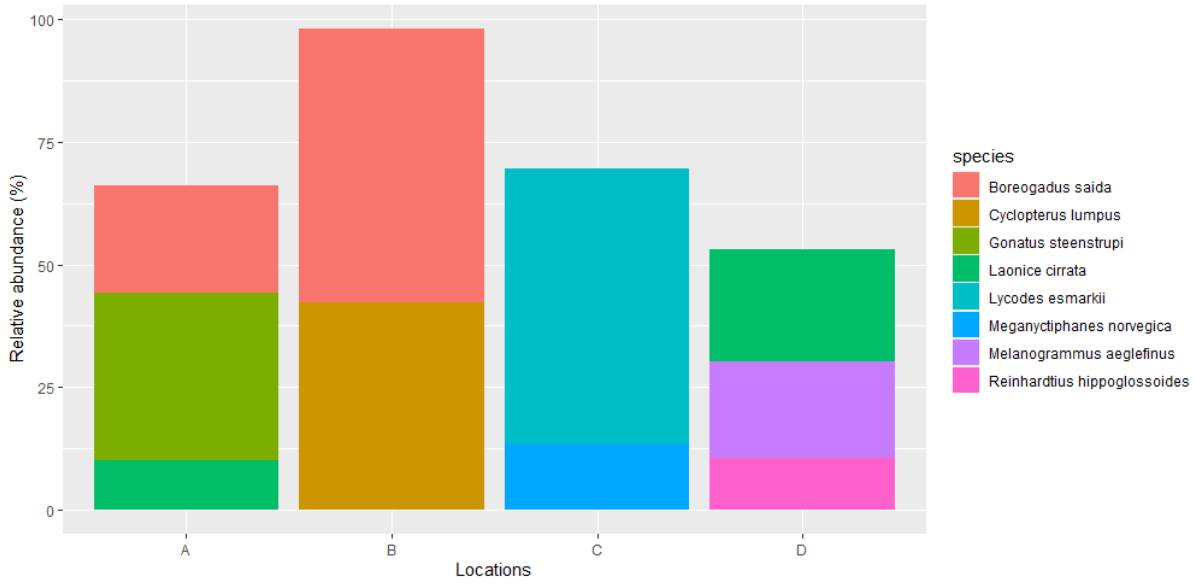

Figure 13. Relative abundance of most important prey items at species level (>10 %) at the various locations. The various locations are described in Figure 1. This are filtered data where prey species with a relative abundance below 10 % are filtered out.

Other noteworthy findings

Five skate stomachs were completely empty (Appendix 3, Figure 1). One belonging to Arctic skate, two to spinytail skate, and two to thorny skate. From these stomach samples it was possible to identify species which would not have been possible in a traditional morphological diet study. AH037 had 198,658 reads, BS010 had 347 reads, BS045 had 1011 reads, AR006 had 177,096 reads, and AR010 had 274,956 reads. A total of 48 different species were found in these stomachs (Appendix 3, Figure 19).

Two juvenile skates were observed with internal yolk sacs, indicating they had hatched recently). The spinytail skate (BS045) had 1,011 reads and the thorny skate (AR003) had 471 reads. A total of 16 different species were found in these two stomachs (Appendix 3, Figure 20). A hydrozoan, *Aglantha digitale* (34 %), was the most important prey item of BS045, and an arthropod, *Eurycope inermis* (34 %), was the most important prey item of AR003.

Some strange reads were encountered. The reads (137) of a fin whale (*Balaenoptera physalus*) where found in one spinytail skate (AL850). Two strange arthropod species, *Clavella adunca* (5,977 reads) where found in one Arctic skate (AH050) and *Balanus balanus* were found in several skates (Appendix 3, Figure 21). Six different Cnidaria species were observed in several skates (Appendix 3, Figure 22). The six different species was *Aglantha digitale, Bougainvillia muscus, Campanularia hincksii, Clytia hemisphaerica, Cyanea*

capillata, and *Obelia dichotoma.* A chaetognath species (*Sagitta elegans*) which might be secondary prey were observed in several skates (Appendix 3, Figure 23–24).

Discussion

Skate diet differences

Spinytail skate, Arctic skate and thorny skate had different diets. The difference in diets reflects the size differences of the skates. Spinytail skate is largest, and feed on larger prey items such as different fishes, Arctic skate is medium-sized and feed on medium sized prey, while thorny skate is the smaller of these three skate species, and feed mainly on smaller prey items such as Annelida. These results are supported both by Dolgov (2005) and Eriksen et al., (2020) that also investigated the diet of spinytail skate, Arctic skate, and thorny skate in the Barents Sea. Arthropoda was the prey group having the highest % frequency of occurrence in all three skate species, also confirmed by Dolgov (2005) and Eriksen et al., (2020). Another relatively large bottom dwelling fish in the Barents Sea, Greenland halibut (*R. hippoglossoides*), have cephalopods, especially *Gonatus fabricii*, and fishes such as herring and blue whiting as the most important prey species (Michalsen et al., 1998), a diet that is comparable to these three skate species. A study on Arctic skate with pop-off tags documented limited horizontal dispersal (Peklova et al., 2014) and another study on thorny skates in the Newfoundland area with tags also revealed limited horizontal dispersal (Templeman, 1984), indicating that Arctic skate and thorny have fed where it was caught. Spinytail skate have most likely the same behaviour as the other two with limited horizontal dispersal.

Ontogenetic shifts were found for spinytail skate, Arctic skate, and thorny skate, but no sex differences in diet. As the skate grew larger, a more piscivores diet was adopted while smaller prey items such as Annelida, Arthropoda, and Mollusca became less important for the larger skates. Dolgov (2005) concluded that the size composition of the prey is decided by the size of the predator, the longer the predator, the longer the prey. The smallest thorny skates (11–15 cm, size class 1) mainly consumed Polychaeta and Gammaridea, while the middle group (36–40 cm, size class 4) preyed upon more shrimp, fish, and fisheries waste than the smaller ones. The larger thorny skates (61–65 cm, larger than size class 6) fed on more cod and other fishes (Dolgov, 2005). Espinoza et al. (2012) compared diet between different stages of the skate *Raja velezi* and the shark *Mustelus henlei* along the Pacific coast of Costa Rica. That

study revealed clear ontogenetic dietary shifts. Crustaceans (mainly shrimps, crabs and stomatopods) dominated the diet of immature individuals, and adults had a higher proportion of fish. In general, skates tend to become increasingly piscivorous and reach a higher trophic level as they grow larger and older (Ebert & Bizzarro, 2007), they are considered marine top predators and reach an estimated trophic level of 3.48–4.22 (Ebert & Bizzarro, 2007).

Pelagic vs Benthic diet

The pelagic diet of Arctic skate separated it from the two other skate species. Arctic skate might be a pelagic feeder, supported by the fact that Arctic skate performs depth changes of more than 150 m per 0.5 hour (Peklova et al., 2014). Data from Dolgov (2005) and Eriksen et al., (2020), indicated similar trends as in the present study (Appendix 3, Figure 12-15). Prey items in (Dolgov, 2005; Eriksen et al., 2020) were decided to be pelagic or benthic based on SeaLifeBase and FishBase (Froese & Pauly, 2022; Palomares & Pauly, 2022). Arctic skate consumed more pelagic prey items than spinytail skate and thorny skate. Some of the most important prey species of Arctic skate were Atlantic gonate squid, Polar cod, crimson pasiphaeid *(Pasiphaea tarda),* lumpfish, and northern krill, all being classified as pelagic species (Froese & Pauly, 2022; Palomares & Pauly, 2022). Other pelagic prey species of Arctic skate were herring, capelin, and blue whiting. This might be fishery waste (Byrkjedal et al., 2015), but the location where the Arctic skate were caught had no fishery activity in 2019–2021 according to Barentswatch (barentswatch.no), indicating that herring, capelin and blue whiting were not fishery waste. Dolgov (2005) found that less than 2 % of the diet of Arctic skate came from fishery waste while in thorny skate 35 % of the mass came from fishery waste.

The diet of Polar cod is one indication that Polar cod is a pelagic species. The main diet of polar cod are pelagic (Palomares & Pauly, 2022) crustacean groups: copepods, euphausiids and amphipods (Ajiad & Gjøsæter, 1990; Dalpadado et al., 2001). Polar cod is not a typical plankton feeder (depending on size), but mostly a consumer of large forms of plankton and ice-associated fauna (Falk-Petersen et al., 1986; Wassmann et al., 2006). Juvenile Polar cod (size range 50–70 mm) mostly feed on zooplankton including *Themisto* spp. and adult *Calanus* spp. (Pechenik et al., 1973). Adult Polar cod's (2+ years) preferred prey items are pelagic hyperiids such as the amphipod *T. libellula*, and calanoid copepods. Epibenthic crustaceans (*Mysis* spp.) are also important for adult Polar cods (Hop et al., 1997). Polar cods stay in the water column between May–September (Ponomarenko, 2000; Shleinik, 1970) and

the age-0 year-class of Polar cod are often planktonic (Baranenkova et al., 1966). After September, they gradually descend towards the seabed, where most individuals stay until March–April the following year (Ponomarenko, 2000). The Arctic skates in the study were caught between September–January when the Polar cod gradually descend from the upper layers and go down to the seabed. Small Polar cod (juveniles) stays mostly in the water column while the adult Polar cod show vertical displacement. A chaetognath, *Sagitta* sp., is only found in small polar cod (Orlova et al., 2009). *Sagitta* sp. could be secondary prey of Arctic skate in this present study, and the predator of *Sagitta* sp. could be Polar cod. By investigating the presence of Polar cod and *Sagitta* sp. in the diet of Arctic skate, it is possible to investigate if Arctic skate fed on small or large Polar cod (if *Sagitta* sp. is secondary prey of Arctic skate). In Appendix 3, Figure 24 it was investigated if polar cod was found together with *Sagitta elegans* in the diet of Arctic skate. On six occasions *S. elegans* and Polar cod were found together in the diet of these 16 Arctic skates, indicating that Arctic skate feeds in the water column.

Environmental variables

The environmental variables depth, temperature, and location strongly correlated with the differences in diet between the skates. Arctic skate were the one caught deeper, colder, and further north than the two other skates, sampled in location A and B, explaining the presence of more cold adapted species in the diet of Arctic skate (such as Polar cod) (Figure 1). Spinytail skate were found in the same locations as Arctic skate and thorny skate, explaining the mix of Arctic and boreal species (such as the Arctic species *Oithona simili* (a copepod) and the Boreal species greater eelpout). Thorny skates were collected furthest south of the three species, in location D, explaining the more boreal diet (such as haddock). Eriksen et al., (2020) saw a similar trend and could split fish (predators) in two groups: fishes of Atlantic origin feeding on copepods and euphausiids, and fishes of Arctic origin feeding on hyperiid amphipods. As a comparison, hyperiids such as *Themisto abyssorum* and *T. libellula* were mostly found in Arctic skate while *C. finmarchicus* was only found in Thorny skate. Typically, *C. finmarchicus* are found in both the Barents and Norwegian Seas, in warmer Atlantic waters (Melle et al., 2004), while *C. hyperboreus* are confined to colder waters in the western Norwegian Sea (in the North-Atlantic between Norway and Greenland) (Melle et al., 2004). So, temperature gradients make some prey species more common in specific locations, like the squid *G. steenstrupi* that was more common in location A, and haddock that was more common in location D.

Other noteworthy findings

The possibility to identify prey items in empty stomachs make metabarcoding a better method at presence/absence studies than morphological methods. With morphological methods, the prey must be seen to identify it, but when there is nothing to see, nothing can be identified. This will also apply to stomachs with content that is fully digested. This fully digested content is impossible to analyse with a morphological method, but with metabarcoding the fully digested content will still contain DNA that can be sequenced. So, molecular methods have an advantage over morphological analysis of diets because it can identify prey items for longer periods after consumption (Waraniak et al., (2019).

The presence of prey items in skates with internal yolk sac means that skates start to feed early. Early feeding and feeding before complete absorption of internal yolk sac is observed in lake trout (*Salvelinus namaycush*) (Ladago et al., 2016), brown trout (*Salmo trutta*) (Skoglund & Barlaup, 2006), and Chinese sturgeon (*Acipenser sinensis*) (Chai et al., 2011). The two most abundant prey items found in the diet of the two skates was a cnidarian *A. digitale* and an arthropod *E. inermis,* two small organisms. This complies with the earlier results that small skates feed on small prey items.

The DNA of fin whale in a single spinytail skate (AL850) is difficult to explain. Skates do not hunt for whales but could feed on a deceased ones (since skates are opportunistic predators). Fin whales' distribution extends to the Barents Sea (Christensen et al., 1992) and whale falls occur regularly in several world oceans functioning as a high source of organic matter for scavengers (Smith & Baco, 2003). That a whale fall occurred in the Barents Sea, and spinytail skate was scavenging from the carcass is plausible, since spinytail skate already eat fisheries waste (Byrkjedal et al., 2015). Other suggestions might be coprophagy, but since there are few, or none reports of coprophagy in elasmobranch species, it is highly unlikely. However, coprophagy are observed in mackerel larvae (*Scomber scombrus*), they are observed to feed on faecal pellets of crustaceans such as copepods (Conway et al., 1999) and parrotfishes (*Scarus* spp.) and surgeon fishes (*Acanthurus* spp.) are observed to eat faecal pellets (Rempel et al., 2022). Coprophagy are therefore not something to exclude. The whale DNA might also come from contaminations, but then there would be expected more whale DNA in the other samples. Also, there were no one working on fin whale tissue in the lab when the samples were prepared.

Barnacles (*Balanus balanus*) was also in the diet of several skates. The act where skates go to the shore to graze on adult barnacles is unlikely, and that skates filter feed on barnacle larvae is not likely either. *Clavella adunca* is an ectoparasite found on Atlantic cod but Atlantic cod was not in the diet of any of the skates. Finding this parasite in the diet of skates could therefore mean that it was ingested passively in the water or together with another fish. The chaetognath species *Sagitta elegans* could also have been ingested as secondary prey, since it is found in the diet of small Polar cod. The consumption of these preys could be the result of secondary predation or passive ingestion.

Cnidaria is a typically soft bodied, easily digestible prey item not observed in these three skate species earlier but are now observed in them (Appendix 3 Figure 22). Medusozoa, a clade in Cnidaria, have two life stages, one which form a free-swimming medusa and the other as polyps (Technau & Steele, 2011). Hydrozoa and Scyphozoa are two classes within Medusozoa. The cnidarians that were found in the diet of these three skate were Hydrozoa (*Bougainvillia muscus, Campanularia hincksii, Obelia dichotoma, Clytia hemisphaerica, Aglantha digitale*) and Scyphozoa (lion's mane jellyfish (*Cyanea capillata)*). All the Hydrozoans in the diet of the skates are relatively small, while the lion's mane jellyfish *C. capillata* is a large organism, up to about 2 meter in diameter in some waters. If the skates have fed on the Medusozoans in the polyp stage or the medusa stage is not possible to see from a metabarcoding study.

The amphipod *Jassa slatteryi* is a cosmopolitan species reported as cryptogenic or invasive throughout the world (Bonifazi et al., 2018). This species was filtered out from the dataset (described in the materials and methods part) since it had no known distribution in the Barents Sea, although this is a species that could be invasive in the Barents Sea.

Metabarcoding vs morphological analysis

DNA metabarcoding is a powerful tool that give far better resolution of prey items than with traditional morphological studies (Riccioni et al., 2018; Waraniak et al., 2019). With fewer samples, there were found congruent results that not only matches with the morphological studies, but also outcompetes the latter ones. Metabarcoding still follow the same trends as morphological methods when comparing the results at higher taxonomic level (Figure 14-15) (Dolgov, 2005; Eriksen et al., 2020). The relative abundance of Chordata are higher in (Dolgov, 2005) compared to (Eriksen et al., 2020) and the present studies results (Figure 14).

Phyla not observed with morphological studies but with metabarcoding were Porifera, Bryozoa and Nemertea, and classes not observed with morphological studies but with metabarcoding were Hydrozoa, Maxillopoda, Caudofoveata, Scaphopoda, Ascidiacea, Mammalia, and Echinoidea (Appendix 2, Table 8). The only phylum not observed with metabarcoding that was observed with morphological analysis was Ctenophora and classes not observed with metabarcoding that were observed with morphological analysis were Thecostraca, Elasmobranchii, Asteroidea, and Holothuroidea (Appendix 2, Table 8).

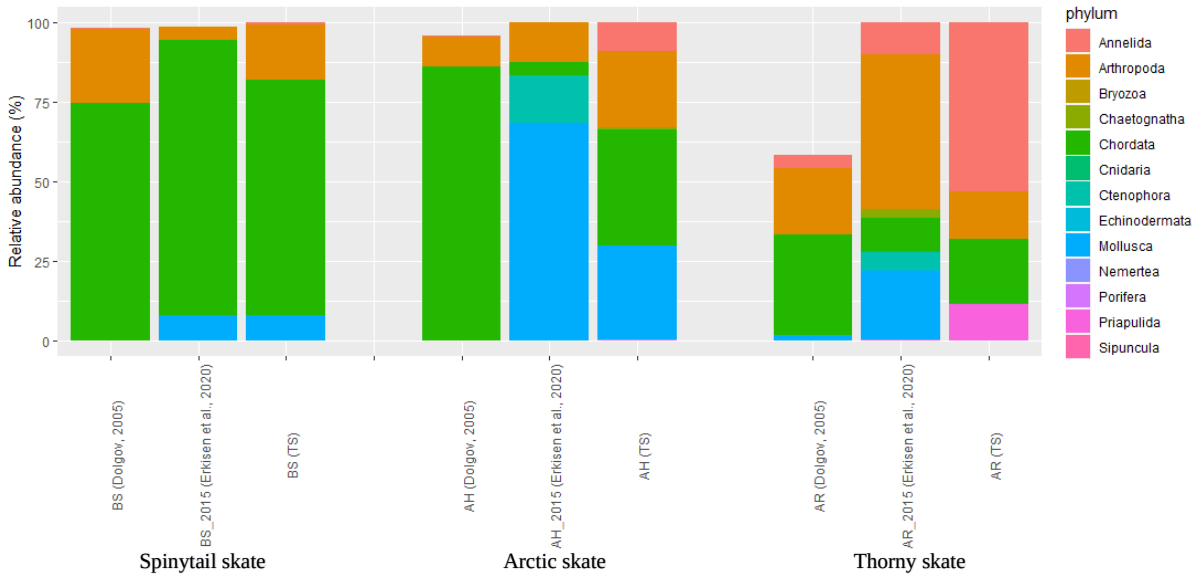

Figure 14. The relative abundance of different prey groups from three different skate species in two morphological studies (Dolgov, 2005; Eriksen et al., 2020) and one molecular study (this study (TS)).This is pooled data with all size classes. The parts of the bars that is missing is fishery waste, other food, and digested food.

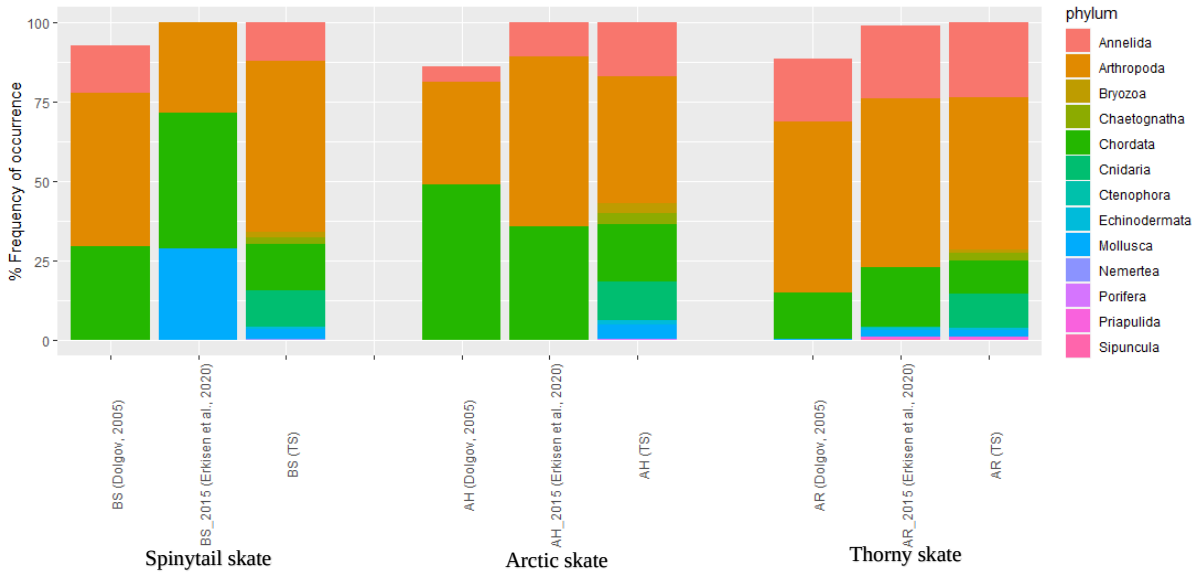

Figure 15. % Frequency of occurrence of different prey groups from three different skate species in two morphological studies (Dolgov, 2005; Eriksen et al., 2020) and one molecular study (this study (TS)).This is pooled data with all size classes. The parts of the bars that is missing is fishery waste, other food, and digested food.

With metabarcoding it is easier to identify soft bodied animals, such as Annelida, Cnidaria and Chaetognatha to species level than with morphology-based methods (Waraniak et al., 2019). With Metabarcoding 26 MOTUs of Annelida were identified to species level, while with morphological methods, only one were identified to species level. In Cnidaria six MOTUs were identified to species level, while none were found with morphological methods (Appendix 2, Table 8). These species are often overlooked or not possible to identify in morphological studies because they are digested faster than for example Arthropods. One problem with metabarcoding was that many sequences did not match with any reference database, but with more species in these databases, more prey items will be identified in the future, making metabarcoding a better and more reliable method (Andersen et al., 2019). What is not possible to determine with metabarcoding alone is what is fishery waste, and what is not, but with morphology based methods it is (Dolgov, 2005). An additional issue is host DNA. During metabarcoding, high concentration of host DNA will be dominant in samples among the targeted prey species (Tercel et al., 2021), making it impossible to determine if e.g. skate DNA comes from the host or the prey. In other words, fisheries waste and cannibalism are two issues a morphological study will handle better than metabarcoding. Coupling metabarcoding with morphological diet studies can reveal higher resolution of diet analysis, as the two methods complement each other. Several studies have shown that a combination of DNA-based and morphological analyses can provide greater resolution of diet

and trophic interactions than either approach used alone (Deagle et al., 2005; Reed et al., 1997).

Dolgov (2005) and Eriksen et al., (2020) did not try to identify every species in the diet of these skates, but rather focused on functional groups. As a result, many species were lumped together at higher taxonomic level, and only the most important species were mentioned. The present study was able to identify 112 unique species, while Dolgov (2005) mentioned 9 unique species and Eriksen et al., (2020) mentioned 47 unique species (Appendix 2, Table 8). The present study's morphological analysis only identified 5 unique species, but not much time or expertise was put into the analysis. A comparison between metabarcoding and morphology-based methods about the number of species found per skate species revealed clear differences between the methods (Figure 16-18). Metabarcoding identified 68 unique species for spinytail skate compared to 3 and 1 species (Dolgov, 2005; Eriksen et al., 2020) respectively (Figure 16). For Arctic skate, 69 different species were identified with metabarcoding, while morphological studies only found 3 and 13 (Dolgov, 2005; Eriksen et al., 2020) respectively (Figure 17). For thorny skate, 89 species were identified with metabarcoding, compared to 8 and 46 species (Dolgov, 2005; Eriksen et al., 2020) respectively (Figure 18). So, for spinytail skate, Arctic skate, and thorny skate (Appendix 2, Table 9), there was a strong difference between the number of species identified from metabarcoding and morphological diet studies respectively, where the former method yielded the largest output of species with a lower number of skates (except for spinytail skate where this study had more skates).

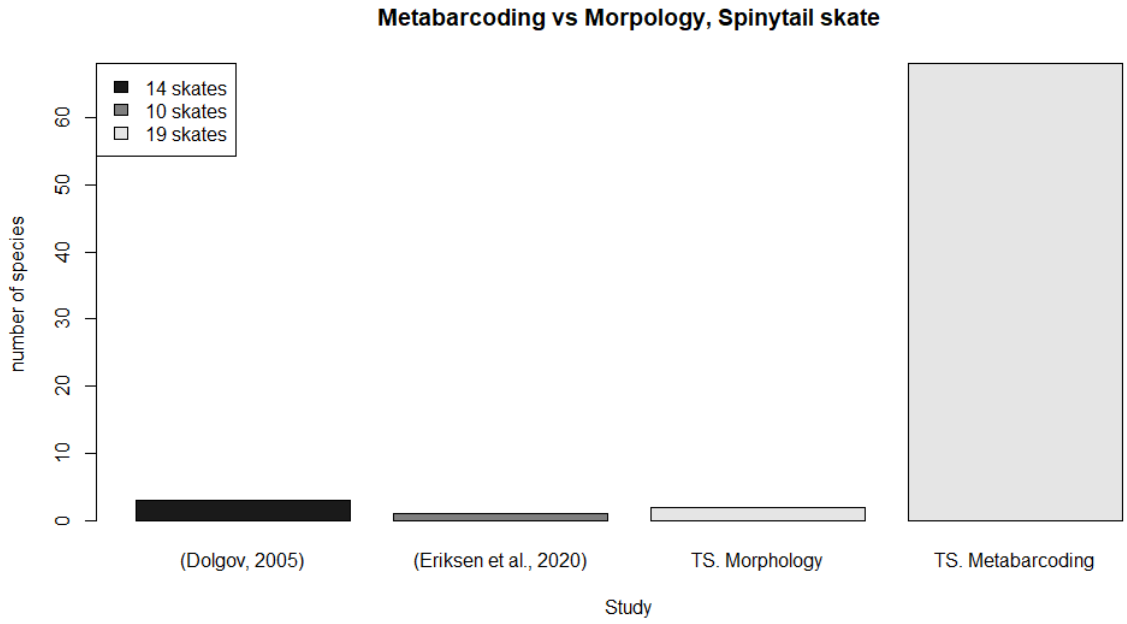

Figure 16. Number of identified species in four different studies on spinytail skate. This study (TS).

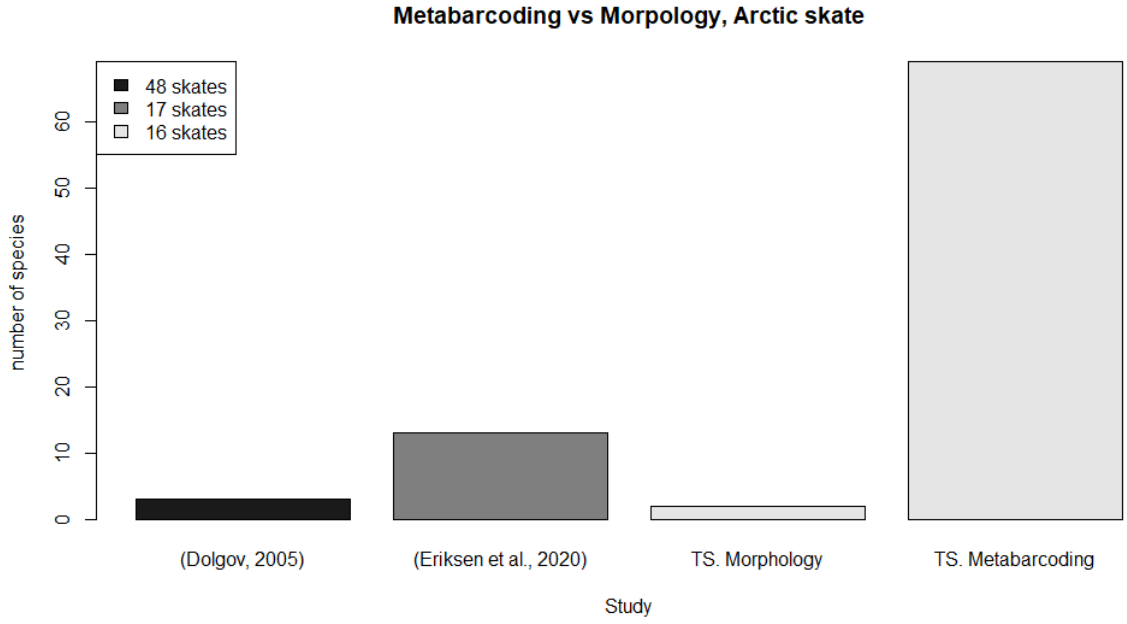

Figure 17. Number of identified species in four different studies on Arctic skate. This study (TS).

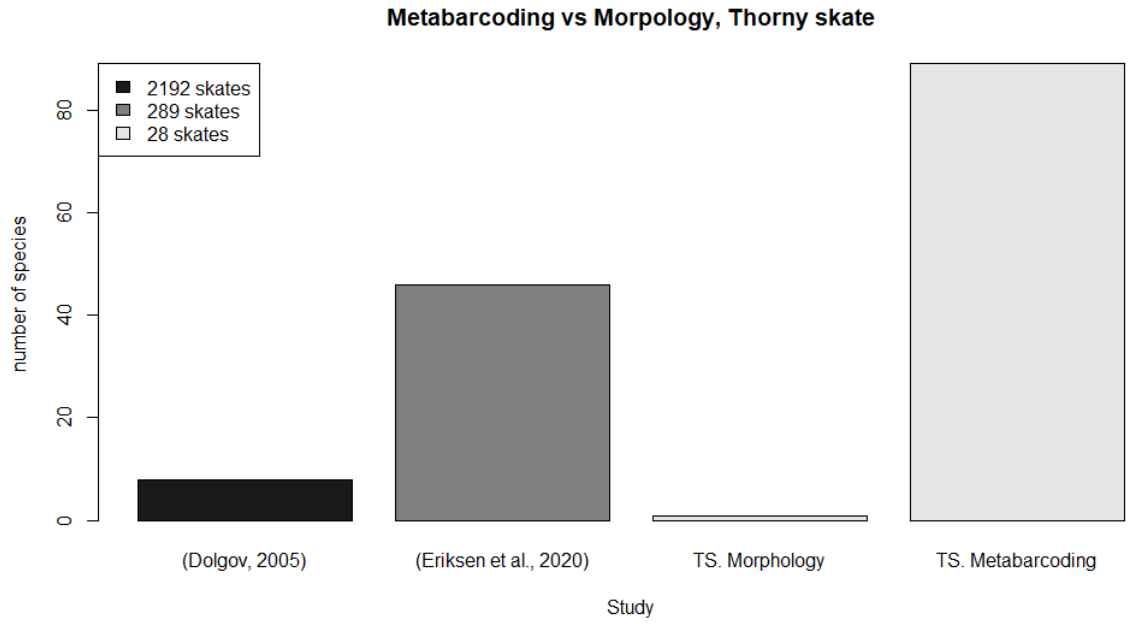

Figure 18. Number of identified species in four different studies on thorny skate. This study (TS).

Limitations of this study

Limitations of this study were the small number of samples, the possibility to assess what was secondary prey, what was passively ingested, if there was cannibalism, and if feeding during the trawl took place.

The number of samples were a limiting factor. Even with a sequencing depth that covered the full diet of each individual skate, the complete variation in diet was not covered with the small sample size of this study. This study only included 63 skates, but more than 35 skates per species would be needed to characteristic the true variation in diet. This is still a low number of samples to truly characterize the diet for each size class within each species. This study will therefore not give a definitive answer of the whole diet of these three species but might give some indications if metabarcoding is a good method to describe the diet of skates compered to morphological methods.

Secondary prey, passive ingestion, cannibalism, feeding in the trawl, DNA contaminations, and tag jumps are difficulties that can be hard to account for. Obvious MOTUs that did not fit in the diet of the skates were easily removed. Other MOTUs had to be looked up to see if they belonged to the Barents Sea. But some MOTUs are more difficult to explain. During two scientific trawls, large and small organisms were separated in different trawl bags. The skates were collected in the codend together with other organisms above ~20 cm. This might have

reduced their feeding during capture. There was no sign of freshly consumed organisms in the skate stomachs during dissection from any of the cruises.

Future perspectives

Future studies should try to find out if Arctic skate is truly a pelagic feeder, and swims actively in the water column compared to other skate species. The three skates should be captured at various locations to see if their diet vary from location to location, and all three should be captured at the same location to see if they have the same, or different diets at the same location. An extensive study that will help future researchers would be to classify what is secondary prey, and what is not in the diet of these skate species. There was a lot of MOTUs that this study did not investigate. In total after the bioinformatic pipeline, it was 897 MOTUs, and this study did only investigate 160 MOTUs. Some of this MOTUs where Amoebozoa, Archaeplastida, Chromalveolata, Excavate, Fungi, and parasites such as Nematoda and Platyhelminthes, and a lot of unidentified Eukaryotic organisms. To further investigate this huge dataset would be an interesting task, to see what sort of parasites skates have, and what sort of other organisms that is accumulated in the stomach of skates.

Conclusion

The aims of this thesis where met. 112 specific prey items within 11 different phyla were identified. The feeding ecology were compared between spinytail skate, Arctic skate, and thorny skate, revelling that spinytail skate were the most piscivores of the three species, Arctic skate had a much larger proportion of their diet that was pelagic compared to the other two skates, and thorny skate mostly fed on annelids. Ontogenetic shifts were discovered for the skates. Skates became more piscivores as they grew larger. There were no sexual differences for the three skate species. The diet varied along environmental gradients, with Arctic prey species being more common in the most northern locations, and boreal prey species being more common in the southern locations. The results were compared with traditional morphology-based diet studies, revelling that metabarcoding detected a wider spectre of prey items compared to morphology-based methods. Metabarcoding was also able to detect soft-bodied animals to a greater extent than morphology-based methods. Some limitations of metabarcoding, that morphology-based methods did not have, was the ability to detect cannibalism and fisheries waste. This makes coupling of metabarcoding and morphology-based methods a better method capable to detect an even broader range of prey items than any of the two methods alone.

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Appendixes

Innhold

Table list

Figure list

1 Metadata

*Table 1. Metadata with station data, skate identification (ID), skate species, skate sex, skate total length (mm), and skate total weight (g). *The identification "AR026" were given to two individuals of the same species, sex, size group and from the same station with a mistake. In the analysis, they were combined as one skate, and the reads were divided on two to be able to compare with other skates. It was better to keep AR026 than to remove it from the analysis since the number of skates already were limiting.*

Ship	Cruise	Date	Stasjon	Serial	Lat	Lon	Depth (m)	Temp (°C)	ID	Species	Sex	Length (mm)	Weight (g)
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	AH028	Arctic skate	f	230	112
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	AH029	Arctic skate	f	185	60
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	AH030	Arctic skate	f	225	100
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	AH031	Arctic skate	m	345	345
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	AH032	Arctic skate	m	157	45
KPH		05.10.2020	2103	2103	80.7789198	3.583316	855	-0,22	AH033	Arctic skate	m	605	2744
KPH		28.09.2020		2062	80.98517	25.457333	188	0,36	AH034	Arctic skate	m	605	2404
KPH		2020		2129	79.94555	6.004825	916	-0,79	AH035	Arctic skate	m	604	2305
KPH	Økosystem	27.09.2020		2057	81.56183	32.366333	824	0,17	AH036	Arctic skate	f	270	184
KPH	Økosystem	27.09.2020		2057	81.56183	32.366333	824	0,17	AH037	Arctic skate	m	230	111
KPH		03.10.2020		2095	81.39217	6.538	784	-0,30	AH038	Arctic skate	f	455	706
KPH		03.10.2020		2095	81.39217	6.538	784	-0,30	AH039	Arctic skate	f	157	56
KPH		03.10.2020		2095	81.39217	6.538	784	-0,30	AH040	Arctic skate	m	109	45
KPH		07.10.2020		2116	80.32333	4.287	912	-0,51	AH041	Arctic skate	m	267	188
19		27.01.2019	24	70324	76.4832131	30.8434625	283		AH049	Arctic skate	f	710	3903
1173		25.01.2019	8	70308	76.0163759	35.8180506	241		AH050	Arctic skate	f	730	4084
	EggaNor2019	06.09.2019		73028	71.8081667	15.5755	724	0,20	AL838	Spinytail skate	f	1220	13000
	EggaNor2019	11.09.2019		73077	76.2306667	14.1076667	800	0,60	AL839	Spinytail skate	m	986	7000
	EggaNor2019	09.09.2019		73061	74.4185	16.2678333	519	3,40	AL841	Spinytail skate	m	995	7000
	EggaNor2019	09.09.2019		73062	74.5878333	16.0966667	586	2,90	AL843	Spinytail skate	f	325	192
	EggaNor2019	06.09.2019		73034	72.2338333	15.8105	681	2,60	AL844	Spinytail skate	f	348	231
	EggaNor2019	10.09.2019		73073	75.5623333	13.8783333	830	-0,10	AL845	Spinytail skate	m	304	154

	EggaNor2019	07.09.2019		73042	72.9393333	14.3128333	906	-0,40	AL846	Spinytail skate	f	512	740
	EggaNor2019	09.09.2019		73059	74.256	16.136	748	2,30	AL847	Spinytail skate	f	610	1205
	EggaNor2019	10.09.2019	ind nr 2	73065	74.9023333	15.4025	834	0,40	AL849	Spinytail skate	f	695	1854
	EggaNor2019	10.09.2019	ind nr 1	73065	74.9023333	15.4025	834	0,40	AL850	Spinytail skate	m	545	820
	EggaNor2019	10.09.2019	ind nr 3	73065	74.9023333	15.4025	834	0,40	AL851	Spinytail skate	m	800	2961
	EggaNor2019	09.09.2019		73061	74.4185	16.2678333	519	3,40	AL852	Spinytail skate	f	580	1066
HH	Forsøk med reketrål	13.10.2020	594		71.3533052	022.5731966	420	8,40	BS018	Spinytail skate	m	1420	16600
HH	Forsøk med reketrål	15.10.2020	613		71.7335658	032.8530201	298	8,30	BS022	Spinytail skate	f	1385	16000
G.O. Sars		20.08.2020		2557	71.575	21.0475	319	4,37	BS042	Spinytail skate	m	1310	13500
KPH		07.10.2020	2118	2118	80.1942024	5.2265788	817	-0,63	BS043	Spinytail skate	m	780	2456
G.O. Sars		19.08.2020	223	2555	71.6783367	23.038635	381	4,41	BS044	Spinytail skate	m	565	970
JH	10.10.2020	10.10.2020	55012		70.563895	30.7953967	90	8,68	BS045	Spinytail skate	m	280	127
HH	Torsketrål	13.01.2021	34		71.5056263	026.6099724	299	6,00	BS046	Spinytail skate	m	1230	11700
HH	Forsøk med reketrål	14.10.2020	605		71.8462389	033.0167527	259	7,80	AR001	Thorny skate	m	560	1629
HH	Forsøk med reketrål	14.10.2020	605		71.8462389	033.0167527	259	7,80	AR002	Thorny skate	m	510	1293
HH	Forsøk med reketrål	14.10.2020	605		71.8462389	033.0167527	259	7,80	AR003	Thorny skate	f	110	12
HH	Forsøk med reketrål	15.10.2020	611		71.7269358	032.7863711	297	8,50	AR004	Thorny skate	m	570	1751
HH	Forsøk med reketrål	15.10.2020	611		71.7269358	032.7863711	297	8,50	AR005	Thorny skate	m	590	1604
HH	Forsøk med reketrål	15.10.2020	611		71.7269358	032.7863711	297	8,50	AR006	Thorny skate	m	420	614
HH	Forsøk med reketrål	15.10.2020	609		71.7311405	033.0921926	270	7,60	AR007	Thorny skate	m	410	568
HH	Forsøk med reketrål	15.10.2020	609		71.7311405	033.0921926	270	7,60	AR008	Thorny skate	m	440	819
HH	Forsøk med reketrål	15.10.2020	609		71.7311405	033.0921926	270	7,60	AR009	Thorny skate	m	500	1232
HH	Forsøk med reketrål	15.10.2020	609		71.7311405	033.0921926	270	7,60	AR010	Thorny skate	m	130	16
HH	Forsøk med reketrål	15.10.2020	607		71.7343600	033.0921134	265	7,70	AR011	Thorny skate	m	340	345
HH	Forsøk med reketrål	15.10.2020	607		71.7343600	033.0921134	265	7,70	AR012	Thorny skate	f	470	948

HH	Forsøk med reketrål	15.10.2020	607	71.7343600	033.0921134	265	7,70	AR013	Thorny skate	f	410	648
HH	Forsøk med reketrål	15.10.2020	607	71.7343600	033.0921134	265	7,70	AR014	Thorny skate	f	400	540
HH	Forsøk med reketrål	15.10.2020	604	71.8330897	033.1723152	261	7,70	AR015	Thorny skate	f	390	604
HH	Forsøk med reketrål	18.10.2020	630	71.8118037	032.8006060	280	7,00	AR016	Thorny skate	f	570	1750
HH	Forsøk med reketrål	17.10.2020	624	71.7550272	032.5814042	298	7,40	AR017	Thorny skate	m	210	90
HH	Forsøk med reketrål	15.10.2020	612	71.7251614	032.9317132	303	8,20	AR019	Thorny skate	f	220	95
HH	Forsøk med reketrål	18.10.2020	628	71.7251614	032.9317132	296	7,80	AR020	Thorny skate	f	510	1241
HH	Forsøk med reketrål	18.10.2020	628	71.7251614	032.9317132	296	7,80	AR021	Thorny skate	f	130	19
HH	Forsøk med reketrål	15.10.2020	613	71.7335658	032.8530201	298	8,30	AR023	Thorny skate	m	150	35
HH	Forsøk med reketrål	15.10.2020	613	71.7335658	032.8530201	298	8,30	AR024	Thorny skate	f	240	122
HH	Forsøk med reketrål	15.10.2020	610	71.7282610	032.9005529	300	8,30	AR025	Thorny skate	f	560	1682
HH	Forsøk med reketrål	15.10.2020	610	71.7282610	032.9005529	300	8,30	AR026*	Thorny skate	m	220	93
HH	Forsøk med reketrål	15.10.2020	610	71.7282610	032.9005529	300	8,30	AR026*	Thorny skate	m	250	127
HH	Forsøk med reketrål	16.10.2020	615	71.7315628	033.1261866	276	7,30	AR027	Thorny skate	f	130	19
HH	Torsketrål	14.01.2021	40	71.5123627	026.6747481	312	5,90	AR047	Thorny skate	f	320	242
HH	Torsketrål	14.01.2021	40	71.5123627	026.6747481	312	5,90	AR048	Thorny skate	f	320	250

2 Tables

Table 2. Permutational analysis of variance. Measurement of the sum of squares within and between groups and F test to compare within-group to between-group variance. Skate species in compared with size classes and sex.

Permanova, Skate * Size class + Sex						
		df	**Sum of squares**	**R2**	**F**	**P**
Relative abundance	Skate	2	3.0	0.1	3.7	0.001
	Size class	1	1.1	0.04	2.7	0.001
	Sex	1	0.3	0.01	0.8	0.8
	Skate: Size class	2	1.2	0.04	1.5	0.02
	Residual	55	22.2	0.8		
	Total	61	27.9	1		
% Frequency of occurrence	Skate	2	2.3	0.1	3.9	0.001
	Size class	1	1.0	0.05	3.5	0.001
	Sex	1	0.3	0.01	0.9	0.6
	Skate: Size class	2	1.1	0.05	1.9	0.001
	Residual	55	15.7	0.8		
	Total	61	20.3	1.0		

Table 3. Permutational analysis of variance. Pairwise comparison between skates.

Permanova, Skate * Size class							
			df	**Sum of squares**	**R2**	**F**	**P**
Arctic skate v Spinytail skate	Relative abundance	Skate	1	1.5	0.1	3.8	0.001
		Size class	1	0.6	0.04	1.5	0.1
		Skate: Size class	1	0.4	0.03	0.9	0.5
		Residual	31	12.6	0.8		
		Total	34	15.2	1.0		
Arctic skate v Spinytail skate	% Frequency of occurrence	Skate	1	1.0	0.09	3.6	0.001
		Size class	1	0.7	0.06	2.5	0.001
		Skate: Size class	1	0.4	0.04	1.6	0.07
		Residual	31	8.5	0.8		
		Total	34	10.6	1.0		
Arctic skate v Thorny skate	Relative abundance	Skate	1	1.8	0.1	4.6	0.001
		Size class	1	1.0	0.05	2.6	0.001
		Skate: Size class	1	0.7	0.04	1.7	0.025
		Residual	39	15.3	0.8		
		Total	42	18.8	1.0		
Arctic skate v Thorny skate	% Frequency of occurrence	Skate	1	1.5	0.1	5.2	0.001
		Size class	1	0.9	0.07	3.3	0.001
		Skate: Size class	1	0.6	0.04	2.2	0.007
		Residual	39	11.0	0.8		
		Total	42	13.9	1.0		
Spinytail skate v	Relative abundance	Skate	1	1.2	0.06	3.0	0.001
		Size class	1	1.3	0.06	3.1	0.001

Thorny skate		Skate: Size class	1	0.7	0.03	1.6	0.03
		Residual	42	17.2	0.8		
		Total	45	20.3	1		
Spinytail skate v Thorny skate	% Frequency of occurrence	Skate	1	0.9	0.06	3.1	0.001
		Size class	1	1.0	0.06	3.2	0.001
		Skate: Size class	1	0.6	0.04	1.8	0.012
		Residual	42	12.6	0.8		
		Total	45	15.0	1.0		

Table 4. Envfit test fits environmental vectors or factors onto an ordination. Depth, temperature, and location have been tested to see if diet varies along environmental gradients.

		Relative abundance		**% Frequency of occurrence**	
		R2	**P**	**R2**	**P**
All skates	Depth	0.5	0.001	0.1	0.02
	Temperature	0.5	0.001	0.2	0.003
	Location	0.003	0.001	0.2	0.001
Arctic skate	Depth	0.2	0.5	0.08	0.7
	Temperature	0.09	0.6	0.1	0.4
	Location	0	1.0	0	1.0
Spinytail skate	Depth	0.2	0.5	0.3	0.06
	Temperature	0.09	0.6	0.1	0.4
	Location	0	1.0	0.2	0.2
Thorny skate	Depth	0.2	0.1	0.1	0.2
	Temperature	0.02	0.8	0.06	0.5
	Location	0	1.0	0	1.0

Table 5. Pelagic prey items in the diet of spinytail skate, Arctic skate,and thorny skate.

Pelagic prey items			
Phylum	**Species**		
Cnidaria	*Aglantha digitale*	*Campanularia hincksii*	*Cyanea capillata*
Arthropoda	*Apherusa glacialis*	*Pseudocalanus acuspes*	*Pasiphaea tarda*
	Arrhis phyllonyx	*Gaetanus tenuispinus*	*Pseudocalanus minutus*
	Boreomysis arctica	*Hymenodora glacialis*	*Scolecithricella minor*
	Boreomysis nobilis	*Meganyctiphanes norvegica*	*Themisto abyssorum*
	Boroecia maxima	*Metridia longa*	*Themisto libellula*
	Calanus hyperboreus	*Microcalanus pusillus*	*Thysanoessa inermis*
	Centropages hamatus	*Oithona similis*	*Thysanoessa longicaudata*
	Chiridius gracilis		
Chaetognatha	*Pseudosagitta maxima*	*Sagitta elegans*	
Mollusca	*Gonatus steenstrupi*		
Chordata	*Boreogadus saida*	*Cyclopterus lumpus*	*Mallotus villosus*
	Clupea harengus		

Table 6. Benthic prey items in the diet of spinytail skate, Arctic skate,and thorny skate.

Benthic prey items			
Phylum	**Species**		
Cnidaria	*Bougainvillia muscus*	*Clytia hemisphaerica*	*Obelia dichotoma*
Priapulida	*Priapulus caudatus*		
Arthropoda	*Aoroides exilis*	*Idotea pelagica*	*Paramphithoe hystrix*
	Anonyx compactus	*Ilyarachna hirticeps*	*Pardalisca abyssi*
	Bathymedon obtusifrons	*Lebbeus polaris*	*Paroediceros curvirostratus*
	Bythocaris irene	*Leucon nathorsti*	*Pontophilus norvegicus*
	Caprella equilibra	*Maera loveni*	*Rhachotropis lomonosovi*
	Chionoecetes opilio	*Monocorophium acherusicum*	*Rostroculodes borealis*
	Cleippides quadricuspis	*Monoculodes packardi*	*Sabinea sarsi*
	Crangon allmanni	*Munnopsis typica*	*Sabinea septemcarinata*
	Diastylis goodsiri	*Mysideis insignis*	*Saduria entomon*
	Dulichia tuberculata	*Neopleustes pulchellus*	*Saduria sabini*
	Erythrops glacialis	*Onisimus litoralis JMG02*	*Schisturella pulchra*
	Eurythenes gryllus	*Pagurus bernhardus*	*Sclerocrangon ferox*
	Eusirus holmi	*Pagurus pubescens*	*Stegocephalus inflatus*
	Gitanopsis bispinosa	*Pandalus borealis*	*Syrrhoe crenulata*
	Halirages fulvocinctus	*Pandalus montagui*	*Tmetonyx cicada*
	Hyas coarctatus		
Annelida	*Aglaophamus malmgreni*	*Galathowenia oculata*	*Nephtys ciliata*
	Brada inhabilis	*Gyptis golikovi*	*Nothria conchylega CMC02*
	Bylgides groenlandicus	*Harmothoe globifera*	*Ophelina acuminata*
	Chaetozone setosa	*Harmothoe sp. CMC01*	*Pholoe baltica*
	Chirimia biceps	*Hydroides elegans*	*Polycirrus arcticus*
	Chone infundibuliformis	*Laonice cirrata*	*Prionospio cirrifera*
	Eunoe nodosa CMC01	*Lysippe labiata*	

		Nephtys caeca	
Sipuncula	*Golfingia margaritacea*		
Mollusca	*Arctinula greenlandica*	*Cryptonatica affinis*	*Siphonodentalium lobatum*
	Bathypolypus arcticus	*Laona finmarchica*	*Yoldiella frigida*
	Chaetoderma nitidulum	*Limacia clavigera*	
Nemertea	*Micrura varicolor*		
Chordata	*Cottunculus microps*	*Leptagonus decagonus*	*Microstomus kitt*
	Diplosoma listerianum	*Leptoclinus maculatus*	*Paraliparis bathybius*
	Gadus morhua	*Lumpenus lampretaeformis*	*Reinhardtius hippoglossoides*
	Gaidropsarus argentatus	*Lycenchelys muraena*	*Sebastes mentella*
	Hippoglossoides platessoides	*Lycodes esmarkii*	*Triglops murrayi*
	Hippoglossus hippoglossus	*Lycodes pallidus*	*Trisopterus esmarkii*
		Melanogrammus aeglefinus	
Echinodermata	*Brisaster fragilis*	*Ophiocten gracilis*	*Ophiopholis aculeata*
	Crossaster papposus	*Ophiocten sericeum*	

Table 7. Prey items with unkown distribution or with both pelagic and benthic distribution in the diet of spinytail skate, Arctic skate,and thorny skate.

Pelagic and/or benthic or unknown prey items			
Phylum	**Species**		
Arthropoda	*Amphilochus sp1Pans*	*Gammaridae sp. KML 32*	*Microphasma agassizi*
	Balanus balanus	*Halirages qvadridentatus*	*Munnopsurus giganteus*
	Clavella adunca	*Ilyarachna torleivi*	*Rhachotropis macropus*
	Eurycope inermis	*Liljeborgia fissicornis*	*Zygomolgus dentatus*
Annelida	*Abyssoninoe scopa*	*Laonice sp. DZMB-HH-57467.65*	*Phyllodoce sp. CMC01*
	Austrolaenilla mollis	*Lumbrineris sp. CMC01*	*SigalionidaeGEN sp. MC*
	Cossura pygodactilata		
Chordata	*Ammodytes marinus*	*Balaenoptera physalus*	*Micromesistius poutassou*

Table 8. Metabarcoding vs. morphology study on diet. X = phylum/class is present.

		Morphology			Metabarcoding
		(Dolgov, 2005)	(Eriksen et al., 2020)	This study	This study
Phylum	**Class**	**Species**	**Species**	**Species**	**Species**
Porifera	1				x
	Demospongiae				x
Ctenophora	1		x		
	Tentaculata		x		
Cnidaria	3		x		6
	Hydrozoa				5
	Scyphozoa		x		1
	Anthozoa		x		x
Priapulida	1		1		1
	Priapulomorpha		1		1
Arthropoda	5	1	22	4	57
	Ostracoda		x		1
	Hexanauplia		1		2
	Maxillopoda				8
	Malacostraca	1	21	4	46
	Thecostraca		x		
Chaetognatha	1		x		1
	Sagittoidea		x		1
Annelida	1	x	1	x	26
	Polychaeta	x	1		26
Sipuncula	1		1		1
	Sipunculidea		1		1
Mollusca	5	x	4	x	4
	Caudofoveata				1

	Bivalvia		1		1
	Scaphopoda				1
	Gastropoda		3		x
	Cephalopoda	x	x	x	1
Bryozoa	1				x
	Gymnolaemata				x
Nemertea	1				x
	Anopla				x
Chordata	4	8	16	1	14
	Ascidiacea				1
	Elasmobranchii	x			
	Actinopterygii	8	16	1	12
	Mammalia				1
Echinodermata	4	x	3		2
	Asteroidea		1		
	Ophiuroidea		2		1
	Echinoidea				1
	Holothuroidea		x		
Sum		9	47	5	112

Table 9. Morphological analysis compared to metabarcoding.

		(Dolgov, 2005)	(Eriksen et al., 2020)	This study. Morphological analysis	This study. Metabarcoding
Arctic skate	Number of skates in analysis	48	17	16	16
	Number MOTUs/OTUs	7	28	8	95
	Number of identified species	3	13	2	69
Spinytail skate	Number of skates in analysis	14	10	19	19
	Number of MOTUs/OTUs	9	5	8	96
	Number of identified species	3	1	2	68
Thorny skate	Number of skates in analysis	2192	289	28	28
	Number of MOTUs/OTUs	17	122	8	121
	Number of identified species	8	46	1	89

3 Figures

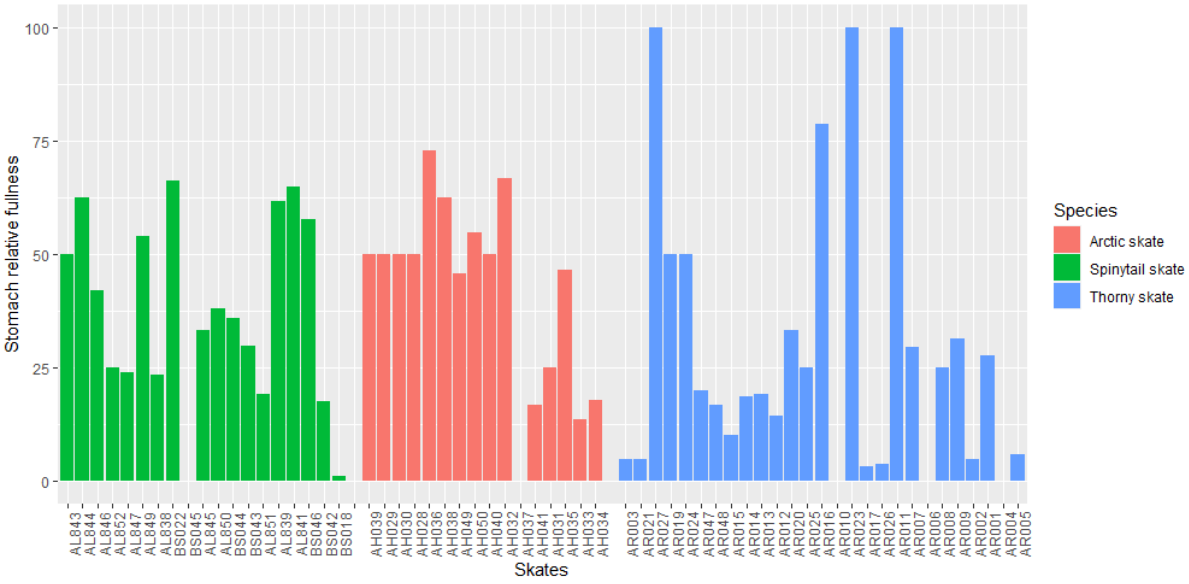

Figure 1. The fullness of stomachs of Arctic skate (red) spinytail skate (green) and thorny skate (blue).

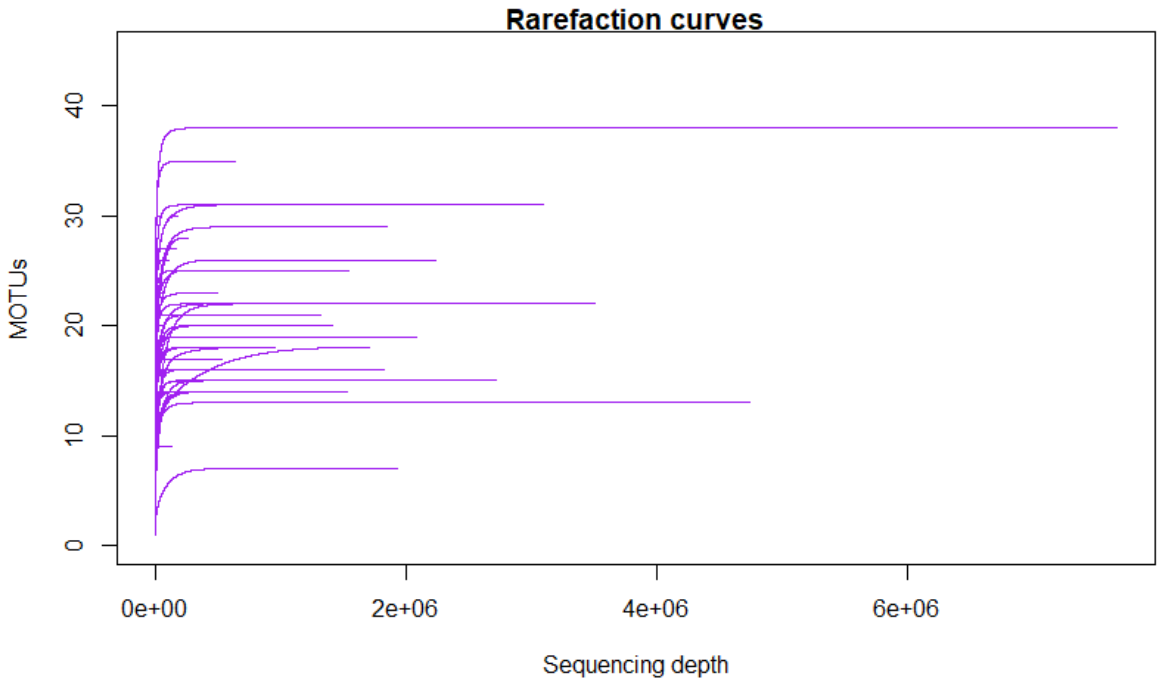

Figure 2. Rarefaction curve of the three skate species.

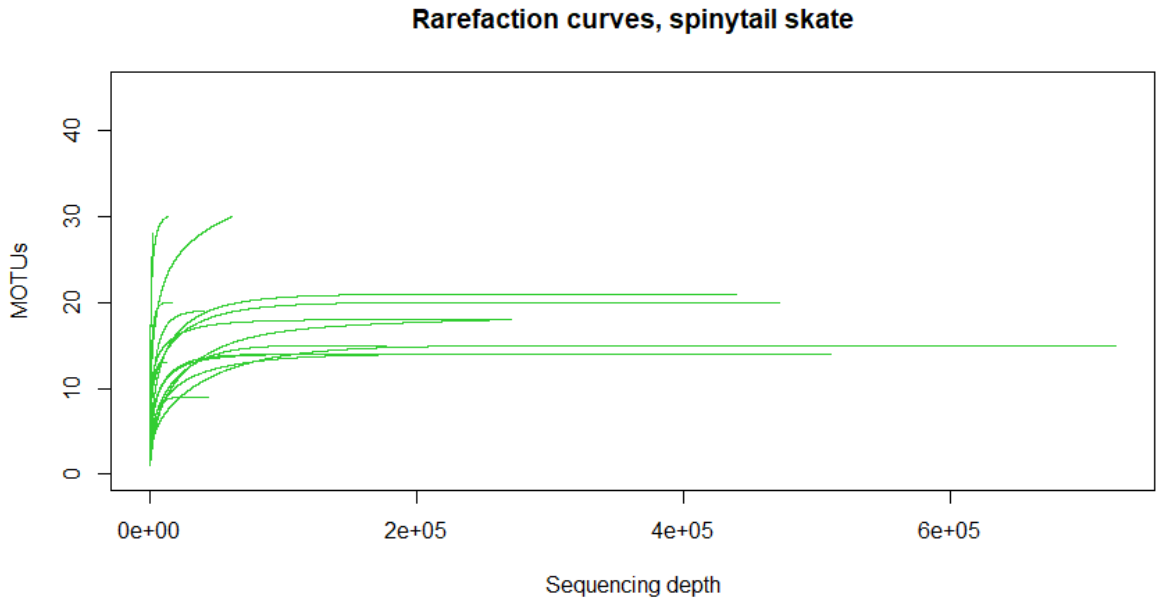

Figure 3. Rarefaction curve of spinytail skate.

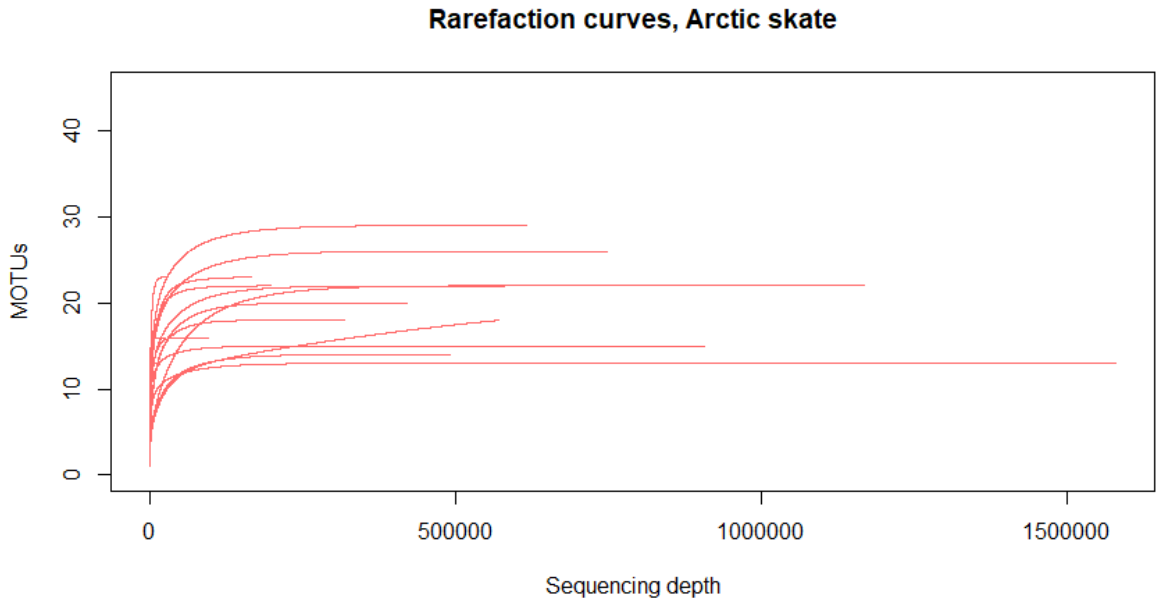

Figure 4. Rarefaction curve of Arctic skate.

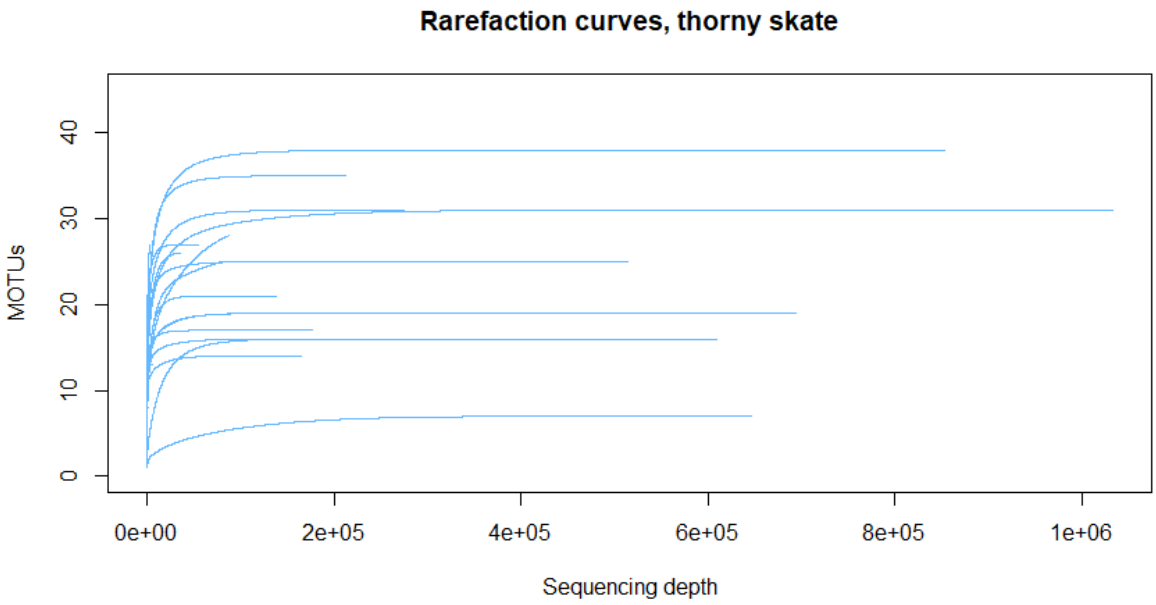

Figure 5. Rarefaction curve of thorny skate.

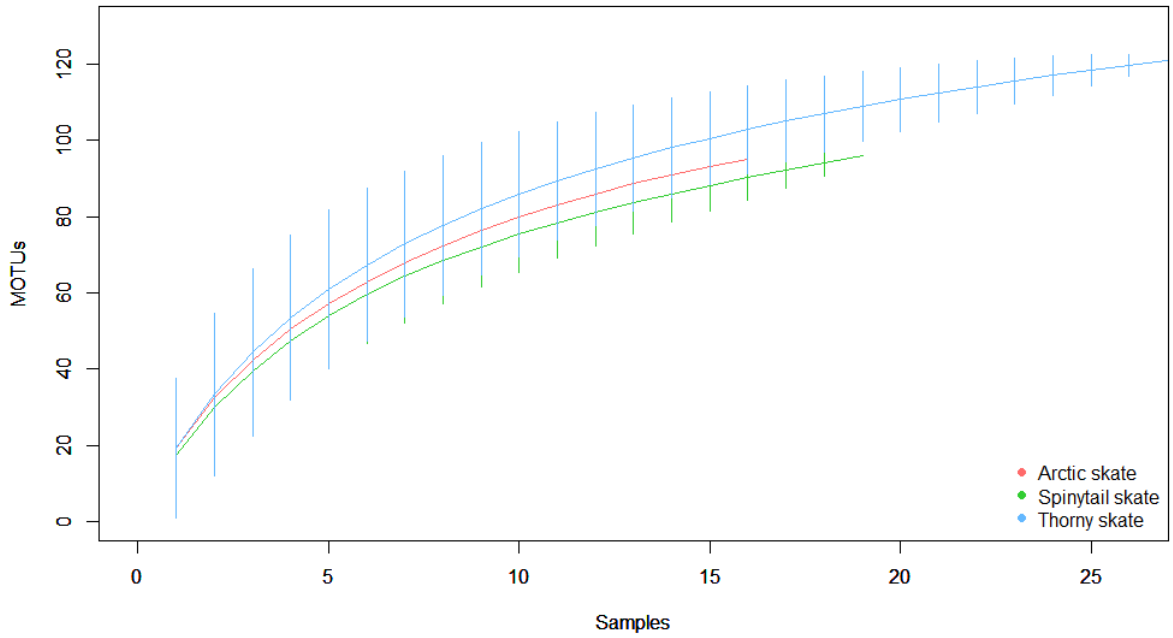

Figure 6. Accumulation curve of the three skate species.

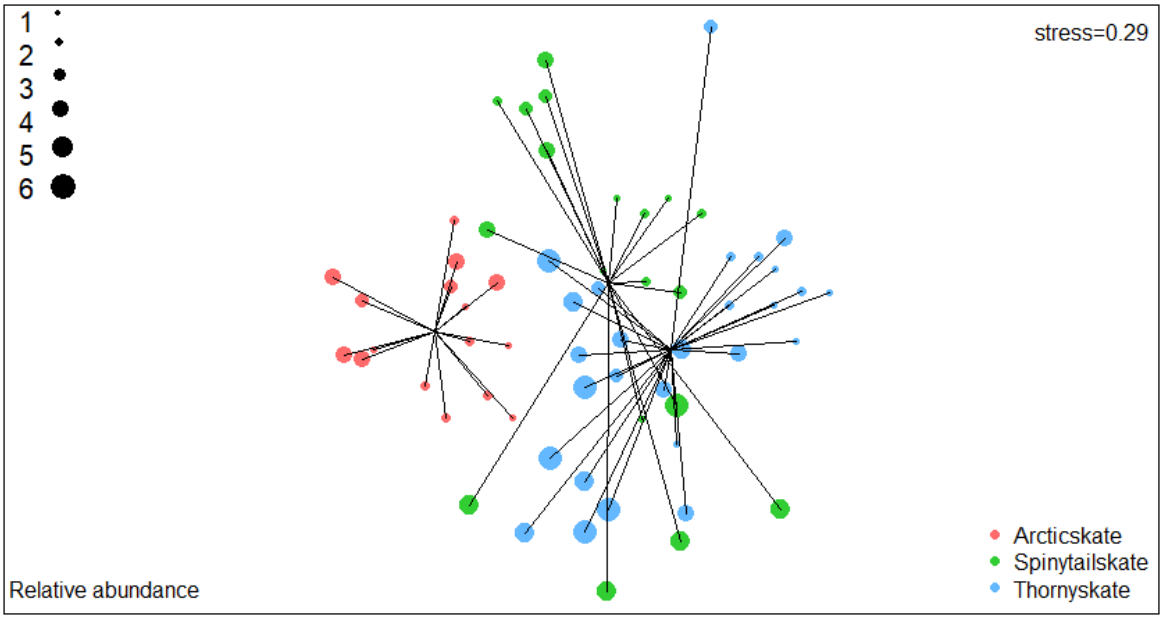

Figure 7. nMDS plot. Relative abundance of prey items. Distances between skate individuals and species and size classes.

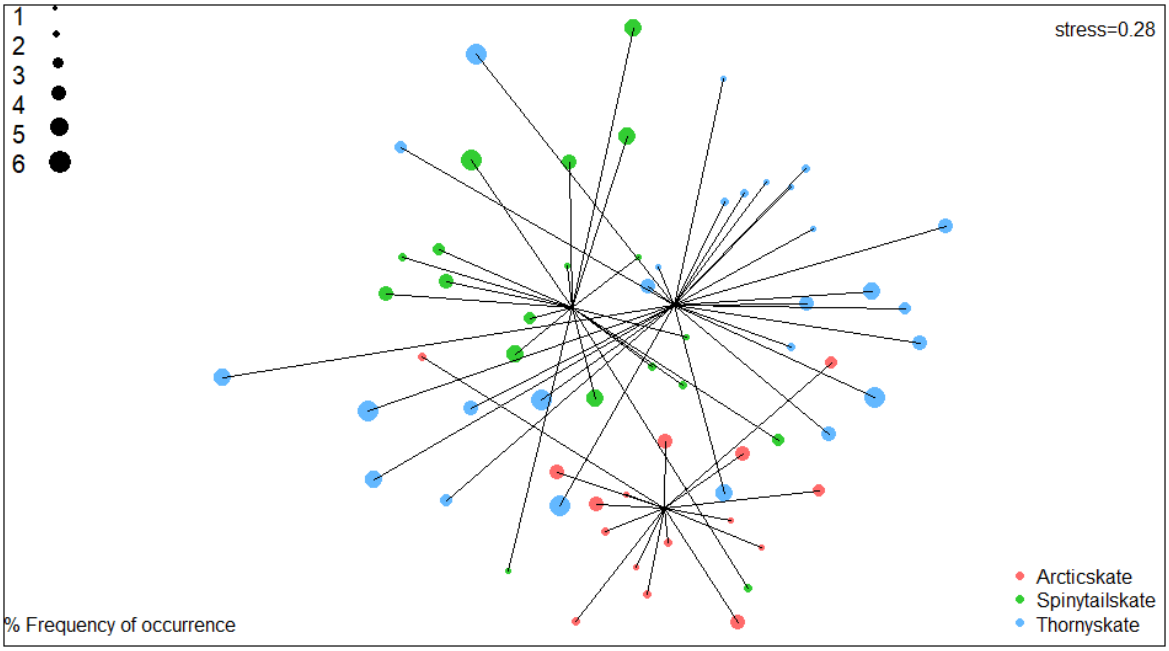

Figure 8. nMDS plot. % Frequency of occurrence of prey items. Distances between skate individuals and species and size classes.

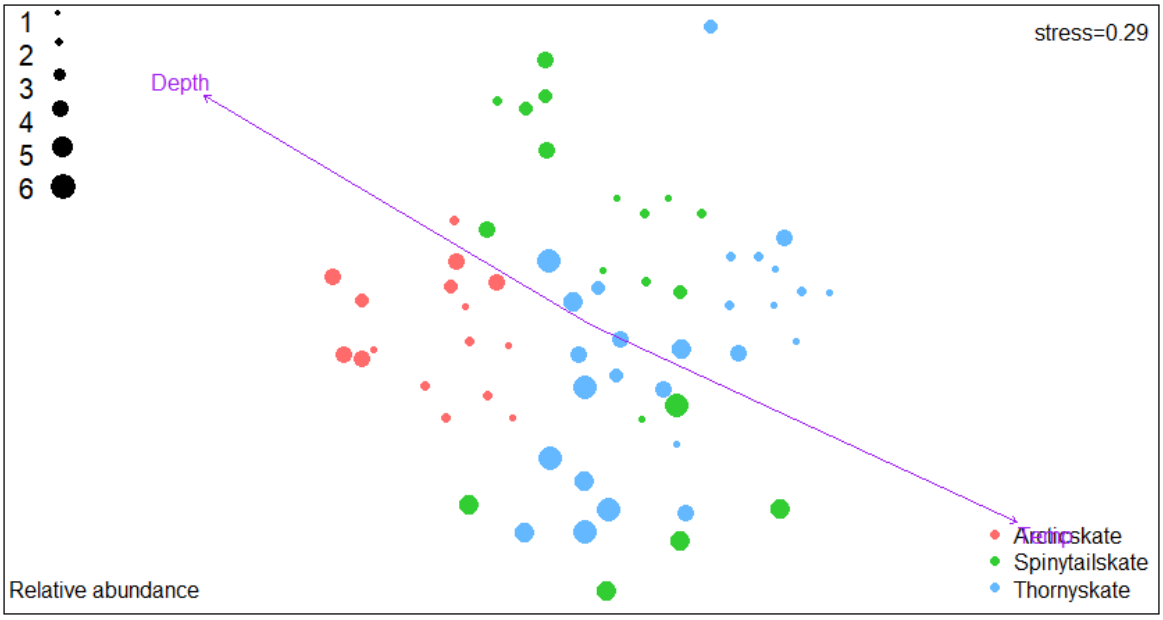

Figure 9. nMDS plot. Relative abundance of prey items. The vectors show how depth and temperature influence the diet of Arctic skate, spinytail skate and thorny skate.

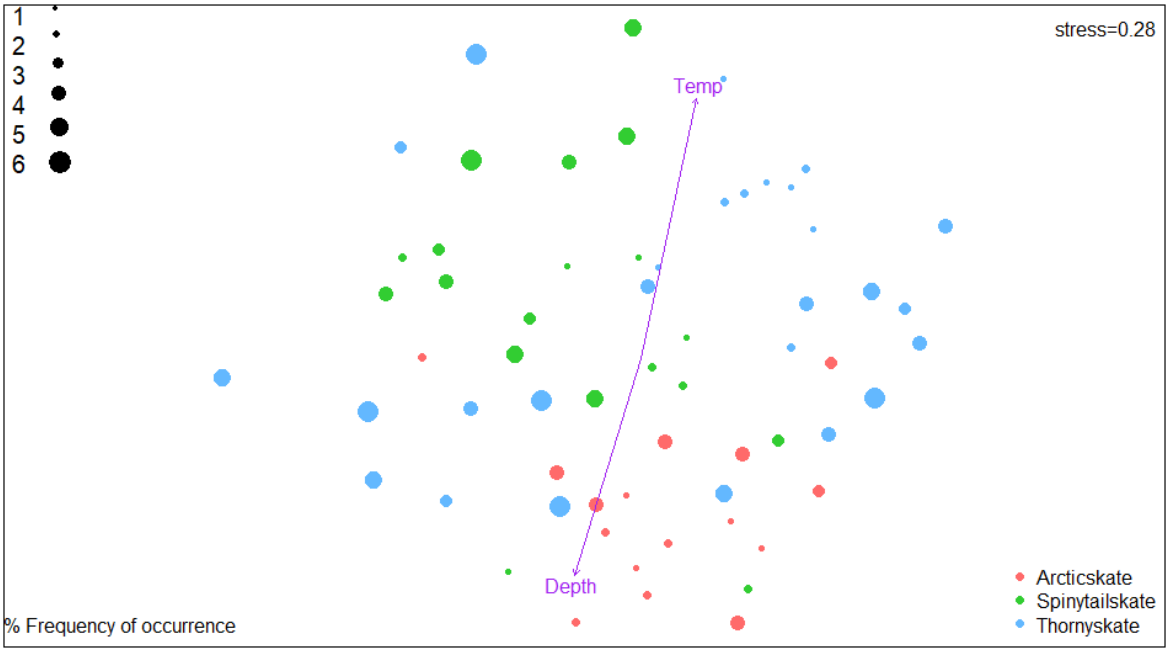

Figure 10. nMDS plot. % Frequency of occurrence of prey items. The vectors show how depth and temperature influence the diet of Arctic skate, spinytail skate and thorny skate.

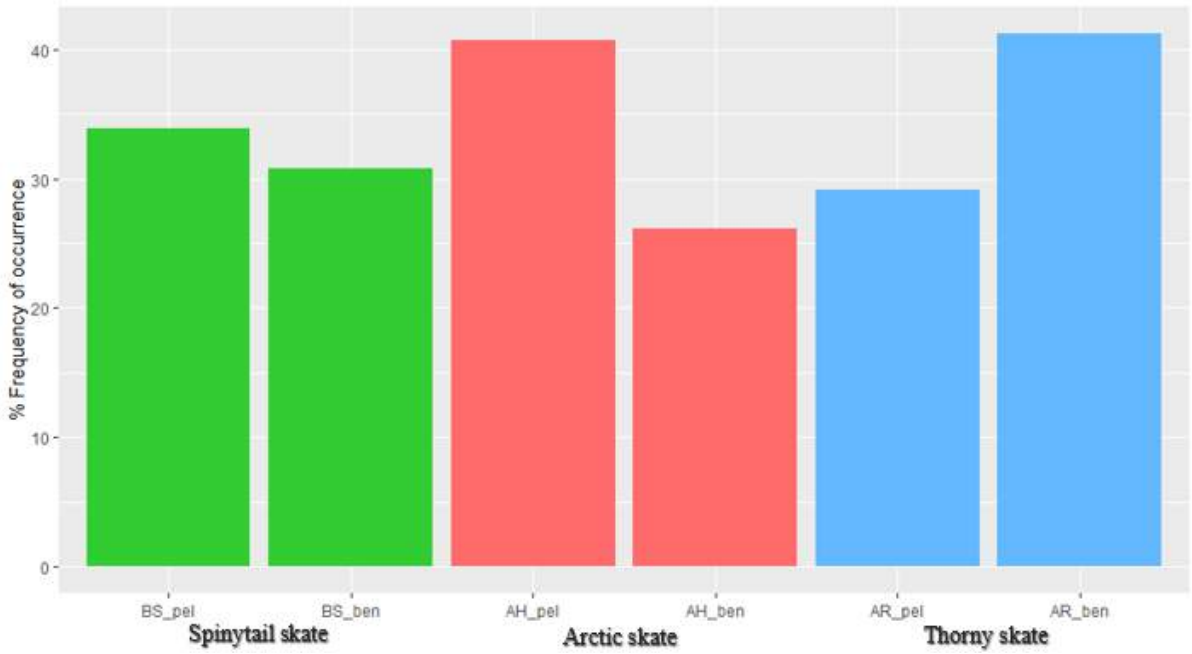

Figure 11. % Frequency of occurrence of pelagic (pel) and benthic (ben) prey items per skate species. Spinytail skate (BS), Arctic skate (AH), and thorny skate (AR).

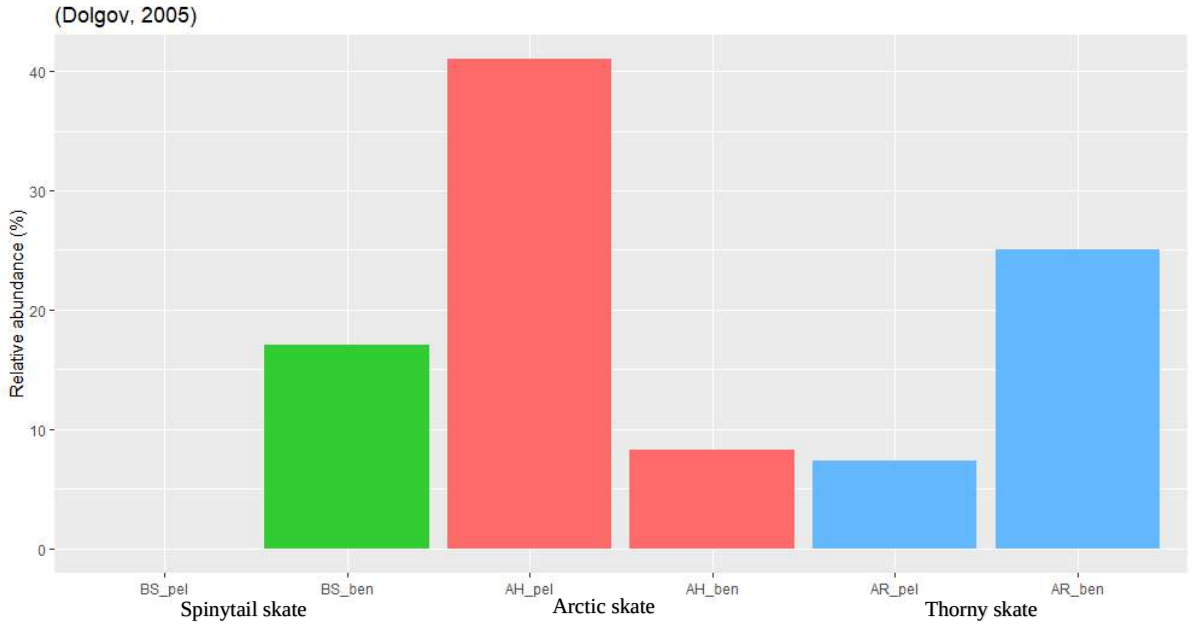

Figure 12. Relative abundance of pelagic and benthic prey items.

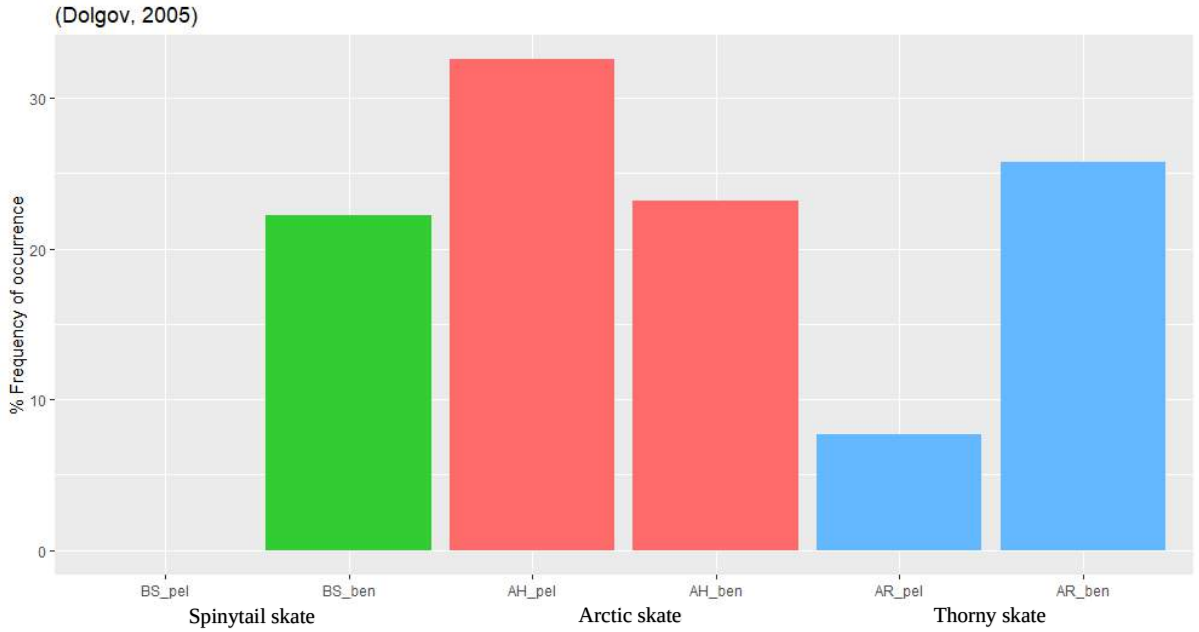

Figure 13. % Frequency of occurrence of pelagic and benthic prey items.

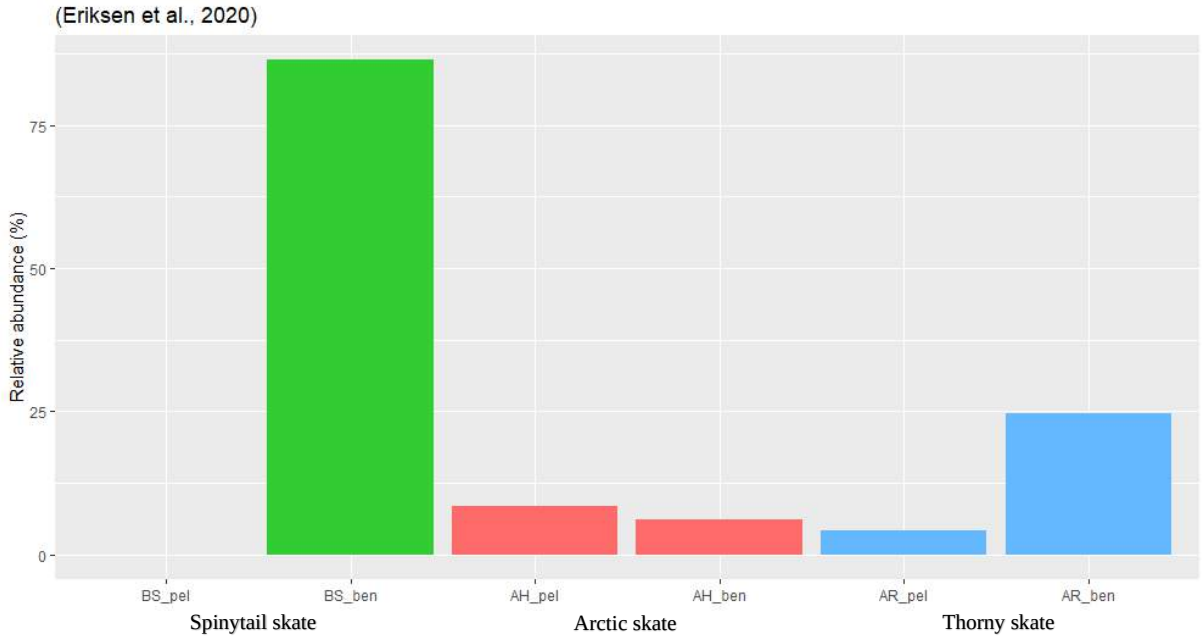

Figure 14. Relative abundance of pelagic and benthic prey items.

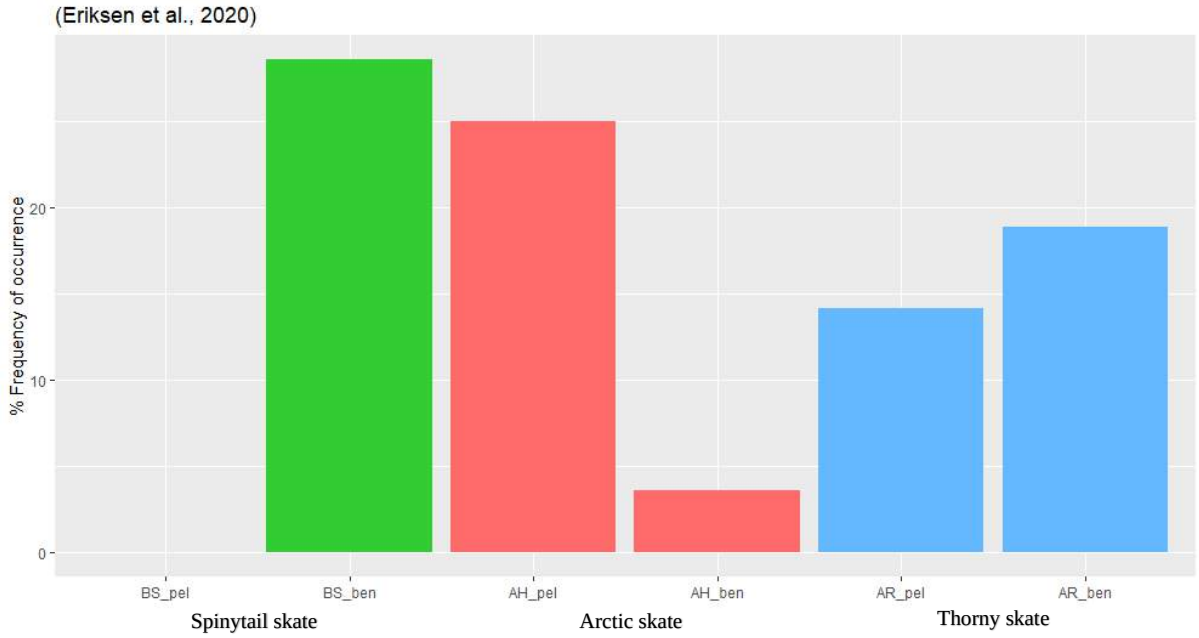

Figure 15. % Frequency of occurrence of pelagic and benthic prey items.

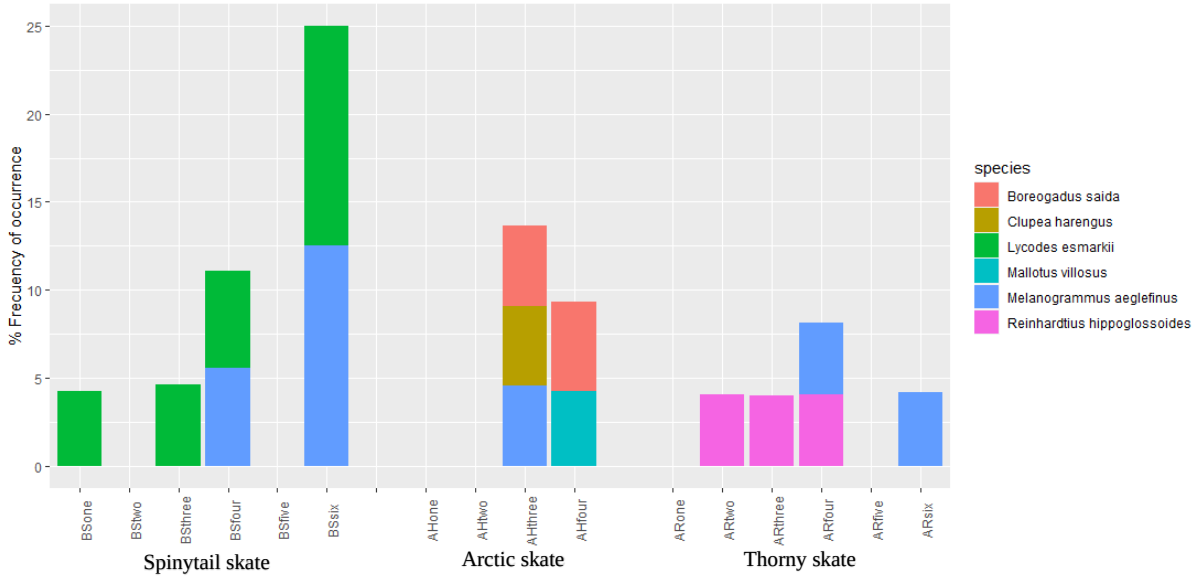

Figure 16. % Frequency of occurrence of most important (>4%) species of Chordata for different size classes of skates. This are filtered data where prey species with a % frequency of occurrence below 4 % are filtered out.

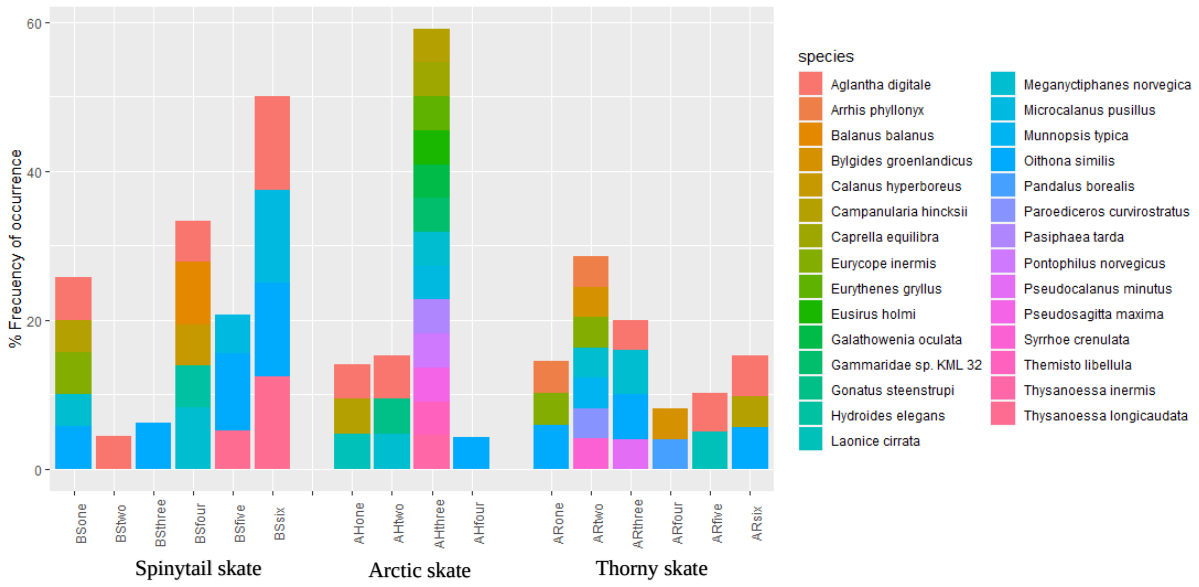

Figure 17. % Frequency of occurrence of most important (>4%) species of other classes than Chordata for different size classes of skates. This are filtered data where prey species with a % frequency of occurrence below 4 % are filtered out.

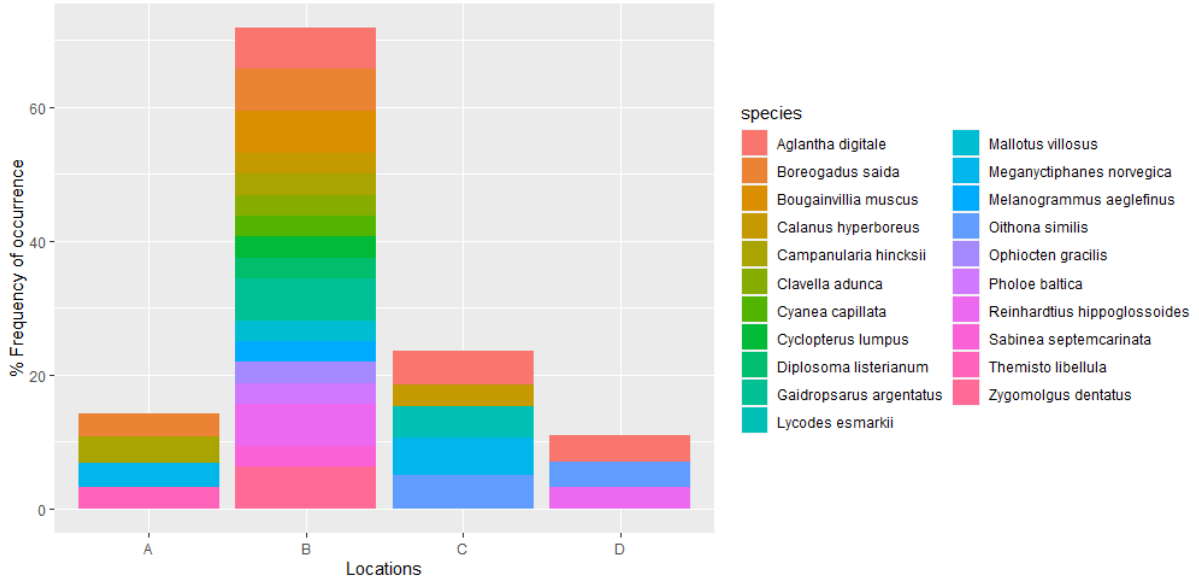

Figure 18. % Frequency of occurrence of most important prey items at species level (>3%, filtered data) at the various locations. The various locations are described in the master's thesis Figure 1. This are filtered data where prey species with a % frequency of occurrence below 3 % are filtered out.

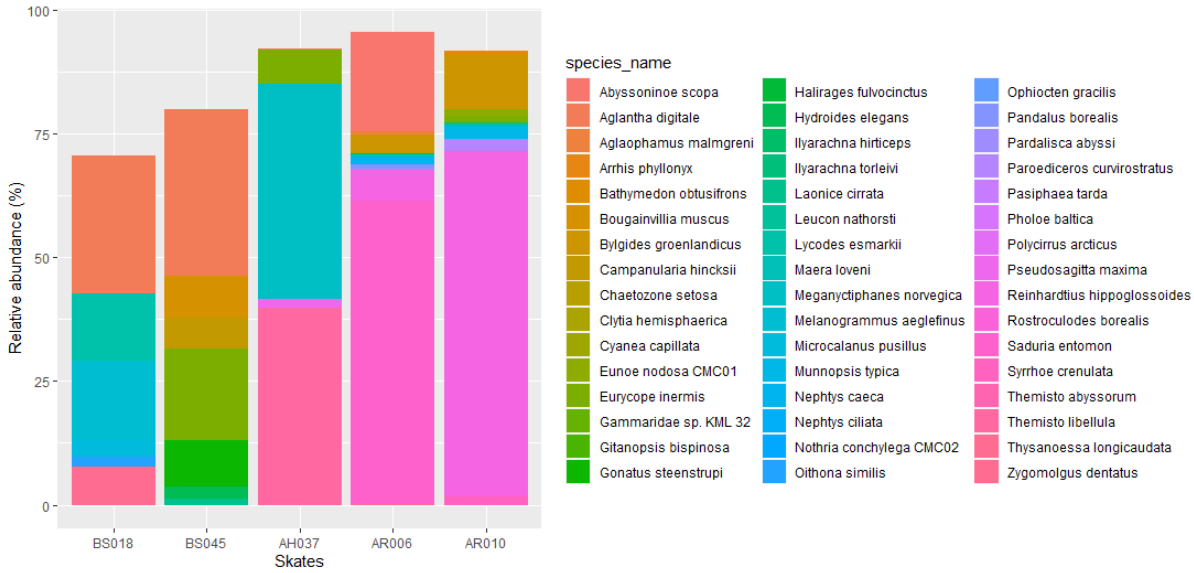

Figure 19. The relative abundance of prey items from skates with empty stomachs.

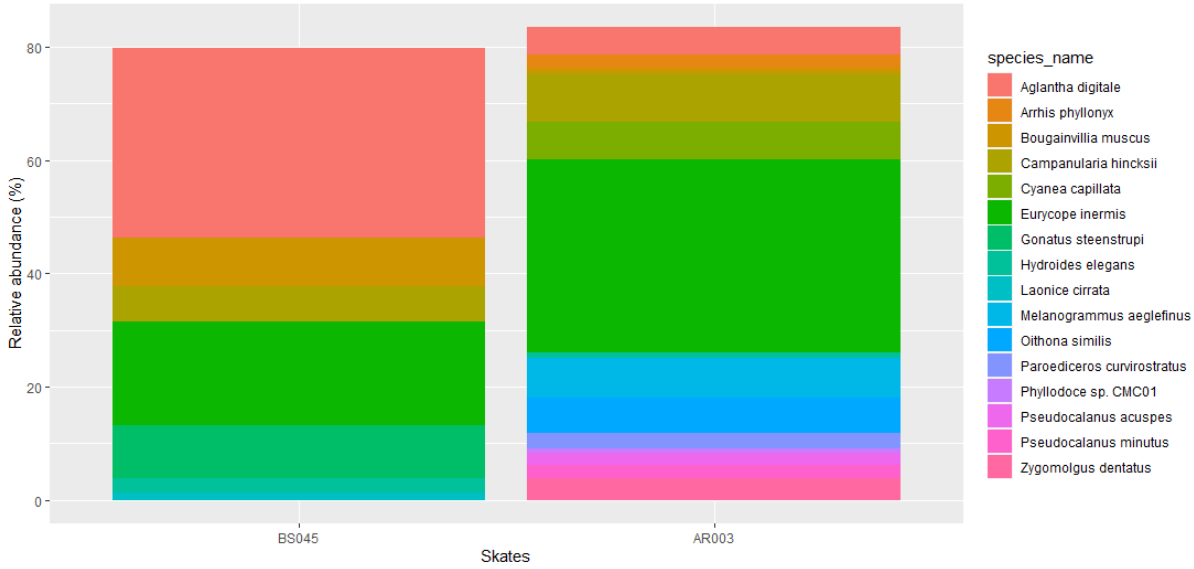

Figure 20. The relative abundance of prey items from skates with internal yolk sac.

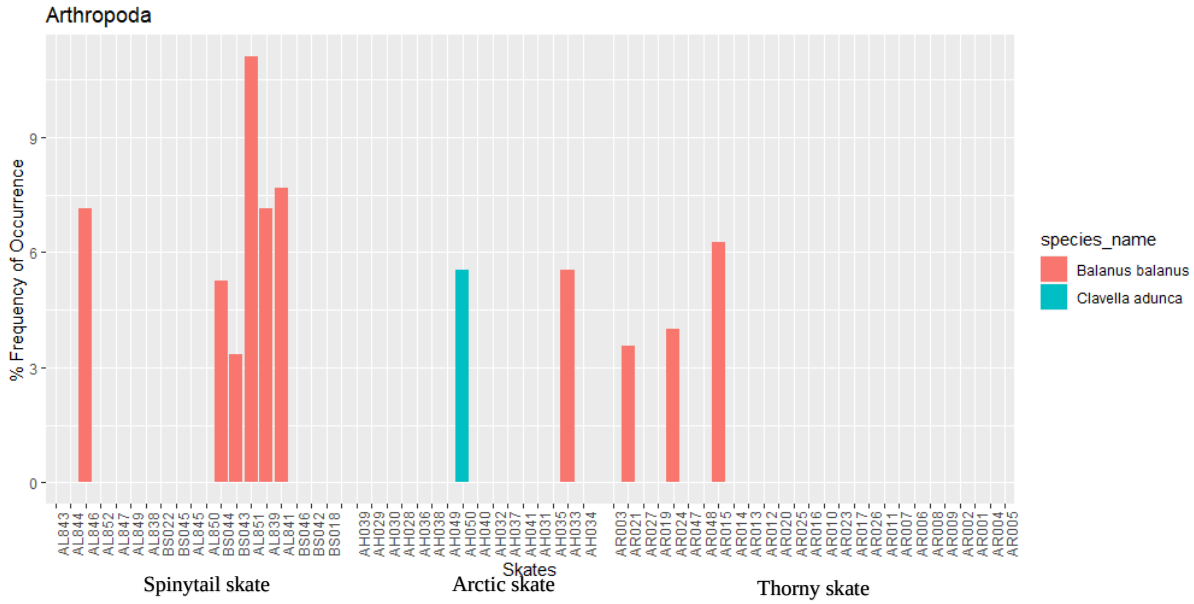

Figure 21. Two arthropod species with unknown origin found in skates.

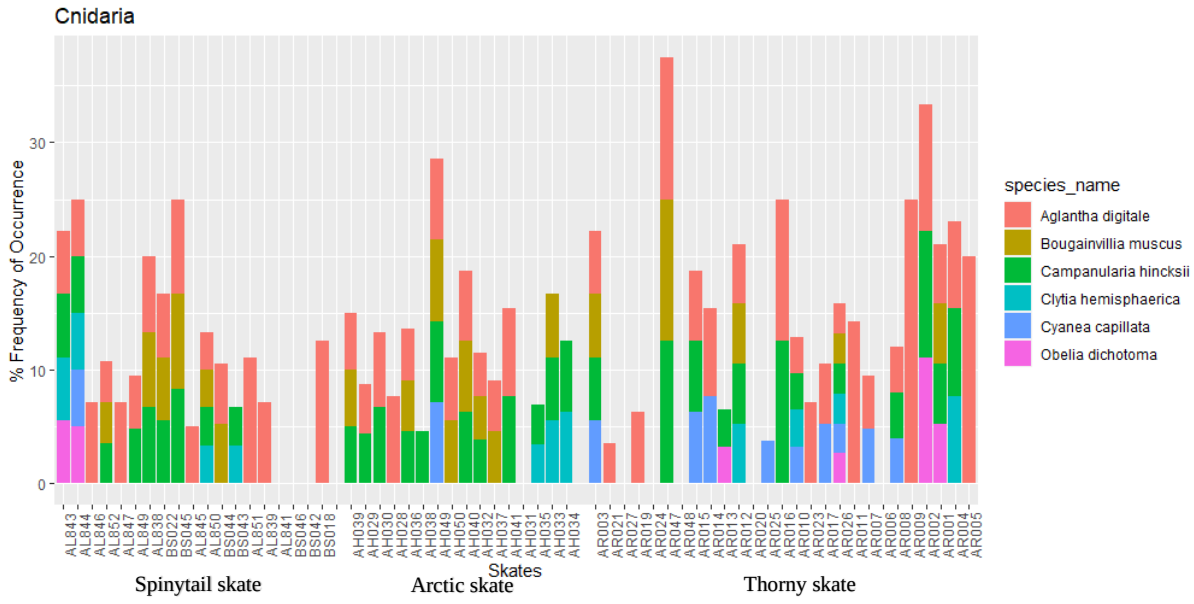

Figure 22. the presence of Cnidaria in skates.

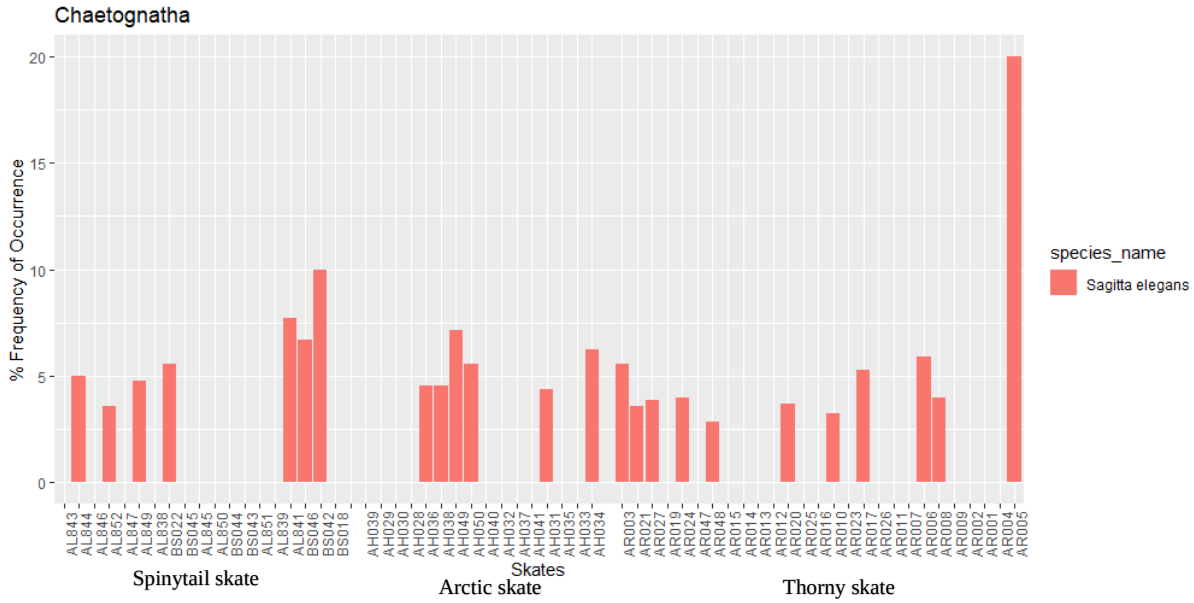

Figure 23. The presence of a chaetognath species in several skates.

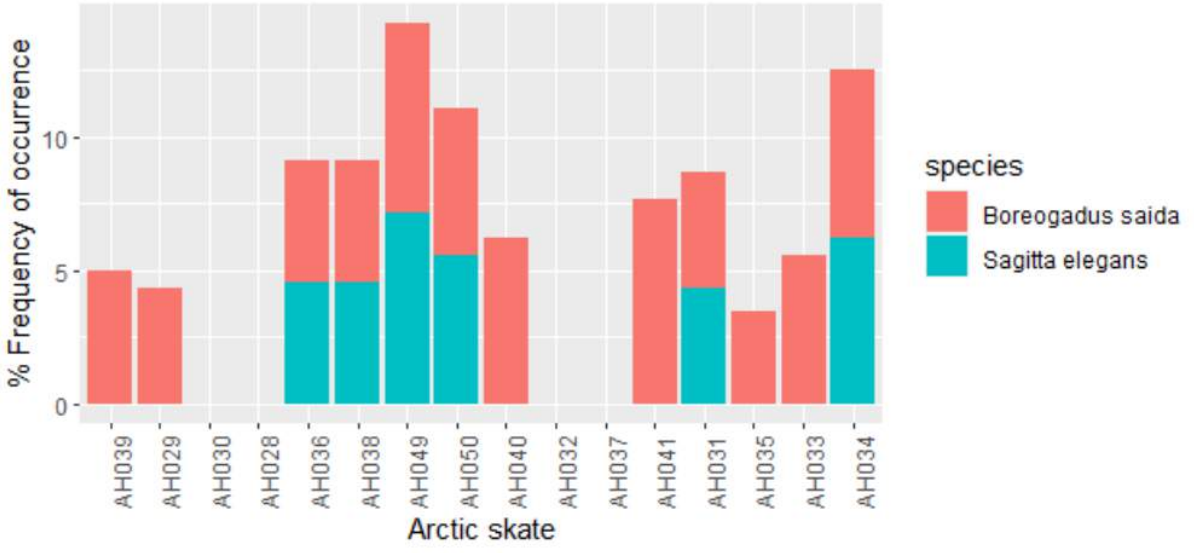

Figure 24. The chaetognath Sagitta elegans *as prey of Polar cod (*Borogadus saida*) and secondary prey of Arctic skate.*

4 Skate sampling procedure for metabarcoding of stomach contents

Note

The samples are to be stored as cold as possible to avoid DNA degradation between and during the different steps. Between the different steps, store at least at -20°C.

Catch

After catch, freeze the skate immediately and mark with the ID and/or station number.

Equipment

Stick mixer, glass bowls (for easy cleaning), scales (weight and length), scalpels, forceps, aluminium trey (for easy cleaning), vinyl gloves (disposed between each sample (van Zinnicq Bergmann et al., 2021)), ethanol (EtOH) , bleach, MilliQ water, containers, Eppendorf tubes, lighter.

Thawing and dissection

Thaw the skates in 4°C freshwater the day before dissection, the stomachs should still be as cold as possible. Note the skates' species, sex, weight (the weight of the skate is higher when thawed in freshwater than when it is thawed in saltwater) and length to the nearest gram and cm, respectively, and take pictures with the ID tag on both the dorsal and ventral side of the skates. Open the skates from the ventral side with a scalpel (Figure 25) and cut as far up on the pharynx as possible and below the pyloric sphincter (Figure 26). Put the stomach in a sterile container and fill it with 96% EtOH and store as cold as possible (at least -20°C) to avoid further degradation of DNA (Barbato et al., 2019). After 24 hours replace the EtOH with new 96% EtOH. In case of big stomachs, change the EtOH again after 24 new hours.

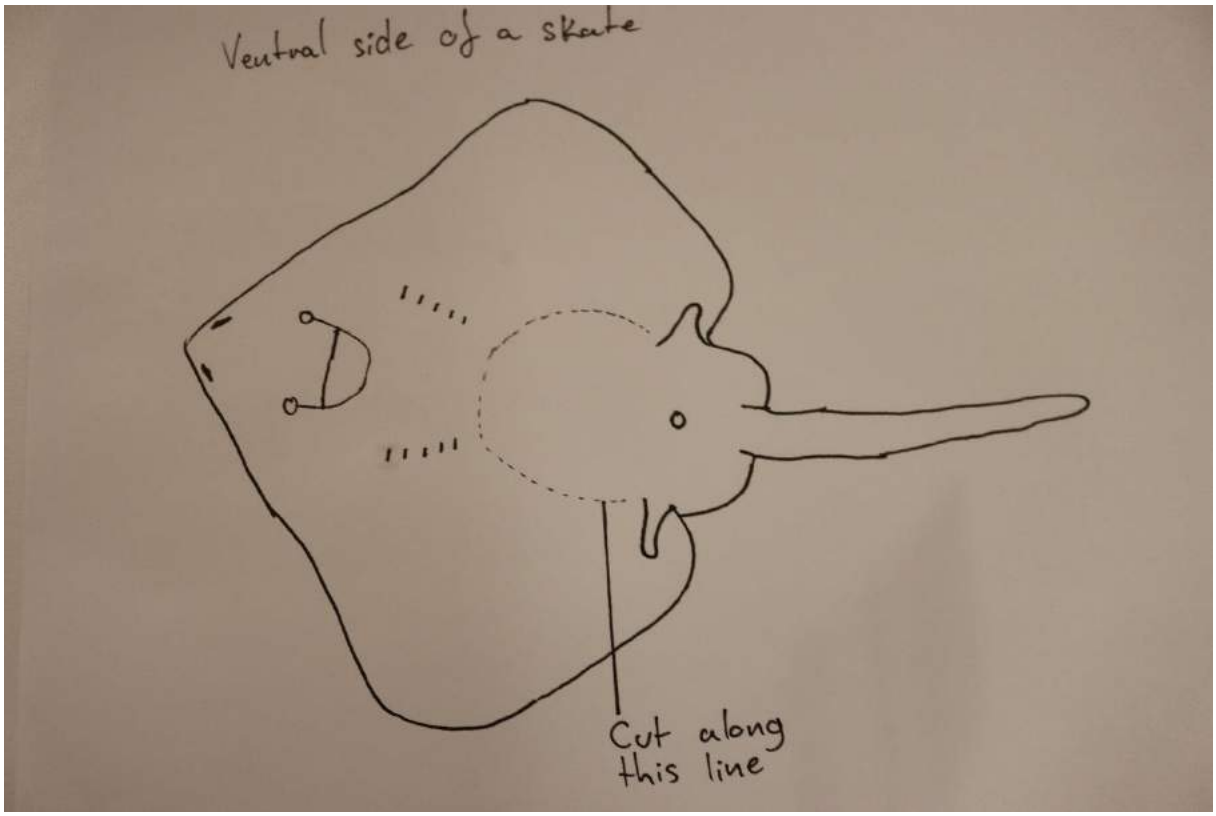

Figure 25. Dissection of a skate. Part 1.

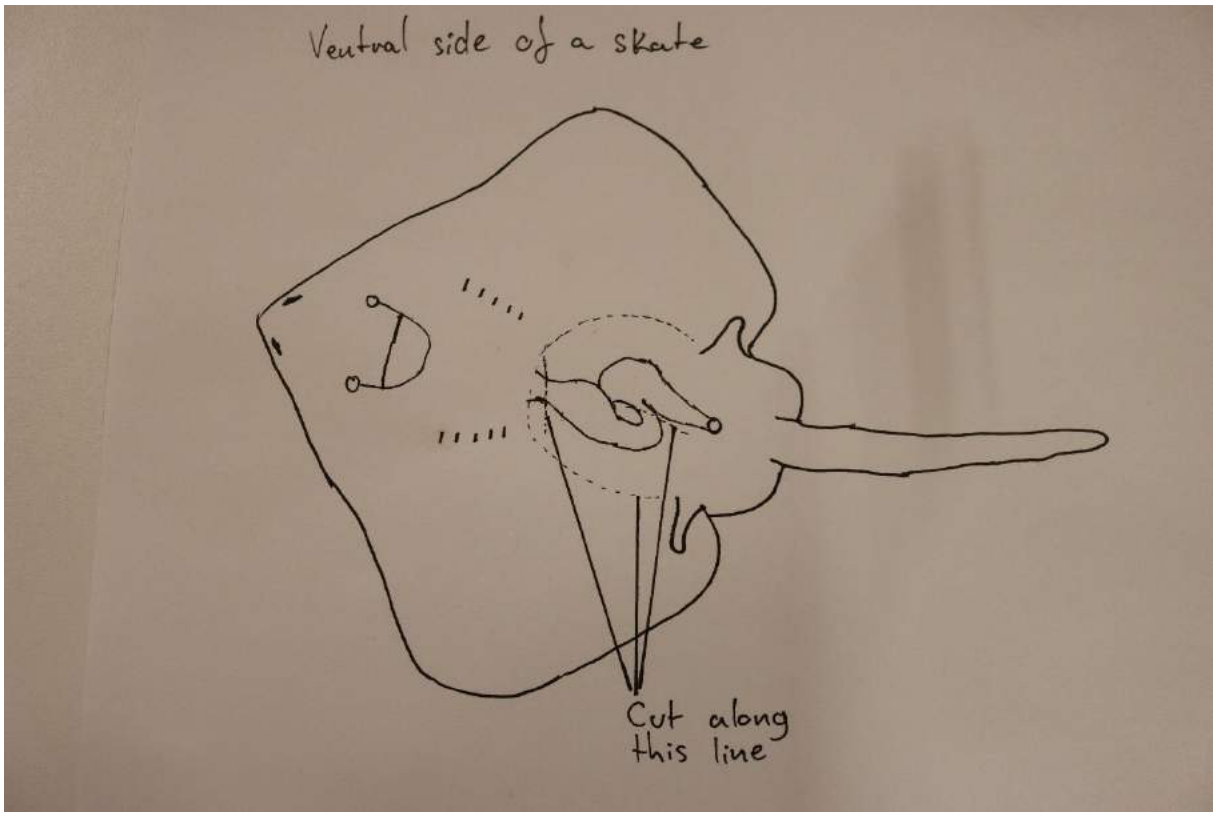

Figure 26. Dissection of a skate. Part 2.

Cleaning and sterilizing

Open the stomachs in a clean lab inside a laminar flow cabinet (Yoon et al., 2017) (van Zinnicq Bergmann et al., 2021). Clean the workspace with hot water and sterilize with bleach (van Zinnicq Bergmann et al., 2021). Rinse with hot water, clean with soap, sterilize with bleach and rinse with MilliQ water the used equipment (Yoon et al., 2017). As well as rinsing, cleaning, and sterilizing, flame sterilize the forceps and scalpel with EtOH. Cleaning and sterilizing of workspace and equipment needs to be done before and after every sample. Dispose vinyl gloves between each sample.

Homogenization of stomach contents

Note the stomachs' weight in gram with and without content. Note the fill grade of the stomachs and degree of decomposition. Take pictures of the stomachs and stomach contents together with the ID of the skates. Rinse the stomach over a glass bowl (glass is easy to clean and sterilize) with MilliQ water to get all the contents out. Homogenize the stomach contents in the bowl with MilliQ water (enough of it to be completely homogenized) with a stick mixer

until the stomach content is completely homogenized (Yoon et al., 2017). Collect three subsamples of the homogenate with a syringe and store in Eppendorf tubes. The syringes are discharged after every sample. Store the subsamples as cold as possible (at least -20°C) right after collection until DNA extraction.

Blanks

Collect blanks from the bottle containing MilliQ water, the tray, the bowl, and the stick mixer between every 5-10 sample. Store the blanks with the subsamples.

5 DNA extraction: Qiagen DNeasy PowerSoil Kit

Note

Take out the samples from the freezer the day before and put them in the fridge.

Change nitril gloves often and between every sample.

Put Solution C1 in the heating cabinet at 60°C.

Cleaning and sterilizing

Clean and sterilize the PCR-workstation and benches surrounding your PCR-workstation with 10% bleach and 70% ethanol. UV-treatment your PCR-workstation for 30 minutes together with the different solutions in the kit, the different tubes (not the PowerBead tubes and the MB Spin Column), the pipettes and filter tips.

Lab protocol

Add 75µL of sample to the PowerBead tubes. Add 60µL of Solution C1 to the PowerBead tubes. Secure the PowerBead tubes to a vortex adapter horizontally and vortex at speed 3 for 1 hour. Centrifuge the PowerBead tubes at 10000 x g for 1 min. Avoiding the beads, transfer the supernatant to a 2ml Collection tube. Add 250µL of Solution C2 and shake the tube for five seconds. Centrifuge the Collection tubes at 10000 x g for 1 min. Avoiding the pellet, transfer up to 600µL of supernatant to a new Collection tube. Add 200µL of Solution C3 and shake for 5 seconds. Centrifuge the Collection tubes at 10000 x g for 1 min. Avoiding the pellet, transfer up to 700µL of supernatant to a large 2ml tube. Shake to mix Solution C4. Add 2x600µL of Solution C4 to the supernatant in the large tube and shake for 5 seconds. Transfer 630µL of supernatant to the MB Spin Column and centrifuge at 10000 x g for 1 min. Discard flow-through. Repeat until all the supernatants have been filtered through the MB Spin Column. Add 500µL of Solution C5 to the MB Spin Column and centrifuge at 10000 x g for 1 min. Discharge the flow through. Dry spin, centrifuge the MB Spin Column at 10000 x g for 1 min. Place MB Spin Column in final 1.5ml tube. Add 100µL of Solution C6 to the centre of the white filter membrane. Do not touch the filter. Centrifuge the 1.5ml tube at 10000 x g for 1 min. Keep the flow-through and discharge the MB Spin Column. Transfer 30µL of the DNA as an aliquot into a PCR-plate. Freeze the remaining 70µL as stock and the PCR-plate at -20°C.

Cleaning and sterilizing

Clean and sterilize the PCR-workstation and benches surrounding your PCR-workstation with 10% bleach, MilliQ water and 70% ethanol. UV-treatment your PCR-workstation for 30 minutes.

6 DNA extraction: Qiagen DNeasy PowerSoil Pro Kit

Note

Take out the samples from the freezer the day before and put them in the fridge.

Change nitril gloves often and between every sample.

Put Solution CD3 in the heating cabinet at 60°C.

Store Solution CD2 at 2-8°C.

Cleaning and sterilizing

Clean and sterilize the PCR-workstation and benches surrounding your PCR-workstation with 10% bleach and 70% ethanol. UV-treatment your PCR-workstation for 30 minutes together with the different solutions in the kit, the different tubes (not the PowerBead tubes and the MB Spin Column), the pipettes and filter tips.

Lab protocol

Spin the PowerBead Pro Tube at 15000 x g for 1 min. Add 75µL of the sample to the PowerBead Pro Tubes and 800µL of Solution CD1. Shake for 5 seconds. Secure the PowerBead Pro Tubes horizontally on a vortex adapter and vortex at full speed for 20 min. Centrifuge the PowerBead Pro Tubes at 15000 x g for 1 min. Transfer supernatant to a 2mL Microcentrifuge Tube. Add 200µL of Solution CD2 and shake for 5 seconds. Centrifuge the Microcentrifuge Tubes at 15000 x g for 1 min. Transfer up to 700µL of supernatant to a clean 2mL Microcentrifuge Tube. Add 600µL of Solution CD3 and shake for 5 seconds. Load 650µL of lysate onto an MB Spin Column and centrifuge at 15000 x g for 1 min. Discharge flow-through and repeat until all lysates have been filtered. Place MB Spin Column in a new 2ml Collection Tube. Add 500µL of Solution EA to the MB Spin Column and centrifuge at 15000 x g for 1 min. Discharge flow-through. Add 500µL of Solution C5 to the MB Spin Column and centrifuge at 15000 x g for 1 min. Discharge flow-through. Put MB Spin Column in a new 2ml Collection Tube and centrifuge at 16000 x g for 2 min. Place the MB Spin Column in final 1.5ml Elution Tube and add 100µL of Solution C6. Centrifuge at 15000 x g for 1 min. Keep the flow-through and discharge the MB Spin Column. Transfer 30µL of the

DNA as an aliquot into a PCR-plate. Freeze the remaining 70µL as stock and the PCR-plate at -20℃.

Cleaning and sterilizing

Clean and sterilize the PCR-workstation and benches surrounding your PCR-workstation with 10% bleach, MilliQ water and 70% ethanol. UV-treatment your PCR-workstation for 30 minutes.

7 DNA amplification: Simple 1-step PCR protocol

Note

We use a simple 1-step PCR protocol to amplify the Leray fragment. The metabarcoding primers have an 8-base sample-tag attached (each tag with at least 3 differences out of 8 bases). Also, we add a variable number (2-4) of leading Ns, to increase sequencing variability to improve Illumina sequenzing. Each forward and reverse primer has the sample-tag attached in both ends. There are 96 such different pairs of forward and reverse primers.

Cleaning

Clean the workstation and equipment with bleach, MilliQ water, and ethanol before and after the lab work. UV-treat the workstation before and after the lab work.

Equipment

Amplitaq Gold 360 master mix

Bovine serum albumin (BSA)

Protocol

Prepare PCR plates and Eppendorf tubes. (n=100 per PCR plate and Eppendorf tube).

Pipette n x 10µL AmpliTaq Gold Master Mix, n x 0.16µL BSA 20µg/µL and n x 5.84µL H_2O into the Eppendorf tubes.

Pipette 9.7µL of the PCR mix into each well of the PCR plates.

Centrifuge the plates with primers.

Pipette 1µL forward and 1µL reverse primer 5 µM into each well of the PCR plates.

Centrifuge the PCR plates with PCR mix.

Centrifuge the PCR plates with DNA template.

Pipette 2µL of DNA template and transfer it into each well of the PCR plates with PCR mix.

The PCR programme is 10 min of 95℃ needed for denaturing the blocking antibody of Taq polymerase. Then 35 cycles of 1 min 94℃, 1 min 45℃, min 72℃ and then 5 min 72℃ extension time. After that the PCR product will be refrigerated.

8 Library pooling and concentration: MinElute PCR Purification Kit

Note before starting

This protocol is for cleanup of up to 5 µg PCR product (70 bp to 4 kb). Add ethanol (96%-100%) to Buffer PE concentrate before use. All centrifugal steps are carried out at 17.900 x g in a microcentrifuge at room temperature. Add 1:250 volume pH indicator I to Buffer PB. Add pH indicator to the entire buffer contents. Do not add pH indicator I to buffer aliquots. The yellow colour of buffer PB with pH indicator I indicates a pH of ≤7.5. The adsorption of DNA to the membrane is efficient only at pH ≤7.5.

Cleaning

Clean the workstation and equipment with bleach, MilliQ water, and ethanol before and after the lab work. UV-treat the workstation before and after the lab work.

Protocol

Pool the PCR product (18 µL per sample) in two Eppendorf tubes (96 samples per tube).

Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix. Check that the colour of the mixture is yellow (like Buffer PB without the PCR sample). If the colour of the mixture is orange or violet, add 10 µL 3 M sodium acetate, pH 5.0, and mix. The colour of the mixture will turn to yellow.

Place a MinElute column in a provided 2 ml collection tube.

Apply the sample to the MinElute column and centrifuge for 1 min. Discard flow-through and place the MinElute column back into the same collection tube.

Add 750 µL Buffer PE to the MinElute column and centrifuge for 1 min. Discard the flow-through and place the MinElute column back in the same collection tube.

Centrifuge the column in a 2 mL collection tube for 1 min. Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

Place each MinElute column in a clean 1.5 mL microcentrifuge tube.

To elute DNA, add 10 µL Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the centre of the MinElute membrane. Let the column stand for 1 min, and then centrifuge the column for 1 min.

Store the DNA at -30℃ to -15℃.

Concentration of the DNA from Qubit 4 fluorometer are in Table 10.

Table 10. Concentration of DNA from the two libraries Raj 1 and Raj 2.

Library	**Qubit C**	**µL DBA pool**	**µL water**
Raj 1	522	5.75	34.25
Raj 2	698	4.30	35.70

9 Library preparation: NEXTflex PCR-Free DNA sequencing Kit

Protocol

Step A: End repair

Add nuclease-free water, fragmented DNA (40 µL of 3000 µg DNA/1 µL water), 7 µL NEXTflex PCR-Free End Repair Buffer Mix and 3 µL NEXTflex PCR-Free End Repair Enzyme Mix to one PCR strip/library.

Mix thoroughly by pipetting.

Apply lids to the PCR strips and incubate on a thermocycler for 30 minutes at 22°C.

Step B: Clean-up

Transfer 50 µL of the DNA to an Eppendorf tube.

Add 42.4 µL of AMPure XP Beads to each sample and mix thoroughly by pipetting.

Incubate the sample at room temperature for 5 minutes.

Place the Eppendorf tubes on a magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.

Set pipette to 90 µL, slowly remove and discard the supernatant taking care not to disturb the beads.

With Eppendorf tubes on a stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipetting.

Repeat the previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.

Remove the Eppendorf tubes from the magnetic stand and let dry at toom temperature for 3 minutes.

Resuspend dried beads with 17.5 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the Eppendorf tube.

Incubate resuspended beads at toom temperature for 2 minutes.

Place Eppendorf tubes on magnetic stand at room temperature for 5 minutes or until the sample appears completely clear.

Do not disturb the sample in this step. Transfer 16 µL of clear sample to a PCR strip.

Add 40 µL of AMPure XP beads to each well containing sample and mix thoroughly by pipetting.

Incubate at room temperature for 5 minutes.

Place the PCR strip on a magnetic stand for 5 minutes at room temperature or until the sample appears completely clear.

Do not discard the sample in this step. Transfer 88 µL of the supernatant to a new PCR strip. Be careful not to disrupt the magnetic bead pellet or transfer any magnetic beads with the sample. The bead pellet binds and removes DNA above 400 bp.

Add 88 µL of AMPure XP Beads to each PCR strip containing sample and mix thoroughly by pipetting.

Incubate at room temperature for 5 minutes.

Place the PCR strip on the magnetic stand at room temperature for 5 minutes or until the supernatant appears clear.

Remove and discard 172 µL of the supernatant taking care not to disturb beads. Some liquid may remain in wells.

With PCR strips on stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.

Repeat the step above for a total of 2 ethanol washes. Ensure all ethanol has been removed.

Remove the PCR strips from the magnetic stand and let dry at room temperature for 3 minutes.

Resuspend dried beads with 17.5 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the wall.

Incubate resuspended beads at room temperature for 2 minutes.

Place PCR strips on magnetic stand at room temperature for 5 minutes or until the sample appears clear.

Transfer 16 µL of clear supernatant to new PCR strip.

Now the DNA can be stored at -20℃ if there is need for a pause. Thaw on ice before next step.

Step C: 3’ Adenylation

Combine the following in PCR strips:

16 µL End-Repaired DNA (from Step B)

4.5 µL NEXTflex PCR-Free Adenylation Mix

Mix thoroughly by pipetting.

Apply lids to PCR strips and incubate on a thermocycler for 30 minutes at 37℃.

Proceed to Step D.

Step D: Adapter Ligation

The following adapter titrations for the given DNA starting input amounts are recommended in Table 11.

Table 11. Recommended adapter titration for the given DNA starting input.

DNA starting input amount:	Adapter amount:
3 µg	2.5 µL
1 µg	1.25 µL

500 ng	0.625 µL

For each sample, combine the following reagents (in this order) in the PCR strips:

20.5 µL 3’ Adenylated DNA (from Step C)

31.5 µL NEXTflex PCR-Free Ligation Mix

_ µL NEXTflex PCR-Free DNA Adapter or NEXTflex PCR-Free Barcode

_ µL Nuclease-free Water

54.5 µL in total

Mix thoroughly by pipetting.

Apply lids to the PCR strips and incubate on a thermocycler for 15 minutes at 22°C.

Proceed to Step E.

Step E: Clean-Up

Add 44 µL of AMPure XP Beads to each sample and mix thoroughly by pipetting.

Incubate at room temperature for 5 minutes.

Place the PCR strips on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.

Remove clear supernatant taking care not to disturb beads. Some liquid may remain in wells.

With PCR strips on stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate PCR strips at room temperature for 30 seconds. Carefully remove ethanol by pipetting.

Repeat the previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.

Remove the PCR strips from the magnetic stand and let dry at room temperature for 3 minutes.

Resuspend dried beads with 57 µL of Resuspension Buffer. Mix thoroughly by pipetting and ensuring beads are no longer attached to the side of the PCR strips.

Incubate resuspended beads at room temperature for 2 minutes.

Place PCR strips on magnetic stand for 5 minutes or until the supernatant appears completely clear.

Transfer 54.5 µL of clear sample to a new PCR strip.

Add 44 µL of AMPure XP Beads to each sample and mix thoroughly by pipetting.

Incubate at room temperature for 5 minutes.

Place the PCR strip on the magnetic stand at roon temperature for 5 minutes or until the supernatant appears completely clear.

Remove clear supernatant taking care not to disturb beads. Some liquid may remain in PCR strips.

With PCR strips on stand, add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipetting.

Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.

Remove the PCR strip from the magnetic stand and let dry at room temperature for 3 minutes.

Resuspend dried beads with 22.5 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the PCR strips.

Incubate resuspended beads at room temperature for 2 minutes.

Place PCR strip on magnetic stand for 5 minutes or until the supernatant appears completely clear.

Transfer 20 µL of clear supernatant to new PCR strips.

Safe to pause the experiment, can be stored at -20°C before next step. Thaw on ice before continuing.

Step F: Quantification

qPCR is recommended to quantitate DNA library templates for optimal cluster density as it selectively measures only those templates with both adapter sequences on either end. qPCR is also a sensitive method for measuring DNA libraries whose concentrations are below the threshold of detection using spectrophotometric methods. NEXTflex PCR-Free library templates will require quantification by qPCR. This can be performed using any qPCR quantification kit with at least three dilutions of a previously used library with known cluster numbers. If performing quantification without a kit, a protocol is provided below.

Choose a control template as similar as possible to your experimental template size, GC content and library type.

Using qPCR dilution buffer, make 6 serial dilutions of the control template in a range from 0.01 pM to 100 pM. Ensure that these dilutions are made fresh for immediate use before qPCR. Make three replicate, independent serial dilutions of each control template. Triplicate results are important for qPCR analysis.

Using qPCR dilution buffer, make a dilution of your library (Ligation Product from Step E) for quantification. Libraries will need to be diluted so that they fall within the range as the control template. Although this range depends on your sample and amount of starting genomic material, recommended dilutions include 1:500, 1:1,000, 1:2,000, 1:5,000, 1:10,000. Make three replicate, independent dilutions for each unknown library.

Prepare 4 µM qPCR Primer 1 and qPCR Primer 2 stock solutions. Mix equal volumes of both primers to achieve a 2 µM qPCR Primer Mix.

For each sample, prepare a master mix by combining the following reagents on ice:

4 µL Nuclease-free Water

10 µL 2X SYBR Master Mix

2 µL qPCR Primer Mix (2 µM)

Add 4 µL of the diluted control template or unknown library dilutions to each well, ensure that you have triplicates for each sample. Add 16 µL of the master mix into each corresponding well.

Mix thoroughly by pipetting

Centrifuge plate for 1 minute at 200 x g.

Quantify the libraries using the following qPCR cycles (note cycle conditions may vary according to SYBR Mix manufacturer):

5 min 95℃

30 sec 95℃

45 sec 60℃

Repeat the 30 sec and 45 sec cycle 35 times.

Analyze the libraries ensuring there is good amplification for each control template. Remove outlying or bad replicates.

Generate a standard curve from the control template by plotting Ct values against the log initial concentration. Efficiency of the amplification should be 90-110% and the R2 should be greater than 0.95. Calculate the initial concentration of your unknown library templates based on the standard curve and the dilution factor of your unknown sample.

Once you have quantified your library, dilute to the appropriate concentration for clustering. If your library concentration is less than 1 nM, follow Bioo Scientific's Denaturation of Sub-nanomolar DNA Libraries Protocol, available by contacting nextgen@biooscientific.com. If multiplexing libraries, transfer equal amounts of each normalized library to be pooled in the well of a new 96-well PCR plate. Mix thoroughly by pipetting.

Proceed to cluster generation

10 Bioinformatic pipeline

The different steps of the bioinformatic pipeline, MJOLNIR are:

RAN prepares for parallel processing.

FREYJA aligns paried-end reads, demultiplexes reads into each sample, and filters by length and retain just ACGT.

HELA dereplicates sequences intra sample, removed chimeras, dereplicates sequences across samples, and generates a table of read abundances.

ODIN clusters sequences into MOTUs and recounts abundances of MOTUs.

THOR assigns taxonomy to each MOTU seed and adds higher taxonomic ranks.

FRIGGA combines taxonomy with abundances.

LOKI removed pseudogenes.

RAGNAROC recovers original sample names from metadata, filters by relative abundance intra sample, filters by minimum total abundance across samples, removed bacterial reads and removes known contaminants.

11 R script

---

title: "SKATE DIET"

output: html_notebook

Packs

```{r}

library(readxl)

library(tidyverse)

library(datasets)

library(mosaic)

library(dplyr)

library(rstatix)

library(ggpubr)

library(broom)

library(ggplot2)

library(reshape2)

library(scales)

library(vegan)

library(MASS)
```

```
library(lattice)

library(permute)

library(circlize)

library(phyloseq)

library(ComplexHeatmap)

library(ggspatial)

library(ggOceanMaps)

library(ggOceanMapsData)

library(devtools)

library(pairwiseAdonis)

library(forcats)
```

Desktop session and dataframes

```{r}
setwd("D:/UiT Office 365/Arve Lynghammar - Metabarkoding skatemager/Excel and R")

TD <- read.delim("D:/UiT Office 365/Arve Lynghammar - Metabarkoding skatemager/Excel and R/RAJX_final_dataset.tsv", header=TRUE)

a <- read_excel("Dataskjema_skater.xlsx",sheet = "Dataskjema_skater")

b <- read_excel("Dataskjema_skater.xlsx",sheet = "Mageinnhold")

c <- read_excel("Dataskjema_skater.xlsx",sheet = "Skater")

Eri <- read_excel("Dolgov and Eriksen.xlsx",sheet = "Eriksen")
```

Dol <- read_excel("Dolgov and Eriksen.xlsx",sheet = "Dolgov")

```

## Laptop session and dataframes

```{r}

setwd("C:/Users/Admin/UiT Office 365/Arve Lynghammar - Metabarkoding skatemager/Excel and R")

TD <- read.delim("C:/Users/Admin/UiT Office 365/Arve Lynghammar - Metabarkoding skatemager/Excel and R/RAJX_final_dataset.tsv", header=TRUE)

a <- read_excel("Dataskjema_skater.xlsx",sheet = "Dataskjema_skater")

b <- read_excel("Dataskjema_skater.xlsx",sheet = "Mageinnhold")

c <- read_excel("Dataskjema_skater.xlsx",sheet = "Skater")

Eri <- read_excel("Dolgov and Eriksen.xlsx",sheet = "Eriksen")

Dol <- read_excel("Dolgov and Eriksen.xlsx",sheet = "Dolgov")

```

MOTUs and species pre filtering

```{r}

TS <- (subset(TD, species_name != ""))

TS <- TS[13]

TS <- aggregate(TS[-1], TS["species_name"], mean)

list(TS$species_name)

```
TS <- TD

TS <- TS[3]

TS <- aggregate(TS[-1], TS["scientific_name"], mean)

list(TD$scientific_name)
```

Filterering

```{r}
TD <- subset(TD, total_reads>100)

TD <- subset(TD, best_identity>0.85)


TD <- subset(TD, superkingdom_name != "Amoebozoa") #Passive diet, or secondary predation

TD <- subset(TD, superkingdom_name != "Archaeplastida") #Passive diet, or secondary predation

TD <- subset(TD, superkingdom_name != "Chromalveolata") #Passive diet, or secondary predation


TD <- subset(TD, kingdom_name != "Fungi") #Passive diet, or secondary predation


TD <- subset(TD, phylum_name != "")

TD <- subset(TD, phylum_name != "Nematoda") #Parasites
```

```
TD <- subset(TD, order_name != "Mytiloida") #Tag jumps

TD <- subset(TD, order_name != "Primates") #Contamination

TD <- subset(TD, order_name != "Rajiformes") #Skate DNA


TD <- subset(TD, species_name != "Leptogorgia sarmentosa") #Not distributed in the Barents Sea

TD <- subset(TD, species_name != "Antedon mediterranea") #Not distributed in the Barents Sea

TD <- subset(TD, species_name != "Caprella scaura") #Not distributed in the Barents Sea

TD <- subset(TD, species_name != "Laticorophium baconi") #Not distributed in the Barents Sea

TD <- subset(TD, species_name != "Cilicaea sp. 72") #No records of distributions the Barents Sea

TD <- subset(TD, species_name != "Eukrohnia bathyantarctica") #No records of distributions the Barents Sea

TD <- subset(TD, species_name != "Jassa slatteryi") #No records of distributions the Barents Sea

TD <- subset(TD, species_name != "Watersipora subovoidea") #No records of distributions the Barents Sea
```

MOTUs and species post filtering

```{r}
```

```
TS <- (subset(TD, species_name != ""))

TS <- (subset(TS, best_identity>0.98))

TS <- TS[13]

TS <- aggregate(TS[-1], TS["species_name"], mean)

list(TS$species_name)



TS <- TD

TS <- TS[3]

TS <- aggregate(TS[-1], TS["scientific_name"], mean)

list(TD$scientific_name)
```

Preparing datasets

```{r}
a <- subset(a,select = -c(2:6,15:37))

b <- subset(b,select = -c(1:2,8:11))

b <- subset(b,ID !="Blank")

b <- aggregate(b[-1], b["ID"], mean)

c <- subset(c,select = -c(2:4))



a <- a[order(a$ID),]

b <- b[order(b$ID),]
```

```
c <- c[order(c$ID),]


a$Species <- (c$Species)
a$spse <- (c$`SpSe`)
a$Size_class <- (c$`Size class`)
a$Stomach_and_cont <- (b$`Stomach + content (g)`)
a$Stomach <- (b$`Stomach (g)`)
a$Stomach_cont <- ((b$`Stomach + content (g)`)-(b$`Stomach (g)`))
a$Capasity_sto <- (b$`Capasity of stomach %`)
a$Decomp <- (b$`Degree of decomposition 0=Complitely, 1=Partly, 2=Non`)
a$Geo <- (c$Geo)
a <- a[!duplicated(a$ID),] #remove the duplicated ID name AR026
a1 <- subset(a,Species != "blank")


#


Eri[is.na(Eri)] <- " "
Dol[is.na(Dol)] <- " "


Eri <- Eri[order(Eri$Phylum),]
Dol <- Dol[order(Dol$Phylum),]
```

```
Eri$AH_m <- (Eri$AH_m/(sum(Eri$AH_m))*100)

Eri$AH_f <- (Eri$AH_f/(sum(Eri$AH_f))*100)

Eri$BS_m <- (Eri$BS_m/(sum(Eri$BS_m))*100)

Eri$BS_f <- (Eri$BS_f/(sum(Eri$BS_f))*100)

Eri$AR_m <- (Eri$AR_m/(sum(Eri$AR_m))*100)

Eri$AR_f <- (Eri$AR_f/(sum(Eri$AR_f))*100)


sum(Eri$AH_m)

sum(Eri$AH_f)

sum(Eri$BS_m)

sum(Eri$BS_f)

sum(Eri$AR_m)

sum(Eri$AR_f)


Dol$AH_m <- (Dol$AH_m/(sum(Dol$AH_m))*100)

Dol$AH_f <- (Dol$AH_f/(sum(Dol$AH_f))*100)

Dol$BS_m <- (Dol$BS_m/(sum(Dol$BS_m))*100)

Dol$BS_f <- (Dol$BS_f/(sum(Dol$BS_f))*100)

Dol$AR_m <- (Dol$AR_m/(sum(Dol$AR_m))*100)

Dol$AR_f <- (Dol$AR_f/(sum(Dol$AR_f))*100)
```

```
sum(Dol$AH_m)

sum(Dol$AH_f)

sum(Dol$BS_m)

sum(Dol$BS_f)

sum(Dol$AR_m)

sum(Dol$AR_f)


#


TDU <- TD

TDS <- subset(TDU, select = c(1:16,209))


TDS$AH028 <- paste((TDU$AH028_84 +TDU$AH028_85 +TDU$AH028_86 )/3)

TDS$AH029 <- paste((TDU$AH029_90 +TDU$AH029_91 +TDU$AH029_92 )/3)

TDS$AH030 <- paste((TDU$AH030_97 +TDU$AH030_98 +TDU$AH030_99 )/3)

TDS$AH031 <- paste((TDU$AH031_25 +TDU$AH031_26 +TDU$AH031_27 )/3)

TDS$AH032 <- paste((TDU$AH032_71 +TDU$AH032_72 +TDU$AH032_73 )/3)

TDS$AH033 <- paste((TDU$AH033_2  +TDU$AH033_3  +TDU$AH033_4  )/3)

TDS$AH034 <- paste((TDU$AH034_15 +TDU$AH034_16 +TDU$AH034_17 )/3)

TDS$AH035 <- paste((TDU$AH035_10 +TDU$AH035_11 +TDU$AH035_9  )/3)
```

```
TDS$AH036 <- paste((TDU$AH036_67 +TDU$AH036_68 +TDU$AH036_69 )/3)

TDS$AH037 <- paste((TDU$AH037_61 +TDU$AH037_62 +TDU$AH037_63 )/3)

TDS$AH038 <- paste((TDU$AH038_12 +TDU$AH038_13 +TDU$AH038_14 )/3)

TDS$AH039 <- paste((TDU$AH039_78 +TDU$AH039_79 +TDU$AH039_80 )/3)

TDS$AH040 <- paste((TDU$AH040_75 +TDU$AH040_76 +TDU$AH040_77 )/3)

TDS$AH041 <- paste((TDU$AH041_94 +TDU$AH041_95 +TDU$AH041_96 )/3)

TDS$AH049 <- paste((TDU$AH049_181+TDU$AH049_182+TDU$AH049_183)/3)

TDS$AH050 <- paste((TDU$AH050_178+TDU$AH050_179+TDU$AH050_180)/3)


TDS$AL838 <- paste((TDU$AL838_171+TDU$AL838_172+TDU$AL838_173)/3)

TDS$AL839 <- paste((TDU$AL839_193+TDU$AL839_194+TDU$AL839_195)/3)

TDS$AL841 <- paste((TDU$AL841_190+TDU$AL841_191+TDU$AL841_192)/3)

TDS$AL843 <- paste((TDU$AL843_203+TDU$AL843_204+TDU$AL843_205)/3)

TDS$AL844 <- paste((TDU$AL844_199+TDU$AL844_200+TDU$AL844_201)/3)

TDS$AL845 <- paste((TDU$AL845_161+TDU$AL845_162+TDU$AL845_163)/3)

TDS$AL846 <- paste((TDU$AL846_152+TDU$AL846_153+TDU$AL846_154)/3)

TDS$AL847 <- paste((TDU$AL847_158+TDU$AL847_159+TDU$AL847_160)/3)

TDS$AL849 <- paste((TDU$AL849_196+TDU$AL849_197+TDU$AL849_198)/3)

TDS$AL850 <- paste((TDU$AL850_164+TDU$AL850_165+TDU$AL850_166)/3)

TDS$AL851 <- paste((TDU$AL851_155+TDU$AL851_156+TDU$AL851_157)/3)

TDS$AL852 <- paste((TDU$AL852_174+TDU$AL852_175+TDU$AL852_176)/3)
```

```
TDS$BS018 <- paste((TDU$BS018_187+TDU$BS018_188+TDU$BS018_189)/3)

TDS$BS022 <- paste((TDU$BS022_184+TDU$BS022_185+TDU$BS022_186)/3)

TDS$BS042 <- paste((TDU$BS042_19 +TDU$BS042_20 +TDU$BS042_21 )/3)

TDS$BS043 <- paste((TDU$BS043_5  +TDU$BS043_6  +TDU$BS043_7  )/3)

TDS$BS044 <- paste((TDU$BS044_87 +TDU$BS044_88 +TDU$BS044_89 )/3)

TDS$BS045 <- paste((TDU$BS045_64 +TDU$BS045_65 +TDU$BS045_66 )/3)

TDS$BS046 <- paste((TDU$BS046_22 +TDU$BS046_23 +TDU$BS046_24 )/3)


TDS$AR001 <- paste((TDU$AR001_168+TDU$AR001_169+TDU$AR001_170)/3)

TDS$AR002 <- paste((TDU$AR002_49 +TDU$AR002_50 +TDU$AR002_51 )/3)

TDS$AR003 <- paste((TDU$AR003_114+TDU$AR003_115+TDU$AR003_116)/3)

TDS$AR004 <- paste((TDU$AR004_110+TDU$AR004_111+TDU$AR004_112)/3)

TDS$AR005 <- paste((TDU$AR005_129+TDU$AR005_130+TDU$AR005_131)/3)

TDS$AR006 <- paste((TDU$AR006_39 +TDU$AR006_40 +TDU$AR006_41 )/3)

TDS$AR007 <- paste((TDU$AR007_45 +TDU$AR007_46 +TDU$AR007_47 )/3)

TDS$AR008 <- paste((TDU$AR008_52 +TDU$AR008_53 +TDU$AR008_54 )/3)

TDS$AR009 <- paste((TDU$AR009_146+TDU$AR009_147+TDU$AR009_148)/3)

TDS$AR010 <- paste((TDU$AR010_120+TDU$AR010_121+TDU$AR010_122)/3)

TDS$AR011 <- paste((TDU$AR011_126+TDU$AR011_127+TDU$AR011_128)/3)

TDS$AR012 <- paste((TDU$AR012_101+TDU$AR012_102+TDU$AR012_103)/3)
```

```
TDS$AR013 <- paste((TDU$AR013_36 +TDU$AR013_37 +TDU$AR013_38 )/3)

TDS$AR014 <- paste((TDU$AR014_58 +TDU$AR014_59 +TDU$AR014_60 )/3)

TDS$AR015 <- paste((TDU$AR015_107+TDU$AR015_108+TDU$AR015_109)/3)

TDS$AR016 <- paste((TDU$AR016_117+TDU$AR016_118+TDU$AR016_119)/3)

TDS$AR017 <- paste((TDU$AR017_136+TDU$AR017_137+TDU$AR017_138)/3)

TDS$AR019 <- paste((TDU$AR019_149+TDU$AR019_150+TDU$AR019_151)/3)

TDS$AR020 <- paste((TDU$AR020_33 +TDU$AR020_34 +TDU$AR020_35 )/3)

TDS$AR021 <- paste((TDU$AR021_143+TDU$AR021_144+TDU$AR021_145)/3)

TDS$AR023 <- paste((TDU$AR023_139+TDU$AR023_140+TDU$AR023_141)/3)

TDS$AR024 <- paste((TDU$AR024_133+TDU$AR024_134+TDU$AR024_135)/3)

TDS$AR025 <- paste((TDU$AR025_42 +TDU$AR025_43 +TDU$AR025_44 )/3)

TDS$AR026 <-
paste((TDU$AR026_104+TDU$AR026_105+TDU$AR026_106+TDU$AR026_55+TDU$A
R026_56+TDU$AR026_57)/6)

TDS$AR027 <- paste((TDU$AR027_123+TDU$AR027_124+TDU$AR027_125)/3)

TDS$AR047 <- paste((TDU$AR047_81 +TDU$AR047_82 +TDU$AR047_83 )/3)

TDS$AR048 <- paste((TDU$AR048_29 +TDU$AR048_30+ TDU$AR048_31 )/3)


TDS$BLANK_HOM_113 <- TDU$Blank_HOM_113

TDS$BLANK_EXT_206 <- TDU$Blank_Ext_206

TDS$BLANK_PCR_207 <- TDU$Blank_PCR_207
```

```
#

TAX <- subset(TDS, select = c(3,6:13))

#

TDF <- TDS

TDF <- subset(TDF, select = -c(1:17))

TDF<-lapply(TDF,as.numeric)

TDF$AH <- paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+
        TDF$AH038+TDF$AH049+TDF$AH050+TDF$AH040+TDF$AH032+
        TDF$AH037+TDF$AH041+TDF$AH031+TDF$AH035+TDF$AH033+
        TDF$AH034)/16)
TDF$BS <- paste((TDF$AL843+TDF$AL844+TDF$AL846+TDF$AL852+TDF$AL847+
        TDF$AL849+TDF$AL838+TDF$BS022+TDF$BS045+TDF$AL845+
        TDF$AL850+TDF$BS044+TDF$BS043+TDF$AL851+TDF$AL839+
        TDF$AL841+TDF$BS046+TDF$BS042+TDF$BS018)/19)
TDF$AR <- paste((TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+
        TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+
```

```
TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016+TDF$AR010+

TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+TDF$AR007+

TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+TDF$AR001+

TDF$AR004+TDF$AR005)/28)


TDF$AHone <- paste((TDF$AH029+TDF$AH032+TDF$AH039+TDF$AH040)/4)

TDF$AHtwo <-
paste((TDF$AH028+TDF$AH030+TDF$AH036+TDF$AH037+TDF$AH041)/5)

TDF$AHthree <- paste((TDF$AH038)/1)

TDF$AHfour <-
paste((TDF$AH031+TDF$AH033+TDF$AH034+TDF$AH035+TDF$AH049+

        TDF$AH050)/6)

TDF$AHfemale <-
paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+

         TDF$AH038+TDF$AH049+TDF$AH050)/8)

TDF$AHmale <-
paste((TDF$AH040+TDF$AH032+TDF$AH037+TDF$AH041+TDF$AH031+

        TDF$AH035+TDF$AH033+TDF$AH034)/8)


TDF$BSone <- paste((TDF$AL843+TDF$AL844+TDF$AL845+TDF$BS045)/4)

TDF$BStwo <- paste((TDF$BS044+TDF$AL846+TDF$AL850+TDF$AL852)/4)

TDF$BSthree <- paste((TDF$BS043+TDF$AL847+TDF$AL849)/3)

TDF$BSfour <- paste((TDF$AL839+TDF$AL841+TDF$AL851)/3)
```

```
TDF$BSfive <- paste((TDF$BS022+TDF$AL838+TDF$BS042+TDF$BS046)/4)

TDF$BSsix <- paste((TDF$BS018)/1)

TDF$BSfemale <-
paste((TDF$AL843+TDF$AL844+TDF$AL846+TDF$AL852+TDF$AL847+

                TDF$AL849+TDF$AL838+TDF$BS022)/8)

TDF$BSmale <-
paste((TDF$BS045+TDF$AL845+TDF$AL850+TDF$BS044+TDF$BS043+

                TDF$AL851+TDF$AL839+TDF$AL841+TDF$BS046+TDF$BS042+

                TDF$BS018)/11)



TDF$ARone <-
paste((TDF$AR003+TDF$AR010+TDF$AR021+TDF$AR023+TDF$AR027)/5)

TDF$ARtwo <- paste((TDF$AR017+TDF$AR019+TDF$AR024+TDF$AR026)/5)

TDF$ARthree <- paste((TDF$AR011+TDF$AR047+TDF$AR048)/3)

TDF$ARfour <-
paste((TDF$AR006+TDF$AR007+TDF$AR008+TDF$AR013+TDF$AR014+

                TDF$AR015)/6)

TDF$ARfive <- paste((TDF$AR002+TDF$AR009+TDF$AR012+TDF$AR020)/4)

TDF$ARsix <-
paste((TDF$AR001+TDF$AR004+TDF$AR005+TDF$AR016+TDF$AR025)/5)

TDF$ARfemale <-
paste((TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+

                TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+

                TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016)/14)
```

```
TDF$ARmale <-
paste((TDF$AR010+TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+
                TDF$AR007+TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+
                TDF$AR001+TDF$AR004+TDF$AR005)/14)


TDF$red <- paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+
              TDF$AH038+TDF$AH040+TDF$AH032+TDF$AH037+TDF$AH041+
              TDF$AH031+TDF$AH035+TDF$AH033+TDF$AH034+TDF$BS043)/15)
TDF$purple <- paste((TDF$AH049+TDF$AH050)/2)
TDF$green <- paste((TDF$AL838+TDF$AL839+TDF$AL841+TDF$AL843+TDF$AL844+
               TDF$AL845+TDF$AL846+TDF$AL847+TDF$AL849+TDF$AL850+
               TDF$AL851+TDF$AL852)/12)
TDF$orange <- paste((TDF$BS018+TDF$BS022+TDF$BS044+TDF$BS045+TDF$BS046+
                TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+
                TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+
                TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016+TDF$AR010+
                TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+TDF$AR007+
                TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+TDF$AR001+
                TDF$AR004+TDF$AR005)/32)


TDF<-lapply(TDF,as.numeric)
TDA<-TDF
```

```
#


TDF <- TDS

TDF <- subset(TDF, select = -c(1:17))


TDF[TDF > 0] <- 1

TDF<-lapply(TDF,as.numeric)


TDF$AH <- paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+
                 TDF$AH038+TDF$AH049+TDF$AH050+TDF$AH040+TDF$AH032+
                 TDF$AH037+TDF$AH041+TDF$AH031+TDF$AH035+TDF$AH033+
                 TDF$AH034)/16)
TDF$BS <- paste((TDF$AL843+TDF$AL844+TDF$AL846+TDF$AL852+TDF$AL847+
                 TDF$AL849+TDF$AL838+TDF$BS022+TDF$BS045+TDF$AL845+
                 TDF$AL850+TDF$BS044+TDF$BS043+TDF$AL851+TDF$AL839+
                 TDF$AL841+TDF$BS046+TDF$BS042+TDF$BS018)/19)
TDF$AR <- paste((TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+
                 TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+
                 TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016+TDF$AR010+
                 TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+TDF$AR007+
```

```
TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+TDF$AR001+

TDF$AR004+TDF$AR005)/28)


TDF$AHone <- paste((TDF$AH029+TDF$AH032+TDF$AH039+TDF$AH040)/4)

TDF$AHtwo <-
paste((TDF$AH028+TDF$AH030+TDF$AH036+TDF$AH037+TDF$AH041)/5)

TDF$AHthree <- paste((TDF$AH038)/1)

TDF$AHfour <-
paste((TDF$AH031+TDF$AH033+TDF$AH034+TDF$AH035+TDF$AH049+

        TDF$AH050)/6)

TDF$AHfemale <-
paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+

         TDF$AH038+TDF$AH049+TDF$AH050)/8)

TDF$AHmale <-
paste((TDF$AH040+TDF$AH032+TDF$AH037+TDF$AH041+TDF$AH031+

        TDF$AH035+TDF$AH033+TDF$AH034)/8)


TDF$BSone <- paste((TDF$AL843+TDF$AL844+TDF$AL845+TDF$BS045)/4)

TDF$BStwo <- paste((TDF$BS044+TDF$AL846+TDF$AL850+TDF$AL852)/4)

TDF$BSthree <- paste((TDF$BS043+TDF$AL847+TDF$AL849)/3)

TDF$BSfour <- paste((TDF$AL839+TDF$AL841+TDF$AL851)/3)

TDF$BSfive <- paste((TDF$BS022+TDF$AL838+TDF$BS042+TDF$BS046)/4)

TDF$BSsix <- paste((TDF$BS018)/1)
```

```
TDF$BSfemale <-
paste((TDF$AL843+TDF$AL844+TDF$AL846+TDF$AL852+TDF$AL847+
                TDF$AL849+TDF$AL838+TDF$BS022)/8)

TDF$BSmale <-
paste((TDF$BS045+TDF$AL845+TDF$AL850+TDF$BS044+TDF$BS043+
                TDF$AL851+TDF$AL839+TDF$AL841+TDF$BS046+TDF$BS042+
                TDF$BS018)/11)



TDF$ARone <-
paste((TDF$AR003+TDF$AR010+TDF$AR021+TDF$AR023+TDF$AR027)/5)

TDF$ARtwo <- paste((TDF$AR017+TDF$AR019+TDF$AR024+TDF$AR026)/5)

TDF$ARthree <- paste((TDF$AR011+TDF$AR047+TDF$AR048)/3)

TDF$ARfour <-
paste((TDF$AR006+TDF$AR007+TDF$AR008+TDF$AR013+TDF$AR014+
                TDF$AR015)/6)

TDF$ARfive <- paste((TDF$AR002+TDF$AR009+TDF$AR012+TDF$AR020)/4)

TDF$ARsix <-
paste((TDF$AR001+TDF$AR004+TDF$AR005+TDF$AR016+TDF$AR025)/5)

TDF$ARfemale <-
paste((TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+
                TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+
                TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016)/14)

TDF$ARmale <-
paste((TDF$AR010+TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+
```

```
                TDF$AR007+TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+
                TDF$AR001+TDF$AR004+TDF$AR005)/14)

TDF$red <- paste((TDF$AH039+TDF$AH029+TDF$AH030+TDF$AH028+TDF$AH036+
               TDF$AH038+TDF$AH040+TDF$AH032+TDF$AH037+TDF$AH041+
               TDF$AH031+TDF$AH035+TDF$AH033+TDF$AH034+TDF$BS043)/15)
TDF$purple <- paste((TDF$AH049+TDF$AH050)/2)
TDF$green <- paste((TDF$AL838+TDF$AL839+TDF$AL841+TDF$AL843+TDF$AL844+
                TDF$AL845+TDF$AL846+TDF$AL847+TDF$AL849+TDF$AL850+
                TDF$AL851+TDF$AL852)/12)
TDF$orange <- paste((TDF$BS018+TDF$BS022+TDF$BS044+TDF$BS045+TDF$BS046+
                TDF$AR003+TDF$AR021+TDF$AR027+TDF$AR019+TDF$AR024+
                TDF$AR047+TDF$AR048+TDF$AR015+TDF$AR014+TDF$AR013+
                TDF$AR012+TDF$AR020+TDF$AR025+TDF$AR016+TDF$AR010+
                TDF$AR023+TDF$AR017+TDF$AR026+TDF$AR011+TDF$AR007+
                TDF$AR006+TDF$AR008+TDF$AR009+TDF$AR002+TDF$AR001+
                TDF$AR004+TDF$AR005)/32)

TDF<-lapply(TDF,as.numeric)

#
```

```
AH <- TDA$AH

BS <- TDA$BS

AR <- TDA$AR


sm <- sum(AH)

AH <- (AH/sm*100)

sm <- sum(BS)

BS <- (BS/sm*100)

sm <- sum(AR)

AR <- (AR/sm*100)


AHone <- TDA$AHone

AHtwo <- TDA$AHtwo

AHthree <- TDA$AHthree

AHfour <- TDA$AHfour


sm <- sum(AHone)

AHone <- (AHone/sm*100)

sm <- sum(AHtwo)

AHtwo <- (AHtwo/sm*100)
```

```
sm <- sum(AHthree)

AHthree <- (AHthree/sm*100)

sm <- sum(AHfour)

AHfour <- (AHfour/sm*100)


BSone <- TDA$BSone

BStwo <- TDA$BStwo

BSthree <- TDA$BSthree

BSfour <- TDA$BSfour

BSfive <- TDA$BSfive

BSsix <- TDA$BSsix


sm <- sum(BSone)

BSone <- (BSone/sm*100)

sm <- sum(BStwo)

BStwo <- (BStwo/sm*100)

sm <- sum(BSthree)

BSthree <- (BSthree/sm*100)

sm <- sum(BSfour)

BSfour <- (BSfour/sm*100)

sm <- sum(BSfive)
```

```
BSfive <- (BSfive/sm*100)

sm <- sum(BSsix)

BSsix <- (BSsix/sm*100)


ARone <- TDA$ARone

ARtwo <- TDA$ARtwo

ARthree <- TDA$ARthree

ARfour <- TDA$ARfour

ARfive <- TDA$ARfive

ARsix <- TDA$ARsix


sm <- sum(ARone)

ARone <- (ARone/sm*100)

sm <- sum(ARtwo)

ARtwo <- (ARtwo/sm*100)

sm <- sum(ARthree)

ARthree <- (ARthree/sm*100)

sm <- sum(ARfour)

ARfour <- (ARfour/sm*100)

sm <- sum(ARfive)

ARfive <- (ARfive/sm*100)
```

```
sm <- sum(ARsix)

ARsix <- (ARsix/sm*100)


AHfemale <- TDA$AHfemale

AHmale <- TDA$AHmale

BSfemale <- TDA$BSfemale

BSmale <- TDA$BSmale

ARfemale <- TDA$ARfemale

ARmale <- TDA$ARmale


sm <- sum(AHfemale)

AHfemale <- (AHfemale/sm*100)

sm <- sum(AHmale)

AHmale <- (AHmale/sm*100)

sm <- sum(BSfemale)

BSfemale <- (BSfemale/sm*100)

sm <- sum(BSmale)

BSmale <- (BSmale/sm*100)

sm <- sum(ARfemale)

ARfemale <- (ARfemale/sm*100)

sm <- sum(ARmale)
```

```
ARmale <- (ARmale/sm*100)


red <- TDA$red

purple <- TDA$purple

green <- TDA$green

orange <- TDA$orange


sm <- sum(red)

red <- (red/sm*100)

sm <- sum(purple)

purple <- (purple/sm*100)

sm <- sum(green)

green <- (green/sm*100)

sm <- sum(orange)

orange <- (orange/sm*100)


best_identity <- TDS$best_identity

sci <- TAX$scientific_name

phylum <- TAX$phylum_name

class <- TAX$class_name

species <- TAX$species_name
```

```
Rdf <- data.frame(best_identity,sci,phylum,class,species,
                  AH,BS,AR,
                  AHone,AHtwo,AHthree,AHfour,
                  BSone,BStwo,BSthree,BSfour,BSfive,BSsix,
                  ARone,ARtwo,ARthree,ARfour,ARfive,ARsix,
                  AHfemale,AHmale,
                  BSfemale,BSmale,
                  ARfemale,ARmale,
                  red,purple,green,orange)
#


AH <- TDF$AH

BS <- TDF$BS

AR <- TDF$AR


sm <- sum(AH)

AH <- (AH/sm*100)

sm <- sum(BS)

BS <- (BS/sm*100)

sm <- sum(AR)
```

```
AR <- (AR/sm*100)


AHone <- TDF$AHone

AHtwo <- TDF$AHtwo

AHthree <- TDF$AHthree

AHfour <- TDF$AHfour


sm <- sum(AHone)

AHone <- (AHone/sm*100)

sm <- sum(AHtwo)

AHtwo <- (AHtwo/sm*100)

sm <- sum(AHthree)

AHthree <- (AHthree/sm*100)

sm <- sum(AHfour)

AHfour <- (AHfour/sm*100)


BSone <- TDF$BSone

BStwo <- TDF$BStwo

BSthree <- TDF$BSthree

BSfour <- TDF$BSfour

BSfive <- TDF$BSfive
```

```
BSsix <- TDF$BSsix


sm <- sum(BSone)

BSone <- (BSone/sm*100)

sm <- sum(BStwo)

BStwo <- (BStwo/sm*100)

sm <- sum(BSthree)

BSthree <- (BSthree/sm*100)

sm <- sum(BSfour)

BSfour <- (BSfour/sm*100)

sm <- sum(BSfive)

BSfive <- (BSfive/sm*100)

sm <- sum(BSsix)

BSsix <- (BSsix/sm*100)


ARone <- TDF$ARone

ARtwo <- TDF$ARtwo

ARthree <- TDF$ARthree

ARfour <- TDF$ARfour

ARfive <- TDF$ARfive

ARsix <- TDF$ARsix
```

```
sm <- sum(ARone)

ARone <- (ARone/sm*100)

sm <- sum(ARtwo)

ARtwo <- (ARtwo/sm*100)

sm <- sum(ARthree)

ARthree <- (ARthree/sm*100)

sm <- sum(ARfour)

ARfour <- (ARfour/sm*100)

sm <- sum(ARfive)

ARfive <- (ARfive/sm*100)

sm <- sum(ARsix)

ARsix <- (ARsix/sm*100)


AHfemale <- TDF$AHfemale

AHmale <- TDF$AHmale

BSfemale <- TDF$BSfemale

BSmale <- TDF$BSmale

ARfemale <- TDF$ARfemale

ARmale <- TDF$ARmale
```

```
sm <- sum(AHfemale)

AHfemale <- (AHfemale/sm*100)

sm <- sum(AHmale)

AHmale <- (AHmale/sm*100)

sm <- sum(BSfemale)

BSfemale <- (BSfemale/sm*100)

sm <- sum(BSmale)

BSmale <- (BSmale/sm*100)

sm <- sum(ARfemale)

ARfemale <- (ARfemale/sm*100)

sm <- sum(ARmale)

ARmale <- (ARmale/sm*100)


red <- TDF$red

purple <- TDF$purple

green <- TDF$green

orange <- TDF$orange


sm <- sum(red)

red <- (red/sm*100)

sm <- sum(purple)
```

```
purple <- (purple/sm*100)

sm <- sum(green)

green <- (green/sm*100)

sm <- sum(orange)

orange <- (orange/sm*100)


Fdf <- data.frame(best_identity,sci,phylum,class,species,

                  AH,BS,AR,

                  AHone,AHtwo,AHthree,AHfour,

                  BSone,BStwo,BSthree,BSfour,BSfive,BSsix,

                  ARone,ARtwo,ARthree,ARfour,ARfive,ARsix,

                  AHfemale,AHmale,

                  BSfemale,BSmale,

                  ARfemale,ARmale,

                  red,purple,green,orange)


#


P <- subset(TDS, select = c(18:79))

P <- lapply(P,as.numeric)
```

```
TDR <- data.frame(TDS$best_identity,
                  TAX$scientific_name,TAX$phylum_name,
                  TAX$class_name,TAX$species_name,
                 P$AH028,P$AH029,P$AH030,P$AH031,
                 P$AH032,P$AH033,P$AH034,P$AH035,
                 P$AH036,P$AH037,P$AH038,P$AH039,
                 P$AH040,P$AH041,P$AH049,P$AH050,
                 P$AL838,P$AL839,P$AL841,P$AL843,
                 P$AL844,P$AL845,P$AL846,P$AL847,
                 P$AL849,P$AL850,P$AL851,P$AL852,
                 P$AR001,P$AR002,P$AR003,P$AR004,
                 P$AR005,P$AR006,P$AR007,P$AR008,
                 P$AR009,P$AR010,P$AR011,P$AR012,
                 P$AR013,P$AR014,P$AR015,P$AR016,
                 P$AR017,P$AR019,P$AR020,P$AR021,
                 P$AR023,P$AR024,P$AR025,P$AR026,
                 P$AR027,P$AR047,P$AR048,P$BS018,
                 P$BS022,P$BS042,P$BS043,P$BS044,
                 P$BS045,P$BS046)

colnames(TDR)<-gsub("P.","",colnames(TDR))
```

```
colnames(TDR)<-gsub("TAX.","",colnames(TDR))

colnames(TDR)<-gsub("TDS.","",colnames(TDR))


#


P$AH028 <- (P$AH028/(sum(P$AH028))*100)

P$AH029 <- (P$AH029/(sum(P$AH029))*100)

P$AH030 <- (P$AH030/(sum(P$AH030))*100)

P$AH031 <- (P$AH031/(sum(P$AH031))*100)

P$AH032 <- (P$AH032/(sum(P$AH032))*100)

P$AH033 <- (P$AH033/(sum(P$AH033))*100)

P$AH034 <- (P$AH034/(sum(P$AH034))*100)

P$AH035 <- (P$AH035/(sum(P$AH035))*100)

P$AH036 <- (P$AH036/(sum(P$AH036))*100)

P$AH037 <- (P$AH037/(sum(P$AH037))*100)

P$AH038 <- (P$AH038/(sum(P$AH038))*100)

P$AH039 <- (P$AH039/(sum(P$AH039))*100)

P$AH040 <- (P$AH040/(sum(P$AH040))*100)

P$AH041 <- (P$AH041/(sum(P$AH041))*100)

P$AH049 <- (P$AH049/(sum(P$AH049))*100)

P$AH050 <- (P$AH050/(sum(P$AH050))*100)
```

```
P$AL838 <- (P$AL838/(sum(P$AL838))*100)

P$AL839 <- (P$AL839/(sum(P$AL839))*100)

P$AL841 <- (P$AL841/(sum(P$AL841))*100)

P$AL843 <- (P$AL843/(sum(P$AL843))*100)

P$AL844 <- (P$AL844/(sum(P$AL844))*100)

P$AL845 <- (P$AL845/(sum(P$AL845))*100)

P$AL846 <- (P$AL846/(sum(P$AL846))*100)

P$AL847 <- (P$AL847/(sum(P$AL847))*100)

P$AL849 <- (P$AL849/(sum(P$AL849))*100)

P$AL850 <- (P$AL850/(sum(P$AL850))*100)

P$AL851 <- (P$AL851/(sum(P$AL851))*100)

P$AL852 <- (P$AL852/(sum(P$AL852))*100)


P$AR001 <- (P$AR001/(sum(P$AR001))*100)

P$AR002 <- (P$AR002/(sum(P$AR002))*100)

P$AR003 <- (P$AR003/(sum(P$AR003))*100)

P$AR004 <- (P$AR004/(sum(P$AR004))*100)

P$AR005 <- (P$AR005/(sum(P$AR005))*100)

P$AR006 <- (P$AR006/(sum(P$AR006))*100)

P$AR007 <- (P$AR007/(sum(P$AR007))*100)
```

```
P$AR008 <- (P$AR008/(sum(P$AR008))*100)

P$AR009 <- (P$AR009/(sum(P$AR009))*100)

P$AR010 <- (P$AR010/(sum(P$AR010))*100)

P$AR011 <- (P$AR011/(sum(P$AR011))*100)

P$AR012 <- (P$AR012/(sum(P$AR012))*100)

P$AR013 <- (P$AR013/(sum(P$AR013))*100)

P$AR014 <- (P$AR014/(sum(P$AR014))*100)

P$AR015 <- (P$AR015/(sum(P$AR015))*100)

P$AR016 <- (P$AR016/(sum(P$AR016))*100)

P$AR017 <- (P$AR017/(sum(P$AR017))*100)

P$AR019 <- (P$AR019/(sum(P$AR019))*100)

P$AR020 <- (P$AR020/(sum(P$AR020))*100)

P$AR021 <- (P$AR021/(sum(P$AR021))*100)

P$AR023 <- (P$AR023/(sum(P$AR023))*100)

P$AR024 <- (P$AR024/(sum(P$AR024))*100)

P$AR025 <- (P$AR025/(sum(P$AR025))*100)

P$AR026 <- (P$AR026/(sum(P$AR026))*100)

P$AR027 <- (P$AR027/(sum(P$AR027))*100)

P$AR047 <- (P$AR047/(sum(P$AR047))*100)

P$AR048 <- (P$AR048/(sum(P$AR048))*100)
```

```
P$BS018 <- (P$BS018/(sum(P$BS018))*100)

P$BS022 <- (P$BS022/(sum(P$BS022))*100)

P$BS042 <- (P$BS042/(sum(P$BS042))*100)

P$BS043 <- (P$BS043/(sum(P$BS043))*100)

P$BS044 <- (P$BS044/(sum(P$BS044))*100)

P$BS045 <- (P$BS045/(sum(P$BS045))*100)

P$BS046 <- (P$BS046/(sum(P$BS046))*100)


TDPR <- data.frame(TDS$best_identity,

                   TAX$scientific_name,TAX$phylum_name,

                   TAX$class_name,TAX$species_name,

                  P$AH028,P$AH029,P$AH030,P$AH031,

                  P$AH032,P$AH033,P$AH034,P$AH035,

                  P$AH036,P$AH037,P$AH038,P$AH039,

                  P$AH040,P$AH041,P$AH049,P$AH050,

                  P$AL838,P$AL839,P$AL841,P$AL843,

                  P$AL844,P$AL845,P$AL846,P$AL847,

                  P$AL849,P$AL850,P$AL851,P$AL852,

                  P$AR001,P$AR002,P$AR003,P$AR004,

                  P$AR005,P$AR006,P$AR007,P$AR008,

                  P$AR009,P$AR010,P$AR011,P$AR012,
```

```
            P$AR013,P$AR014,P$AR015,P$AR016,
            P$AR017,P$AR019,P$AR020,P$AR021,
            P$AR023,P$AR024,P$AR025,P$AR026,
            P$AR027,P$AR047,P$AR048,P$BS018,
            P$BS022,P$BS042,P$BS043,P$BS044,
            P$BS045,P$BS046)

colnames(TDPR)<-gsub("P.","",colnames(TDPR))
colnames(TDPR)<-gsub("TAX.","",colnames(TDPR))
colnames(TDPR)<-gsub("TDS.","",colnames(TDPR))

#

P <- TDPR
P[P > 0] <- 1
TDF1 <- P

TDF1$best_identity <- TDS$best_identity
TDF1$scientific_name <- TAX$scientific_name
TDF1$phylum_name <- TAX$phylum_name
TDF1$class_name <- TAX$class_name
```

```
TDF1$species_name <- TAX$species_name


#


P$AH028 <- (P$AH028/(sum(P$AH028))*100)

P$AH029 <- (P$AH029/(sum(P$AH029))*100)

P$AH030 <- (P$AH030/(sum(P$AH030))*100)

P$AH031 <- (P$AH031/(sum(P$AH031))*100)

P$AH032 <- (P$AH032/(sum(P$AH032))*100)

P$AH033 <- (P$AH033/(sum(P$AH033))*100)

P$AH034 <- (P$AH034/(sum(P$AH034))*100)

P$AH035 <- (P$AH035/(sum(P$AH035))*100)

P$AH036 <- (P$AH036/(sum(P$AH036))*100)

P$AH037 <- (P$AH037/(sum(P$AH037))*100)

P$AH038 <- (P$AH038/(sum(P$AH038))*100)

P$AH039 <- (P$AH039/(sum(P$AH039))*100)

P$AH040 <- (P$AH040/(sum(P$AH040))*100)

P$AH041 <- (P$AH041/(sum(P$AH041))*100)

P$AH049 <- (P$AH049/(sum(P$AH049))*100)

P$AH050 <- (P$AH050/(sum(P$AH050))*100)
```

```
P$AL838 <- (P$AL838/(sum(P$AL838))*100)

P$AL839 <- (P$AL839/(sum(P$AL839))*100)

P$AL841 <- (P$AL841/(sum(P$AL841))*100)

P$AL843 <- (P$AL843/(sum(P$AL843))*100)

P$AL844 <- (P$AL844/(sum(P$AL844))*100)

P$AL845 <- (P$AL845/(sum(P$AL845))*100)

P$AL846 <- (P$AL846/(sum(P$AL846))*100)

P$AL847 <- (P$AL847/(sum(P$AL847))*100)

P$AL849 <- (P$AL849/(sum(P$AL849))*100)

P$AL850 <- (P$AL850/(sum(P$AL850))*100)

P$AL851 <- (P$AL851/(sum(P$AL851))*100)

P$AL852 <- (P$AL852/(sum(P$AL852))*100)


P$AR001 <- (P$AR001/(sum(P$AR001))*100)

P$AR002 <- (P$AR002/(sum(P$AR002))*100)

P$AR003 <- (P$AR003/(sum(P$AR003))*100)

P$AR004 <- (P$AR004/(sum(P$AR004))*100)

P$AR005 <- (P$AR005/(sum(P$AR005))*100)

P$AR006 <- (P$AR006/(sum(P$AR006))*100)

P$AR007 <- (P$AR007/(sum(P$AR007))*100)

P$AR008 <- (P$AR008/(sum(P$AR008))*100)
```

```
P$AR009 <- (P$AR009/(sum(P$AR009))*100)

P$AR010 <- (P$AR010/(sum(P$AR010))*100)

P$AR011 <- (P$AR011/(sum(P$AR011))*100)

P$AR012 <- (P$AR012/(sum(P$AR012))*100)

P$AR013 <- (P$AR013/(sum(P$AR013))*100)

P$AR014 <- (P$AR014/(sum(P$AR014))*100)

P$AR015 <- (P$AR015/(sum(P$AR015))*100)

P$AR016 <- (P$AR016/(sum(P$AR016))*100)

P$AR017 <- (P$AR017/(sum(P$AR017))*100)

P$AR019 <- (P$AR019/(sum(P$AR019))*100)

P$AR020 <- (P$AR020/(sum(P$AR020))*100)

P$AR021 <- (P$AR021/(sum(P$AR021))*100)

P$AR023 <- (P$AR023/(sum(P$AR023))*100)

P$AR024 <- (P$AR024/(sum(P$AR024))*100)

P$AR025 <- (P$AR025/(sum(P$AR025))*100)

P$AR026 <- (P$AR026/(sum(P$AR026))*100)

P$AR027 <- (P$AR027/(sum(P$AR027))*100)

P$AR047 <- (P$AR047/(sum(P$AR047))*100)

P$AR048 <- (P$AR048/(sum(P$AR048))*100)


P$BS018 <- (P$BS018/(sum(P$BS018))*100)
```

```
P$BS022 <- (P$BS022/(sum(P$BS022))*100)

P$BS042 <- (P$BS042/(sum(P$BS042))*100)

P$BS043 <- (P$BS043/(sum(P$BS043))*100)

P$BS044 <- (P$BS044/(sum(P$BS044))*100)

P$BS045 <- (P$BS045/(sum(P$BS045))*100)

P$BS046 <- (P$BS046/(sum(P$BS046))*100)


TDPF1 <- P

TDPF1$best_identity <- TDS$best_identity

TDPF1$scientific_name <- TAX$scientific_name

TDPF1$phylum_name <- TAX$phylum_name

TDPF1$class_name <- TAX$class_name

TDPF1$species_name <- TAX$species_name


#DATASETS

TAX <- subset(TDS,select = c(3,6:13))

OTU <- subset(TDR,select = c(6:67))

OTU <- OTU*3

OTU$AR026 <- OTU$AR026*2

OTU.r <- subset(TDPR,select = c(6:67))

OTU.p <- subset(TDPF1,select = c(6:67))
```

```
META <- subset(a1, select = c(1,4:5,7:10,12,18))


#Dimensions

dim(TAX)

dim(OTU.r)

dim(OTU.p)

dim(META)
```

Map

```{r}
#install.packages(c("ggOceanMapsData", "ggOceanMaps"),

#                 repos = #c("https://cloud.r-project.org","https://mikkovihtakari.github.io/drat"))


map=a1


map$Species <- as.character(map$Species)

map$Species[map$Species == "Thornyskate"] <- "Thorny skate"


map$Species <- as.character(map$Species)

map$Species[map$Species == "Arcticskate"] <- "Arctic skate"
```

```
map$Species <- as.character(map$Species)

map$Species[map$Species == "Spinytailskate"] <- "Spinytail skate"


map <- subset(map,select = -c(6,13:16))


map<-transform(map,

        Lat=as.numeric(Lat),

        Lon=as.numeric(Lon))


# start lon, slutt lon, start lat, slutt lat


map1 = basemap(limits = c(65), bathymetry = TRUE, land.border.col = NA) +

  geom_spatial_point(data = map, shape = 20, alpha = 0.5,

                aes(x = Lon, y = Lat, size = 1, color=Species)) +

  labs(subtitle = paste("n = ", nrow(map))) +

  labs(title = paste("Map"))


map1 # the whole Arctic


map2 = basemap(limits = c(-2, 35, 70, 83), bathymetry = TRUE, land.border.col = NA) +

  geom_spatial_point(data = map, shape = 20, alpha = 0.5,
```

```
                aes(x = Lon, y = Lat, color=Species, size = 1)) +
  labs(subtitle = paste("n = ", nrow(map))) +
  labs(title = paste("Map"))

map2 # small map
```

BARPLOTS Abundance, Relative abundance, Frequency of occurrence, % Frequency of occurrence

```{r}
positions <- c(
"AL843","AL844","AL846","AL852","AL847","AL849","AL838","BS022","BS045","AL845","AL850","BS044",
"BS043","AL851","AL839","AL841","BS046","BS042","BS018","",
"AH039","AH029","AH030","AH028","AH036","AH038","AH049","AH050","AH040","AH032","AH037","AH041",
"AH031","AH035","AH033","AH034","",
"AR003","AR021","AR027","AR019","AR024","AR047","AR048","AR015","AR014","AR013","AR012","AR020",
"AR025","AR016","AR010","AR023","AR017","AR026","AR011","AR007","AR006","AR008","AR009","AR002",
"AR001","AR004","AR005")
```

```
TDRx <- TDR

TDRx <- aggregate(TDRx[c(-1,-2,-3,-4,-5)], TDRx["phylum_name"], sum)

TDRx <- melt(TDRx,id.vars="phylum_name")

names(TDRx)[1] <- "phylum"


ggplot(TDRx,aes(variable,log(value),fill=phylum))+

  geom_bar(stat="identity")+

  xlab("")+ylab("log(reads)")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)


TDPRx <- TDPR

TDPRx <- aggregate(TDPRx[c(-1,-2,-3,-4,-5)], TDPRx["phylum_name"], sum)

TDPRx <- melt(TDPRx,id.vars="phylum_name")

names(TDPRx)[1] <- "phylum"


ggplot(TDPRx,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+

  xlab("")+ylab("Relative abundance (%)")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)
```

```
TDF1x <- TDF1

TDF1x <- aggregate(TDF1x[c(-1,-2,-3,-4,-5)], TDF1x["phylum_name"], sum)

TDF1x <- melt(TDF1x,id.vars="phylum_name")

names(TDF1x)[1] <- "phylum"


ggplot(TDF1x,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+

  xlab("")+ylab("Frequency of occurrence")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)


TDPF1x <- TDPF1

TDPF1x <- aggregate(TDPF1x[c(-1,-2,-3,-4,-5)], TDPF1x["phylum_name"], sum)

TDPF1x <- melt(TDPF1x,id.vars="phylum_name")

names(TDPF1x)[1] <- "phylum"


ggplot(TDPF1x,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+

  xlab("")+ylab("% Frequency of occurrence")+

  theme(axis.text.x = element_text(angle = 90))+
```

```
  scale_x_discrete(limits = positions)
```

Pelagic and benthic diet realtive abundance

```{r}
df <- subset(Rdf, select = c(1:12,25:26))

df <- (subset(df, best_identity>0.98))

df <- subset(df, select = c(3:14))



df <- subset(df, species != "")



df <- subset(df, species != "Abyssoninoe scopa")

df <- subset(df, species != "Lumbrineris sp. CMC01")

df <- subset(df, species != "Phyllodoce sp. CMC01")

df <- subset(df, species != "Austrolaenilla mollis")

df <- subset(df, species != "SigalionidaeGEN sp. MC")

df <- subset(df, species != "Cossura pygodactilata")

df <- subset(df, species != "Laonice sp. DZMB-HH-57467.65")

df <- subset(df, species != "Zygomolgus dentatus")

df <- subset(df, species != "Clavella adunca") #Ectoparasite on Gadus morhua

df <- subset(df, species != "Amphilochus sp1Pans")

df <- subset(df, species != "Halirages qvadridentatus")
```

```
df <- subset(df, species != "Rhachotropis macropus")

df <- subset(df, species != "Gammaridae sp. KML 32")

df <- subset(df, species != "Liljeborgia fissicornis")

df <- subset(df, species != "Microphasma agassizi")

df <- subset(df, species != "Eurycope inermis")

df <- subset(df, species != "Ilyarachna torleivi")

df <- subset(df, species != "Munnopsurus giganteus")

df <- subset(df, species != "Balanus balanus") #two life stages

df <- subset(df, species != "Balaenoptera physalus") #Whale fall?


#Pelagic species, removing benthic species

pel <- df

pel <- subset(pel,species != "Nothria conchylega CMC02")

pel <- subset(pel,species != "Ophelina acuminata")

pel <- subset(pel,species != "Gyptis golikovi")

pel <- subset(pel,species != "Aglaophamus malmgreni")

pel <- subset(pel,species != "Nephtys caeca")

pel <- subset(pel,species != "Nephtys ciliata")

pel <- subset(pel,species != "Pholoe baltica")

pel <- subset(pel,species != "Bylgides groenlandicus")

pel <- subset(pel,species != "Eunoe nodosa CMC01")
```

```
pel <- subset(pel,species != "Galathowenia oculata")

pel <- subset(pel,species != "Chone infundibuliformis")

pel <- subset(pel,species != "Hydroides elegans")

pel <- subset(pel,species != "Chirimia biceps")

pel <- subset(pel,species != "Laonice cirrata")

pel <- subset(pel,species != "Prionospio cirrifera")

pel <- subset(pel,species != "Lysippe labiata")

pel <- subset(pel,species != "Chaetozone setosa")

pel <- subset(pel,species != "Polycirrus arcticus")

pel <- subset(pel,species != "Golfingia margaritacea")

pel <- subset(pel,species != "Gitanopsis bispinosa")

pel <- subset(pel,species != "Aoroides exilis")

pel <- subset(pel,species != "Cleippides quadricuspis")

pel <- subset(pel,species != "Halirages fulvocinctus")

pel <- subset(pel,species != "Caprella equilibra")

pel <- subset(pel,species != "Monocorophium acherusicum")

pel <- subset(pel,species != "Dulichia tuberculata")

pel <- subset(pel,species != "Paramphithoe hystrix")

pel <- subset(pel,species != "Eurythenes gryllus")

pel <- subset(pel,species != "Eusirus holmi")

pel <- subset(pel,species != "Rhachotropis lomonosovi")
```

```
pel <- subset(pel,species != "Maera loveni")

pel <- subset(pel,species != "Bathymedon obtusifrons")

pel <- subset(pel,species != "Monoculodes packardi")

pel <- subset(pel,species != "Paroediceros curvirostratus")

pel <- subset(pel,species != "Rostroculodes borealis")

pel <- subset(pel,species != "Pardalisca abyssi")

pel <- subset(pel,species != "Neopleustes pulchellus")

pel <- subset(pel,species != "Syrrhoe crenulata")

pel <- subset(pel,species != "Schisturella pulchra")

pel <- subset(pel,species != "Anonyx compactus")

pel <- subset(pel,species != "Onisimus itoralis JMG02")

pel <- subset(pel,species != "Tmetonyx cicada")

pel <- subset(pel,species != "Diastylis goodsiri")

pel <- subset(pel,species != "Leucon nathorsti")

pel <- subset(pel,species != "Pontophilus norvegicus")

pel <- subset(pel,species != "Sabinea septemcarinata")

pel <- subset(pel,species != "Bythocaris irene")

pel <- subset(pel,species != "Pandalus borealis")

pel <- subset(pel,species != "Saduria entomon")

pel <- subset(pel,species != "Ilyarachna hirticeps")

pel <- subset(pel,species != "Munnopsis typica")
```

```
pel <- subset(pel,species != "Erythrops glacialis")

pel <- subset(pel,species != "Mysideis insignis")

pel <- subset(pel,species != "Watersipora subovoidea")

pel <- subset(pel,species != "Melanogrammus aeglefinus")

pel <- subset(pel,species != "Gaidropsarus argentatus")

pel <- subset(pel,species != "Paraliparis bathybius")

pel <- subset(pel,species != "Cottunculus microps")

pel <- subset(pel,species != "Leptoclinus maculatus")

pel <- subset(pel,species != "Lumpenus lampretaeformis")

pel <- subset(pel,species != "Lycenchelys muraena")

pel <- subset(pel,species != "Lycodes esmarkii")

pel <- subset(pel,species != "Reinhardtius hippoglossoides")

pel <- subset(pel,species != "Diplosoma listerianum")

pel <- subset(pel,species != "Obelia dichotoma") #Sessile

pel <- subset(pel,species != "Clytia hemisphaerica") #Sessile

pel <- subset(pel,species != "Brisaster fragilis")

pel <- subset(pel,species != "Ophiocten gracilis")

pel <- subset(pel,species != "Yoldiella frigida")

pel <- subset(pel,species != "Chaetoderma nitidulum")

pel <- subset(pel,species != "Bathypolypus arcticus")

pel <- subset(pel,species != "Siphonodentalium lobatum")
```

```
pel <- subset(pel,species != "Micrura varicolor")

pel <- subset(pel,species != "Priapulus caudatus")

pel <- subset(pel,species != "Harmothoe globifera")

pel <- subset(pel,species != "Harmothoe sp. CMC01")

pel <- subset(pel,species != "Bougainvillia muscus") #hydroid and medusa stage


#Benthic species, removing pelagic species

ben <- df

ben <- subset(ben,species !="Apherusa glacialis") #ice-associated amphipod, pelagic occurrences of A. glacialis have also been reported (Kunisch et al., 2020) Pelagic occurrences of the ice amphipod Apherusa glacialis throughout the Arctic

ben <- subset(ben,species !="Themisto abyssorum")

ben <- subset(ben,species !="Themisto libellula")

ben <- subset(ben,species !="Arrhis phyllonyx")

ben <- subset(ben,species !="Hymenodora glacialis")

ben <- subset(ben,species !="Pasiphaea tarda")

ben <- subset(ben,species !="Meganyctiphanes norvegica")

ben <- subset(ben,species !="Thysanoessa inermis")

ben <- subset(ben,species !="Thysanoessa longicaudata")

ben <- subset(ben,species !="Boreomysis nobilis")

ben <- subset(ben,species !="Chiridius gracilis")

ben <- subset(ben,species !="Gaetanus tenuispinus")
```

```
ben <- subset(ben,species !="Calanus hyperboreus")

ben <- subset(ben,species !="Microcalanus pusillus")

ben <- subset(ben,species !="Pseudocalanus acuspes")

ben <- subset(ben,species !="Pseudocalanus minutus")

ben <- subset(ben,species !="Metridia longa")

ben <- subset(ben,species !="Scolecithricella minor")

ben <- subset(ben,species !="Oithona similis")

ben <- subset(ben,species !="Boroecia maxima")

ben <- subset(ben,species !="Pseudosagitta maxima")

ben <- subset(ben,species !="Sagitta elegans")

ben <- subset(ben,species !="Clupea harengus")

ben <- subset(ben,species !="Boreogadus saida") #Polartorsken beiter semipelagisk og ned til havbunnen

ben <- subset(ben,species !="Mallotus villosus")

ben <- subset(ben,species !="Cyclopterus lumpus")

ben <- subset(ben,species !="Campanularia hincksii") #medusa?

ben <- subset(ben,species !="Aglantha digitale") #medusa?

ben <- subset(ben,species !="Cyanea capillata") #medusa?

ben <- subset(ben,species !="Gonatus steenstrupi")
```

BARPLOT AH v BS v AR

```{r}

pel1 <- subset(pel, select = (1:6))

ben1 <- subset(ben, select = (1:6))


pel1 <- aggregate(pel1[c(-1,-2,-3)], pel1["species"], sum)

ben1 <- aggregate(ben1[c(-1,-2,-3)], ben1["species"], sum)


pel1 <- melt(pel1,id.vars="species")

ben1 <- melt(ben1,id.vars="species")


anova <- aov(value ~ variable, pel1)

summary(anova)


anova <- aov(value ~ variable, ben1)

summary(anova)


pel2 <- subset(pel, select = (1:6))

ben2 <- subset(ben, select = (1:6))


names(pel2)[4] <- "AH_pel"

names(pel2)[5] <- "BS_pel"
```

```
names(pel2)[6] <- "AR_pel"


names(ben2)[4] <- "AH_ben"

names(ben2)[5] <- "BS_ben"

names(ben2)[6] <- "AR_ben"


pel2 <- aggregate(pel2[c(-1,-2,-3)], pel2["species"], sum)

ben2 <- aggregate(ben2[c(-1,-2,-3)], ben2["species"], sum)


pel2 <- melt(pel2,id.vars="species")

ben2 <- melt(ben2,id.vars="species")


df <- rbind(pel2, ben2)


df %>%

  mutate(name = fct_relevel(variable,

           "BS_pel","BS_ben",

           "AH_pel","AH_ben",

           "AR_pel","AR_ben" )) %>%

  ggplot( aes(x=name, y=value, fill=variable)) +

    geom_bar(stat="identity")+
```

```
   xlab("Skates")+ylab("Relative abundance")+

     scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))
```

BARPLOT phylum AH

```{r}
pel1 <- subset(pel, select = c(1:3,7:10))


pel1 <- aggregate(pel1[c(-1,-2,-3)], pel1["phylum"], sum)

pel1 <- melt(pel1,id.vars="phylum")


anova <- aov(value ~ variable, pel1)

summary(anova)


ggplot(pel1,aes(variable,value,fill=phylum))+

 geom_bar(stat="identity")+

 xlab("Arctic skate")+ylab("Relative abundance")
```

Barplot specis AH

```{r}
```

```
pel1 <- subset(pel, select = c(3,7:10))

pel1 <- aggregate(pel1[-1], pel1["species"], sum)

pel1 <- melt(pel1,id.vars="species")

pel1 <- subset(pel1, value>5)


ggplot(pel1,aes(variable,value,fill=species))+

  geom_bar(stat="identity")+

  xlab("Arctic skate")+ylab("Relative abundance")
```

Pelagic and benthic diet % frequency of occurrence

```{r}
df <- subset(Fdf, select = c(1:12,25:26))

df <- (subset(df, best_identity>0.98))

df <- subset(df, select = c(3:14))


df <- subset(df, species != "")


df <- subset(df, species != "Abyssoninoe scopa")

df <- subset(df, species != "Lumbrineris sp. CMC01")

df <- subset(df, species != "Phyllodoce sp. CMC01")

df <- subset(df, species != "Austrolaenilla mollis")
```

```
df <- subset(df, species != "SigalionidaeGEN sp. MC")

df <- subset(df, species != "Cossura pygodactilata")

df <- subset(df, species != "Laonice sp. DZMB-HH-57467.65")

df <- subset(df, species != "Zygomolgus dentatus")

df <- subset(df, species != "Clavella adunca") #Ectoparasite on Gadus morhua

df <- subset(df, species != "Amphilochus sp1Pans")

df <- subset(df, species != "Halirages qvadridentatus")

df <- subset(df, species != "Rhachotropis macropus")

df <- subset(df, species != "Gammaridae sp. KML 32")

df <- subset(df, species != "Liljeborgia fissicornis")

df <- subset(df, species != "Microphasma agassizi")

df <- subset(df, species != "Eurycope inermis")

df <- subset(df, species != "Ilyarachna torleivi")

df <- subset(df, species != "Munnopsurus giganteus")

df <- subset(df, species != "Balanus balanus") #two life stages

df <- subset(df, species != "Balaenoptera physalus") #Whale fall?


#Pelagic species, removing benthic species

pel <- df

pel <- subset(pel,species != "Nothria conchylega CMC02")

pel <- subset(pel,species != "Ophelina acuminata")
```

```
pel <- subset(pel,species != "Gyptis golikovi")

pel <- subset(pel,species != "Aglaophamus malmgreni")

pel <- subset(pel,species != "Nephtys caeca")

pel <- subset(pel,species != "Nephtys ciliata")

pel <- subset(pel,species != "Pholoe baltica")

pel <- subset(pel,species != "Bylgides groenlandicus")

pel <- subset(pel,species != "Eunoe nodosa CMC01")

pel <- subset(pel,species != "Galathowenia oculata")

pel <- subset(pel,species != "Chone infundibuliformis")

pel <- subset(pel,species != "Hydroides elegans")

pel <- subset(pel,species != "Chirimia biceps")

pel <- subset(pel,species != "Laonice cirrata")

pel <- subset(pel,species != "Prionospio cirrifera")

pel <- subset(pel,species != "Lysippe labiata")

pel <- subset(pel,species != "Chaetozone setosa")

pel <- subset(pel,species != "Polycirrus arcticus")

pel <- subset(pel,species != "Golfingia margaritacea")

pel <- subset(pel,species != "Gitanopsis bispinosa")

pel <- subset(pel,species != "Aoroides exilis")

pel <- subset(pel,species != "Cleippides quadricuspis")

pel <- subset(pel,species != "Halirages fulvocinctus")
```

```
pel <- subset(pel,species != "Caprella equilibra")

pel <- subset(pel,species != "Monocorophium acherusicum")

pel <- subset(pel,species != "Dulichia tuberculata")

pel <- subset(pel,species != "Paramphithoe hystrix")

pel <- subset(pel,species != "Eurythenes gryllus")

pel <- subset(pel,species != "Eusirus holmi")

pel <- subset(pel,species != "Rhachotropis lomonosovi")

pel <- subset(pel,species != "Maera loveni")

pel <- subset(pel,species != "Bathymedon obtusifrons")

pel <- subset(pel,species != "Monoculodes packardi")

pel <- subset(pel,species != "Paroediceros curvirostratus")

pel <- subset(pel,species != "Rostroculodes borealis")

pel <- subset(pel,species != "Pardalisca abyssi")

pel <- subset(pel,species != "Neopleustes pulchellus")

pel <- subset(pel,species != "Syrrhoe crenulata")

pel <- subset(pel,species != "Schisturella pulchra")

pel <- subset(pel,species != "Anonyx compactus")

pel <- subset(pel,species != "Onisimus itoralis JMG02")

pel <- subset(pel,species != "Tmetonyx cicada")

pel <- subset(pel,species != "Diastylis goodsiri")

pel <- subset(pel,species != "Leucon nathorsti")
```

```
pel <- subset(pel,species != "Pontophilus norvegicus")

pel <- subset(pel,species != "Sabinea septemcarinata")

pel <- subset(pel,species != "Bythocaris irene")

pel <- subset(pel,species != "Pandalus borealis")

pel <- subset(pel,species != "Saduria entomon")

pel <- subset(pel,species != "Ilyarachna hirticeps")

pel <- subset(pel,species != "Munnopsis typica")

pel <- subset(pel,species != "Erythrops glacialis")

pel <- subset(pel,species != "Mysideis insignis")

pel <- subset(pel,species != "Watersipora subovoidea")

pel <- subset(pel,species != "Melanogrammus aeglefinus")

pel <- subset(pel,species != "Gaidropsarus argentatus")

pel <- subset(pel,species != "Paraliparis bathybius")

pel <- subset(pel,species != "Cottunculus microps")

pel <- subset(pel,species != "Leptoclinus maculatus")

pel <- subset(pel,species != "Lumpenus lampretaeformis")

pel <- subset(pel,species != "Lycenchelys muraena")

pel <- subset(pel,species != "Lycodes esmarkii")

pel <- subset(pel,species != "Reinhardtius hippoglossoides")

pel <- subset(pel,species != "Diplosoma listerianum")

pel <- subset(pel,species != "Obelia dichotoma") #Sessile
```

```
pel <- subset(pel,species != "Clytia hemisphaerica") #Sessile

pel <- subset(pel,species != "Brisaster fragilis")

pel <- subset(pel,species != "Ophiocten gracilis")

pel <- subset(pel,species != "Yoldiella frigida")

pel <- subset(pel,species != "Chaetoderma nitidulum")

pel <- subset(pel,species != "Bathypolypus arcticus")

pel <- subset(pel,species != "Siphonodentalium lobatum")

pel <- subset(pel,species != "Micrura varicolor")

pel <- subset(pel,species != "Priapulus caudatus")

pel <- subset(pel,species != "Harmothoe globifera")

pel <- subset(pel,species != "Harmothoe sp. CMC01")

pel <- subset(pel,species != "Bougainvillia muscus") #hydroid and medusa stage


#Benthic species, removing pelagic species

ben <- df

ben <- subset(ben,species !="Apherusa glacialis") #ice-associated amphipod, pelagic occurrences of A. glacialis have also been reported (Kunisch et al., 2020) Pelagic occurrences of the ice amphipod Apherusa glacialis throughout the Arctic

ben <- subset(ben,species !="Themisto abyssorum")

ben <- subset(ben,species !="Themisto libellula")

ben <- subset(ben,species !="Arrhis phyllonyx")

ben <- subset(ben,species !="Hymenodora glacialis")
```

```
ben <- subset(ben,species !="Pasiphaea tarda")

ben <- subset(ben,species !="Meganyctiphanes norvegica")

ben <- subset(ben,species !="Thysanoessa inermis")

ben <- subset(ben,species !="Thysanoessa longicaudata")

ben <- subset(ben,species !="Boreomysis nobilis")

ben <- subset(ben,species !="Chiridius gracilis")

ben <- subset(ben,species !="Gaetanus tenuispinus")

ben <- subset(ben,species !="Calanus hyperboreus")

ben <- subset(ben,species !="Microcalanus pusillus")

ben <- subset(ben,species !="Pseudocalanus acuspes")

ben <- subset(ben,species !="Pseudocalanus minutus")

ben <- subset(ben,species !="Metridia longa")

ben <- subset(ben,species !="Scolecithricella minor")

ben <- subset(ben,species !="Oithona similis")

ben <- subset(ben,species !="Boroecia maxima")

ben <- subset(ben,species !="Pseudosagitta maxima")

ben <- subset(ben,species !="Sagitta elegans")

ben <- subset(ben,species !="Clupea harengus")

ben <- subset(ben,species !="Boreogadus saida") #Polartorsken beiter semipelagisk og ned til havbunnen

ben <- subset(ben,species !="Mallotus villosus")

ben <- subset(ben,species !="Cyclopterus lumpus")
```

```
ben <- subset(ben,species !="Campanularia hincksii") #medusa?

ben <- subset(ben,species !="Aglantha digitale") #medusa?

ben <- subset(ben,species !="Cyanea capillata") #medusa?

ben <- subset(ben,species !="Gonatus steenstrupi")
```

BARPLOT AH v BS v AR

```{r}
pel1 <- subset(pel, select = (1:6))

ben1 <- subset(ben, select = (1:6))


pel1 <- aggregate(pel1[c(-1,-2,-3)], pel1["species"], sum)

ben1 <- aggregate(ben1[c(-1,-2,-3)], ben1["species"], sum)


pel1 <- melt(pel1,id.vars="species")

ben1 <- melt(ben1,id.vars="species")


anova <- aov(value ~ variable, pel1)

summary(anova)


anova <- aov(value ~ variable, ben1)

summary(anova)
```

```
pel2 <- subset(pel, select = (1:6))

ben2 <- subset(ben, select = (1:6))


names(pel2)[4] <- "AH_pel"

names(pel2)[5] <- "BS_pel"

names(pel2)[6] <- "AR_pel"


names(ben2)[4] <- "AH_ben"

names(ben2)[5] <- "BS_ben"

names(ben2)[6] <- "AR_ben"


pel2 <- aggregate(pel2[c(-1,-2,-3)], pel2["species"], sum)

ben2 <- aggregate(ben2[c(-1,-2,-3)], ben2["species"], sum)


pel2 <- melt(pel2,id.vars="species")

ben2 <- melt(ben2,id.vars="species")


df <- rbind(pel2, ben2)


df %>%
```

```
  mutate(name = fct_relevel(variable,

            "BS_pel","BS_ben",

            "AH_pel", "AH_ben",

            "AR_pel", "AR_ben" )) %>%

  ggplot( aes(x=name, y=value, fill=variable)) +

    geom_bar(stat="identity")+

    xlab("Skates")+ylab("% Frequency of occurrence")+

      scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))
```

BARPLOTS Ontogenetic shifts class species

```{r}
df <- subset(Rdf, select = c(1,3,4,5,9:24))

df <- aggregate(df[c(-1,-2,-3,-4)], df[c("best_identity","phylum","class","species")], sum)


df1 <- aggregate(df[c(-1,-2,-3,-4)], df[c("phylum","class","species")], sum)

df1 <- melt(df1,id.vars=c("phylum","class","species"))


positions <- c("BSone","BStwo","BSthree","BSfour","BSfive","BSsix",

              "",

              "AHone","AHtwo","AHthree","AHfour",
```

```
            "",
            "ARone","ARtwo","ARthree","ARfour","ARfive","ARsix")

df1 <- subset(df1, value>4)

ggplot(df1,aes(variable,value,fill=class))+
  geom_bar(stat="identity")+
  xlab("Size classes")+ylab("Relative abundance")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

df1 <- subset(df,best_identity>0.98)
df1 <- subset(df1,species !="")
df1 <- aggregate(df1[c(-1,-2,-3,-4)], df1[c("phylum","class","species")], sum)
df1 <- melt(df1,id.vars=c("phylum","class","species"))
df1 <- subset(df1, value>4)

df2 <- subset(df1,phylum == "Chordata")

ggplot(df2,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
```

```
  xlab("Size classes")+ylab("Relative abundance")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

df2 <- subset(df1,phylum != "Chordata")

ggplot(df2,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
  xlab("Size classess")+ylab("Relative abundance")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

#

df <- subset(Fdf, select = c(1,3,4,5,9:24))
df <- aggregate(df[c(-1,-2,-3,-4)], df[c("best_identity","phylum","class","species")], sum)

df1 <- aggregate(df[c(-1,-2,-3,-4)], df[c("phylum","class","species")], sum)
df1 <- melt(df1,id.vars=c("phylum","class","species"))

df1 <- subset(df1, value>4)
```

```
ggplot(df1,aes(variable,value,fill=class))+
  geom_bar(stat="identity")+
  xlab("Size classes")+ylab("% Frecuency of occurrence")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

df1 <- subset(df,best_identity>0.98)
df1 <- subset(df1,species !="")
df1 <- aggregate(df1[c(-1,-2,-3,-4)], df1[c("phylum","class","species")], sum)
df1 <- melt(df1,id.vars=c("phylum","class","species"))
df1 <- subset(df1, value>4)

df2 <- subset(df1,phylum == "Chordata")

ggplot(df2,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
  xlab("Size classes")+ylab("% Frecuency of occurrence")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)
```

```
df2 <- subset(df1,phylum != "Chordata")


ggplot(df2,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
  xlab("Size classes")+ylab("% Frecuency of occurrence")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)
```

Barplots locations phylum species

```{r}
df <- subset(Rdf, select = c(3,31:34))
df <- subset(df, phylum != "")
df <- aggregate(df[-1], df["phylum"], sum)
names(df)[2] <- "A"
names(df)[3] <- "B"
names(df)[4] <- "C"
names(df)[5] <- "D"
df <- melt(df,id.vars="phylum")


ggplot(df,aes(variable,value,fill=phylum))+
  geom_bar(stat="identity")+
```

```
  xlab("Locations")+ylab("Relative abundance")


df <- subset(Fdf, select = c(3,31:34))

df <- subset(df, phylum != "")

df <- aggregate(df[-1], df["phylum"], sum)

names(df)[2] <- "A"

names(df)[3] <- "B"

names(df)[4] <- "C"

names(df)[5] <- "D"

df <- melt(df,id.vars="phylum")


ggplot(df,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+

  xlab("Locations")+ylab("% Frecuency of occurrence")


df <- subset(Rdf, select = c(1,3,5,31:34))

df <- (subset(df, best_identity>0.98))

df <- (subset(df, species != ""))

df <- subset(df, select = c(2:7))

df <- aggregate(df[c(-1,-2)], df[c("phylum","species")], sum)

names(df)[3] <- "A"
```

```
names(df)[4] <- "B"

names(df)[5] <- "C"

names(df)[6] <- "D"

df <-melt(df,id.vars=c("phylum","species"))

df <- subset(df, value>10)


ggplot(df,aes(variable,value,fill=species))+

  geom_bar(stat="identity")+

  xlab("Locations")+ylab("Relative abundance")


df <- subset(Fdf, select = c(1,3,5,31:34))

df <- (subset(df, best_identity>0.98))

df <- (subset(df, species != ""))

df <- subset(df, select = c(2:7))

df <- aggregate(df[c(-1,-2)], df[c("phylum","species")], sum)

names(df)[3] <- "A"

names(df)[4] <- "B"

names(df)[5] <- "C"

names(df)[6] <- "D"

df <-melt(df,id.vars=c("phylum","species"))

df <- subset(df, value>3)
```

```
ggplot(df,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
  xlab("Locations")+ylab("% Frequency of occurrence")
```

Metabarcoding vs morphology

```{r}
skates <- c(48,17,16,16)
MOTUs <- c(7,28,8,95)
species <- c(3,13,2,69)
study <- c("(Dolgov, 2005)","(Eriksen et al., 2020)","TS. Morphology","TS. Metabarcoding")

barplot(MOTUs,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Arctic skate",
        xlab = "Study",
        ylab = "number of MOTUs/OTUs",
        col = c("grey10","grey50","grey90","grey90"))

legend("topleft",
       c("48 skates","17 skates","16 skates"),
```

```
       fill = c("grey10","grey50","grey90"))


barplot(species,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Arctic skate",
        xlab = "Study",
        ylab = "number of species",
        col = c("grey10","grey50","grey90","grey90"))


legend("topleft",
       c("48 skates","17 skates","16 skates"),
       fill = c("grey10","grey50","grey90"))


skates <- c(14,10,19,19)
MOTUs <- c(9,5,8,96)
species <- c(3,1,2,68)


barplot(MOTUs,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Spinytail skate",
        xlab = "Study",
```

```
        ylab = "number of MOTUs/OTUs",
        col = c("grey10","grey50","grey90","grey90"))

legend("topleft",
       c("14 skates","10 skates","19 skates"),
       fill = c("grey10","grey50","grey90"))

barplot(species,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Spinytail skate",
        xlab = "Study",
        ylab = "number of species",
        col = c("grey10","grey50","grey90","grey90"))

legend("topleft",
       c("14 skates","10 skates","19 skates"),
       fill = c("grey10","grey50","grey90"))

skates <- c(2192,289,28,28)
MOTUs <- c(17,122,8,121)
```

```
species <- c(8,46,1,89)


barplot(MOTUs,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Thorny skate",
        xlab = "Study",
        ylab = "number of MOTUs/OTUs",
        col = c("grey10","grey50","grey90","grey90"))


legend("topleft",
       c("2192 skates","289 skates","28 skates"),
       fill = c("grey10","grey50","grey90"))



barplot(species,
        names.arg = study,
        main = "Metabarcoding vs Morpology, Thorny skate",
        xlab = "Study",
        ylab = "number of species",
        col = c("grey10","grey50","grey90","grey90"))
```

```
legend("topleft",
       c("2192 skates","289 skates","28 skates"),
       fill = c("grey10","grey50","grey90"))
```

Dolgov, Eriksen, This study, phylum

```{r}
df <- subset(Rdf, select = c(3,6:8))
names(df)[2] <- "AH_2019-2021"
names(df)[3] <- "BS_2019-2021"
names(df)[4] <- "AR_2019-2021"
df <- subset(df, phylum != "")
df <- aggregate(df[-1], df["phylum"], sum)
df <- melt(df,id.vars="phylum")
aa <- df

df <- subset(Eri, select = c(2,5:7))
names(df)[2] <- "AH_2015"
names(df)[3] <- "BS_2015"
names(df)[4] <- "AR_2015"
df <- subset(df, Phylum != " ")
```

```
df <- aggregate(df[-1], df["Phylum"], sum)

df <- melt(df,id.vars="Phylum")

names(df)[1] <- "phylum"

bb <- df


df <- subset(Dol, select = c(2,5:7))

names(df)[2] <- "AH_1994-2000"

names(df)[3] <- "BS_1994-2000"

names(df)[4] <- "AR_1994-2000"

df <- subset(df, Phylum != " ")

df <- aggregate(df[-1], df["Phylum"], sum)

df <- melt(df,id.vars="Phylum")

names(df)[1] <- "phylum"

cc <- df


# %F


df <- subset(Fdf, select = c(3,6:8))

names(df)[2] <- "AH_2019-2021"

names(df)[3] <- "BS_2019-2021"

names(df)[4] <- "AR_2019-2021"
```

```
df <- subset(df, phylum != "")

df <- aggregate(df[-1], df["phylum"], sum)

df <- melt(df,id.vars="phylum")

dd <- df


df <- subset(Eri, select = c(2,8:10))

names(df)[2] <- "AH_2015"

names(df)[3] <- "BS_2015"

names(df)[4] <- "AR_2015"

df <- subset(df, Phylum != " ")

df <- aggregate(df[-1], df["Phylum"], sum)

df <- melt(df,id.vars="Phylum")

names(df)[1] <- "phylum"

ee <- df


df <- subset(Dol, select = c(2,8:10))

names(df)[2] <- "AH_1994-2000"

names(df)[3] <- "BS_1994-2000"

names(df)[4] <- "AR_1994-2000"

df <- subset(df, Phylum != " ")

df <- aggregate(df[-1], df["Phylum"], sum)
```

```
df <- melt(df,id.vars="Phylum")

names(df)[1] <- "phylum"

ff <- df


#


df <- rbind(cc, bb, aa)

positions <- c("BS_1994-2000","BS_2015","BS_2019-2021","",

               "AH_1994-2000","AH_2015","AH_2019-2021","",

               "AR_1994-2000","AR_2015","AR_2019-2021")


ggplot(df,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+

  xlab("Skates")+ylab("Relative abundance")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)


df <- rbind(ff, ee, dd)


ggplot(df,aes(variable,value,fill=phylum))+

  geom_bar(stat="identity")+
```

```
  xlab("Skates")+ylab("% Frequency of occurrence")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)
```

Stomach fullness

```{r}
a2 <- subset(a1, select = c(1,10,16))


a2$Species[a2$Species == "Thornyskate"] <- "Thorny skate"

a2$Species[a2$Species == "Arcticskate"] <- "Arctic skate"

a2$Species[a2$Species == "Spinytailskate"] <- "Spinytail skate"


positions <- c(

"AL843","AL844","AL846","AL852","AL847","AL849","AL838","BS022","BS045","AL845","AL850","BS044",

"BS043","AL851","AL839","AL841","BS046","BS042","BS018","",

"AH039","AH029","AH030","AH028","AH036","AH038","AH049","AH050","AH040","AH032","AH037","AH041",

"AH031","AH035","AH033","AH034","",

"AR003","AR021","AR027","AR019","AR024","AR047","AR048","AR015","AR014","AR013","AR012","AR020",
```

"AR025","AR016","AR010","AR023","AR017","AR026","AR011","AR007","AR006","AR008","AR009","AR002",

"AR001","AR004","AR005")

a2$Capasity_sto <- a2$Capasity_sto*100

ggplot(a2,aes(ID,Capasity_sto,fill=Species))+

  geom_bar(stat="identity")+

  xlab("Skates")+ylab("Stomach relative fullness")+

  theme(axis.text.x = element_text(angle = 90))+

  scale_x_discrete(limits = positions)

```

## Rarefaction curves

```{r}

mx<-max(colSums(OTU))

my<-sum(rowSums(OTU)>1)

mxm <- 0

rarecurve(t(OTU), step = 40,

        label = F,

        ylab=c("MOTUs"),

```
        xlab=c("Sequencing depth"),
        main= c("Rarefaction curves"),
        ylim=c(0,45), xlim=c(0,mx),
        col = "purple")

OTU.ah <- subset(OTU,select = c(1:16))
mx<-max(colSums(OTU.ah))
my<-sum(rowSums(OTU.ah)>1)
mxm <- 0
rarecurve(t(OTU.ah), step = 40,
        label = F,
        ylab=c("MOTUs"),
        xlab=c("Sequencing depth"),
        main= c("Rarefaction curves, Arctic skate"),
        ylim=c(0,45), xlim=c(0,mx),
        col = "indianred1")

OTU.bs <- subset(OTU,select = c(17:28,56:62))
mx<-max(colSums(OTU.bs))
my<-sum(rowSums(OTU.bs)>1)
mxm <- 0
```

```
rarecurve(t(OTU.bs), step = 40,
        label = F,
        ylab=c("MOTUs"),
        xlab=c("Sequencing depth"),
        main= c("Rarefaction curves, spinytail skate"),
        ylim=c(0,45), xlim=c(0,mx),
        col = "limegreen")

OTU.ar <- subset(OTU,select = c(29:55))

mx<-max(colSums(OTU.ar))
my<-sum(rowSums(OTU.ar)>1)
mxm <- 0
rarecurve(t(OTU.ar), step = 40,
        label = F,
        ylab=c("MOTUs"),
        xlab=c("Sequencing depth"),
        main= c("Rarefaction curves, thorny skate"),
        ylim=c(0,45), xlim=c(0,mx),
        col = "steelblue1")
```

Accumulation curve

```{r}

par(mar=c(5,5,1,1))

META$Species[META$Species == "Spinytailskate"] <- "Spinytail skate"

META$Species[META$Species == "Arcticskate"] <- "Arctic skate"

META$Species[META$Species == "Thornyskate"] <- "Thorny skate"


plot(specaccum(t(OTU.r),subset = META$Species == "Spinytail
skate"),ylab="MOTUs",xlab="Samples",col = "limegreen",ylim=c(0,130),xlim=c(0,26))


par(new=TRUE)


plot(specaccum(t(OTU.r),subset = META$Species == "Arctic
skate"),ylab="MOTUs",xlab="Samples", col = "indianred1",ylim=c(0,130),xlim=c(0,26))


par(new=TRUE)


plot(specaccum(t(OTU.r),subset = META$Species == "Thorny
skate"),ylab="MOTUs",xlab="Samples",col = "steelblue1",ylim=c(0,130),xlim=c(0,26))


legend("bottomright",legend=unique(META$Species),col =
c("indianred1","limegreen","steelblue1"),pch = 16,bty="n") #labels skates
```

```

## nMDS Relative abundance

```{r}

#First nMDS

dist.r  <- vegdist(t(OTU.r),distance = "bray")

set.seed(123)

nmds  <- metaMDS(dist.r, k=2)

plot(nmds, type="n", xlab=NA, ylab=NA, xaxt="n", yaxt="n")

points(nmds, display="si",col="red",pch=20)


#Second nMDS

par(mar=c(1,1,1,1))

plot(nmds, type="n", xlab=NA, ylab=NA, xaxt="n", yaxt="n") #Blank plot

points(nmds, display="si",

    col=c("indianred1","limegreen","steelblue1")[factor(META$Species)],

    pch=20,

    cex=c(seq(1,3.5,by=0.5))[META$Size_class]) #Points with species and size classes

ordispider(nmds,groups = META$Species,label=F) #Lines

#ordiellipse(nmds,groups = META$Species,label=F,col=c("red","green","blue")) #Circles
```
```

```
#text(nmds$points[,1],nmds$points[,2], display="si",labels = META$ID,cex=0.4) #ID on points (SKATEID)

#ordispider(nmds,groups = paste(META$Species,META$Size_class),label=F) #Lines between size classes


nmds$stress


legend("bottomright",legend=unique(META$Species),
       col = c("indianred1","limegreen","steelblue1"),pch = 16,bty="n") #labels skates
legend("topleft",inset = c(0.03,-0.03),
       legend=c(rep(NA,6)),col = c("black"),pch = 19,bty="n",
       cex = c(seq(1,1.25,by=0.05)),pt.cex= c(seq(0.5,2.5,by=0.4))) #labels size dots
legend("topleft",inset = c(-0.03,-0.03),
       legend=sort(unique(META$Size_class)),
       col = c("black"),pch = NA,bty="n",
       cex = 1.25) #labels size number
legend("topright",legend=paste0("stress=",round(nmds$stress,2)),bty="n") #stress
legend("bottomleft",inset = c(-0.03,0),
       legend=paste0("Relative abundance"),bty="n")


env <- subset(META, select = c(2:3))
en = envfit(nmds, env, permutations = 999, na.rm = TRUE)
```

```
en


plot(en,col = "purple")

ordisurf(nmds,env$Depth,add=TRUE,col = "purple")

ordisurf(nmds,env$Temp,add=TRUE,col = "purple")


#Statistical test

set.seed(123)

permanova <- adonis2(dist.r~META$Species*META$Size_class+META$Sex,,permutations = 999)

permanova


pairwise.adonis2(dist.r~Species*Size_class,data=META,p.adjust.m="BY")


#Statistical test for continuous variables enivronment

env <- subset(META, select = c(2:3,9))

en = envfit(nmds, env, permutations = 999, na.rm = TRUE)

en


#


OTU.r.ah <- subset(OTU.r,select = c(1:16))
```

```
dist.r.ah  <- vegdist(t(OTU.r.ah),distance = "bray")

set.seed(123)

nmds.ah  <- metaMDS(dist.r.ah, k=2)


env.ah <- subset(META, Species == "Arcticskate")

env.ah <- subset(env.ah, select = c(2,3,9))

en = envfit(nmds.ah, env.ah, permutations = 999, na.rm = TRUE)

en


#


OTU.r.bs <- subset(OTU.r,select = c(17:28,56:62))


dist.r.bs  <- vegdist(t(OTU.r.bs),distance = "bray")

set.seed(123)

nmds.bs  <- metaMDS(dist.r.bs, k=2)


env.bs <- subset(META, Species == "Spinytailskate")

env.bs <- subset(env.bs, select = c(2,3,9))

en = envfit(nmds.bs, env.bs, permutations = 999, na.rm = TRUE)
```

```
en

#

OTU.r.ar <- subset(OTU.r,select = c(29:55))

dist.r.ar  <- vegdist(t(OTU.r.ar),distance = "bray")
set.seed(123)
nmds.ar  <- metaMDS(dist.r.ar, k=2)

env.ar <- subset(META, Species == "Thornyskate")
env.ar <- subset(env.ar, select = c(2,3,9))
en = envfit(nmds.ar, env.ar, permutations = 999, na.rm = TRUE)
en
```

nMDS % frequency of occurrence

```{r}
#First nMDS
dist.p  <- vegdist(t(OTU.p),distance = "bray")
set.seed(123)
nmds  <- metaMDS(dist.p, k=2)
```

```
plot(nmds, type="n", xlab=NA, ylab=NA, xaxt="n", yaxt="n")

points(nmds, display="si",col="red",pch=20)


#Second nMDS

par(mar=c(1,1,1,1))

plot(nmds, type="n", xlab=NA, ylab=NA, xaxt="n", yaxt="n") #Blank plot

points(nmds, display="si",

    col=c("indianred1","limegreen","steelblue1")[factor(META$Species)],

    pch=20,

    cex=c(seq(1,3.5,by=0.5))[META$Size_class]) #Points with species and size classes

ordispider(nmds,groups = META$Species,label=F) #Lines

#ordiellipse(nmds,groups = META$Species,label=F,col=c("red","green","blue")) #Circles


#text(nmds$points[,1],nmds$points[,2], display="si",labels = META$ID,cex=0.4) #ID on points (SKATEID)

#ordispider(nmds,groups = paste(META$Species,META$Size_class),label=F) #Lines between size classes


nmds$stress


legend("bottomright",legend=unique(META$Species),

    col = c("indianred1","limegreen","steelblue1"),pch = 16,bty="n") #labels skates
```

```
legend("topleft",inset = c(0.03,-0.03),
       legend=c(rep(NA,6)),col = c("black"),pch = 19,bty="n",
       cex = c(seq(1,1.25,by=0.05)),pt.cex= c(seq(0.5,2.5,by=0.4))) #labels size dots
legend("topleft",inset = c(-0.03,-0.03),
       legend=sort(unique(META$Size_class)),
       col = c("black"),pch = NA,bty="n",
       cex = 1.25) #labels size number
legend("topright",legend=paste0("stress=",round(nmds$stress,2)),bty="n") #stress
legend("bottomleft",inset = c(-0.03,0),
       legend=paste0("% Frequency of occurrence"),bty="n")


env <- subset(META, select = c(2:3))
en = envfit(nmds, env, permutations = 999, na.rm = TRUE)
en


plot(en,col = "purple")
ordisurf(nmds,env$Depth,add=TRUE,col = "purple")
ordisurf(nmds,env$Temp,add=TRUE,col = "purple")


#Statistical test
set.seed(123)
```

```
permanova <- adonis2(dist.p~META$Species*META$Size_class+META$Sex,,permutations = 999)

permanova


pairwise.adonis2(dist.p~Species*Size_class,data=META,p.adjust.m="BY")


#Statistical test for continuous variables enivronment

env <- subset(META, select = c(2:3,9))

en = envfit(nmds, env, permutations = 999, na.rm = TRUE)

en


#


OTU.p.ah <- subset(OTU.p,select = c(1:16))


dist.p.ah  <- vegdist(t(OTU.p.ah),distance = "bray")

set.seed(123)

nmds.ah  <- metaMDS(dist.p.ah, k=2)


env.ah <- subset(META, Species == "Arcticskate")

env.ah <- subset(env.ah, select = c(2,3,9))

en = envfit(nmds.ah, env.ah, permutations = 999, na.rm = TRUE)
```

```
en

#

OTU.p.bs <- subset(OTU.p,select = c(17:28,56:62))

dist.p.bs  <- vegdist(t(OTU.p.bs),distance = "bray")
set.seed(123)
nmds.bs  <- metaMDS(dist.p.bs, k=2)

env.bs <- subset(META, Species == "Spinytailskate")
env.bs <- subset(env.bs, select = c(2,3,9))
en = envfit(nmds.bs, env.bs, permutations = 999, na.rm = TRUE)
en

#

OTU.p.ar <- subset(OTU.p,select = c(29:55))

dist.p.ar  <- vegdist(t(OTU.p.ar),distance = "bray")
set.seed(123)
```

```
nmds.ar  <- metaMDS(dist.p.ar, k=2)

env.ar <- subset(META, Species == "Thornyskate")

env.ar <- subset(env.ar, select = c(2,3,9))

en = envfit(nmds.ar, env.ar, permutations = 999, na.rm = TRUE)

en
```

BARPLOTS Size classes

```{r}
META1 <- subset(META, Species == "Arctic skate")

ggplot(META1,aes(x = reorder(ID, `Length (mm)`),y=`Length (mm)`,fill=Size_class))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("Length (mm)")+ggtitle("Arctic skate")+
  theme(axis.text.x = element_text(angle = 90))

META2 <- subset(META, Species == "Spinytail skate")

ggplot(META2,aes(x = reorder(ID, `Length (mm)`),y=`Length (mm)`,fill=Size_class))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("Length (mm)")+ggtitle("Spinytail skate")+
```

```
  theme(axis.text.x = element_text(angle = 90))


META3 <- subset(META, Species == "Thorny skate")


ggplot(META3,aes(x = reorder(ID, `Length (mm)`),y=`Length (mm)`,fill=Size_class))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("Length (mm)")+ggtitle("Thorny skate")+
  theme(axis.text.x = element_text(angle = 90))
```

Circular bar plots

```{r}
#Relative abundance
df<- subset(Rdf, select = c(1,5,6:8))
df <- subset(df, species != "")
df <- subset(df, best_identity>0.98)
df <- subset(df, select = -1)
df <- aggregate(df[-1], df["species"], sum)
df <- melt(df,id.vars="species")
names(df)[1] <- "individual"
names(df)[2] <- "group"
df <- subset(df, value != 0)
```

```
df <- subset(df, value>4)


data <- df

data = data %>% arrange(group, value)


empty_bar <- 3

to_add <- data.frame( matrix(NA, empty_bar*nlevels(data$group), ncol(data)) )

colnames(to_add) <- colnames(data)

to_add$group <- rep(levels(data$group), each=empty_bar)

data <- rbind(data, to_add)

data <- data %>% arrange(group)

data$id <- seq(1, nrow(data))


label_data <- data

number_of_bar <- nrow(label_data)

angle <- 90 - 360 * (label_data$id-0.5) /number_of_bar

label_data$hjust <- ifelse( angle < -90, 1, 0)

label_data$angle <- ifelse(angle < -90, angle+180, angle)


#
```

```
base_data <- data %>%
  group_by(group) %>%
  summarize(start=min(id), end=max(id) - empty_bar) %>%
  rowwise() %>%
  mutate(title=mean(c(start, end)))

grid_data <- base_data
grid_data$end <- grid_data$end[ c( nrow(grid_data), 1:nrow(grid_data)-1)] + 1
grid_data$start <- grid_data$start - 1
grid_data <- grid_data[-1,]

ggplot(data, aes(x=as.factor(id), y=value, fill=group)) +
  geom_bar(aes(x=as.factor(id), y=value, fill=group), stat="identity", alpha=0.5) +
  geom_segment(data=grid_data, aes(x = end, y = 80, xend = start, yend = 80), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +
  geom_segment(data=grid_data, aes(x = end, y = 60, xend = start, yend = 60), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +
  geom_segment(data=grid_data, aes(x = end, y = 40, xend = start, yend = 40), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +
  geom_segment(data=grid_data, aes(x = end, y = 20, xend = start, yend = 20), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +
  annotate("text", x = rep(max(data$id),4), y = c(20, 40, 60, 80), label = c("20", "40", "60",
"80") , color="grey", size=3 , angle=0, fontface="bold", hjust=1) +
```

```
  geom_bar(aes(x=as.factor(id), y=value, fill=group), stat="identity", alpha=0.5) +

  ylim(-100,120) +

  theme_minimal() +

  theme(

    legend.position = "none",

    axis.text = element_blank(),

    axis.title = element_blank(),

    panel.grid = element_blank(),

    plot.margin = unit(rep(-1,4), "cm")) +

  coord_polar() +

  geom_text(data=label_data, aes(x=id, y=value+10, label=individual, hjust=hjust),
color="black", fontface="bold",alpha=0.6, size=2.5, angle= label_data$angle, inherit.aes =
FALSE ) +

  geom_segment(data=base_data, aes(x = start, y = -5, xend = end, yend = -5), colour =
"black", alpha=0.8, size=0.6 , inherit.aes = FALSE )#  +

#  geom_text(data=base_data, aes(x = title, y = -18, label=group), hjust=c(1,1,0,0), #colour =
"black", alpha=0.8, size=4, fontface="bold", inherit.aes = FALSE)



#% Frequency of occurance

df<- subset(Fdf, select = c(1,5,6:8))

df <- subset(df, species != "")

df <- subset(df, best_identity>0.98)

df <- subset(df, select = -1)
```

```
df <- aggregate(df[-1], df["species"], sum)

df <- melt(df,id.vars="species")

names(df)[1] <- "individual"

names(df)[2] <- "group"

df <- subset(df, value != 0)

df <- subset(df, value>3)


data <- df

data = data %>% arrange(group, value)


empty_bar <- 3

to_add <- data.frame( matrix(NA, empty_bar*nlevels(data$group), ncol(data)) )

colnames(to_add) <- colnames(data)

to_add$group <- rep(levels(data$group), each=empty_bar)

data <- rbind(data, to_add)

data <- data %>% arrange(group)

data$id <- seq(1, nrow(data))


label_data <- data

number_of_bar <- nrow(label_data)

angle <- 90 - 360 * (label_data$id-0.5) /number_of_bar
```

```
label_data$hjust <- ifelse( angle < -90, 1, 0)

label_data$angle <- ifelse(angle < -90, angle+180, angle)


#


base_data <- data %>%

  group_by(group) %>%

  summarize(start=min(id), end=max(id) - empty_bar) %>%

  rowwise() %>%

  mutate(title=mean(c(start, end)))


grid_data <- base_data

grid_data$end <- grid_data$end[ c( nrow(grid_data), 1:nrow(grid_data)-1)] + 1

grid_data$start <- grid_data$start - 1

grid_data <- grid_data[-1,]


ggplot(data, aes(x=as.factor(id), y=value, fill=group)) +

  geom_bar(aes(x=as.factor(id), y=value, fill=group), stat="identity", alpha=0.5) +

  geom_segment(data=grid_data, aes(x = end, y = 80, xend = start, yend = 80), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +

  geom_segment(data=grid_data, aes(x = end, y = 60, xend = start, yend = 60), colour =
"grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +
```

```
  geom_segment(data=grid_data, aes(x = end, y = 40, xend = start, yend = 40), colour = "grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +

  geom_segment(data=grid_data, aes(x = end, y = 20, xend = start, yend = 20), colour = "grey", alpha=1, size=0.3 , inherit.aes = FALSE ) +

  annotate("text", x = rep(max(data$id),4), y = c(20, 40, 60, 80), label = c("20", "40", "60", "80") , color="grey", size=3 , angle=0, fontface="bold", hjust=1) +

  geom_bar(aes(x=as.factor(id), y=value, fill=group), stat="identity", alpha=0.5) +

  ylim(-100,120) +

  theme_minimal() +

  theme(

    legend.position = "none",

    axis.text = element_blank(),

    axis.title = element_blank(),

    panel.grid = element_blank(),

    plot.margin = unit(rep(-1,4), "cm")) +

  coord_polar() +

  geom_text(data=label_data, aes(x=id, y=value+10, label=individual, hjust=hjust), color="black", fontface="bold",alpha=0.6, size=2.5, angle= label_data$angle, inherit.aes = FALSE ) +

  geom_segment(data=base_data, aes(x = start, y = -5, xend = end, yend = -5), colour = "black", alpha=0.8, size=0.6 , inherit.aes = FALSE )#  +

#  geom_text(data=base_data, aes(x = title, y = -18, label=group), hjust=c(1,1,0,0), #colour = "black", alpha=0.8, size=4, fontface="bold", inherit.aes = FALSE)

```

(Dolgov 2005) Species %F %M

```{r}

Dol1 <- subset(Dol, Species != "")



M1 <- subset(Dol, select = c(1:4,5:7))

M2 <- subset(M1, select = c(4:7))

M2 <- subset(M2, Species != " ")



F1 <- subset(Dol, select = c(1:4,8:10))

F2 <- subset(F1, select = c(4:7))

F2 <- subset(F2, Species != " ")

```

Pelagic vs benthic diet, (Dolgov 2005) %M

```{r}



#What is not certain

M3 <- subset(M2, Species != "Micromesistius poutassou")



#What is not pelagic

Pel <- M3
```

```
Pel <- subset(Pel, Species != "Pandalus borealis")

Pel <- subset(Pel, Species != "Gadus morhua")

Pel <- subset(Pel, Species != "Melanogrammus aeglefinus")

Pel <- subset(Pel, Species != "Trisopterus esmarkii")

Pel <- subset(Pel, Species != "Hippoglossoides platessoides")


#What is not benthic

Ben <- M3

Ben <- subset(Ben, Species != "Clupea harengus")

Ben <- subset(Ben, Species != "Mallotus villosus")

Ben <- subset(Ben, Species != "Boreogadus saida")


names(Pel)[2] <- "AH_pel"

names(Pel)[3] <- "BS_pel"

names(Pel)[4] <- "AR_pel"


names(Ben)[2] <- "AH_ben"

names(Ben)[3] <- "BS_ben"

names(Ben)[4] <- "AR_ben"


Pel <- melt(Pel,id.vars="Species")
```

```
Ben <- melt(Ben,id.vars="Species")


pel2 <- Pel

ben2 <- Ben


df <- rbind(pel2, ben2)


positions <- c("BS_pel","BS_ben","AH_pel","AH_ben","AR_pel","AR_ben")


df %>%
  mutate(name = fct_relevel(variable,
           "AH_pel", "AH_ben", "BS_pel",
           "BS_ben", "AR_pel", "AR_ben" )) %>%
  ggplot( aes(x=name, y=value, fill=variable)) +
    geom_bar(stat="identity")+
  xlab("Skates")+ylab("Relative abundance")+ggtitle("(Dolgov, 2005)")+
      scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))+
  scale_x_discrete(limits = positions)
```

Pelagic vs benthic diet, (Dolgov 2005) %F

```{r}

#What is not certain

F3 <- subset(F2, Species != "Micromesistius poutassou")



#What is not pelagic

Pel <- F3

Pel <- subset(Pel, Species != "Pandalus borealis")

Pel <- subset(Pel, Species != "Gadus morhua")

Pel <- subset(Pel, Species != "Melanogrammus aeglefinus")

Pel <- subset(Pel, Species != "Trisopterus esmarkii")

Pel <- subset(Pel, Species != "Hippoglossoides platessoides")



#What is not benthic

Ben <- F3

Ben <- subset(Ben, Species != "Clupea harengus")

Ben <- subset(Ben, Species != "Mallotus villosus")

Ben <- subset(Ben, Species != "Boreogadus saida")



names(Pel)[2] <- "AH_pel"

names(Pel)[3] <- "BS_pel"

names(Pel)[4] <- "AR_pel"
```

```
names(Ben)[2] <- "AH_ben"

names(Ben)[3] <- "BS_ben"

names(Ben)[4] <- "AR_ben"


Pel <- melt(Pel,id.vars="Species")

Ben <- melt(Ben,id.vars="Species")


pel2 <- Pel

ben2 <- Ben


df <- rbind(pel2, ben2)


positions <- c("BS_pel","BS_ben","AH_pel","AH_ben","AR_pel","AR_ben")


df %>%

  mutate(name = fct_relevel(variable,

           "AH_pel", "AH_ben", "BS_pel",

           "BS_ben", "AR_pel", "AR_ben" )) %>%

  ggplot( aes(x=name, y=value, fill=variable)) +

    geom_bar(stat="identity")+
```

```
  xlab("Skates")+ylab("% Frequency of occurrence")+ggtitle("(Dolgov, 2005)")+

     scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))+

  scale_x_discrete(limits = positions)
```

(Eriksen et al., 2020) Species %F %M

```{r}
Eri1 <- subset(Eri, Species != " ")


M1 <- subset(Eri, select = c(1:4,5:7))

M2 <- subset(M1, select = c(4:7))

M2 <- subset(M2, Species != " ")


F1 <- subset(Eri, select = c(1:4,8:10))

F2 <- subset(F1, select = c(4:7))

F2 <- subset(F2, Species != " ")
```

Pelagic vs benthic diet, (Eriksen et al., 2020) %M

```{r}
#What is not certain
```

```
M3 <- subset(M2, Species = "Ammodytes marinus") #Benthopelagic

M3 <- subset(M3, Species != "Micromesistius poutassou") # Kolmule


#What is not pelagic

Pel <- M3

Pel <- subset(Pel, Species != "Arctinula greenlandica")

Pel <- subset(Pel, Species != "Brada inhabilis")

Pel <- subset(Pel, Species != "Chionoecetes opilio")

Pel <- subset(Pel, Species != "Crangon allmanni")

Pel <- subset(Pel, Species != "Crossaster papposus")

Pel <- subset(Pel, Species != "Cryptonatica affinis")

Pel <- subset(Pel, Species != "Gadus morhua")

Pel <- subset(Pel, Species != "Golfingia margaritacea")

Pel <- subset(Pel, Species != "Hippoglossoides platessoides")

Pel <- subset(Pel, Species != "Hippoglossus hippoglossus")

Pel <- subset(Pel, Species != "Hyas coarctatus")

Pel <- subset(Pel, Species != "Idotea pelagica")

Pel <- subset(Pel, Species != "Laona finmarchica")

Pel <- subset(Pel, Species != "Lebbeus polaris")

Pel <- subset(Pel, Species != "Leptagonus decagonus")

Pel <- subset(Pel, Species != "Leptoclinus maculatus")
```

```
Pel <- subset(Pel, Species != "Limacia clavigera")

Pel <- subset(Pel, Species != "Lumpenus lampretaeformis")

Pel <- subset(Pel, Species != "Lycodes pallidus")

Pel <- subset(Pel, Species != "Melanogrammus aeglefinus")

Pel <- subset(Pel, Species != "Microstomus kitt")

Pel <- subset(Pel, Species != "Ophiocten sericeum")

Pel <- subset(Pel, Species != "Ophiopholis aculeata")

Pel <- subset(Pel, Species != "Pagurus bernhardus")

Pel <- subset(Pel, Species != "Pagurus pubescens")

Pel <- subset(Pel, Species != "Pandalus borealis")

Pel <- subset(Pel, Species != "Pandalus montagui")

Pel <- subset(Pel, Species != "Pontophilus norvegicus")

Pel <- subset(Pel, Species != "Priapulus caudatus")

Pel <- subset(Pel, Species != "Sabinea sarsi")

Pel <- subset(Pel, Species != "Sabinea septemcarinata")

Pel <- subset(Pel, Species != "Saduria sabini")

Pel <- subset(Pel, Species != "Sclerocrangon ferox")

Pel <- subset(Pel, Species != "Stegocephalus inflatus")

Pel <- subset(Pel, Species != "Triglops murrayi")

Pel <- subset(Pel, Species != "Sebastes mentella")
```

```
#What is not Benthic

Ben <- M3

Ben <- subset(Ben, Species != "Boreogadus saida")

Ben <- subset(Ben, Species != "Boreomysis arctica")

Ben <- subset(Ben, Species != "Centropages humatus")

Ben <- subset(Ben, Species != "Claupea harengus")

Ben <- subset(Ben, Species != "Mallotus villosus")

Ben <- subset(Ben, Species != "Meganyctiphanes norvegica")

Ben <- subset(Ben, Species != "Themisto abyssorum")

Ben <- subset(Ben, Species != "Thysanoessa inermis")

Ben <- subset(Ben, Species != "Thysanoessa longicaudata")


names(Pel)[2] <- "AH_pel"

names(Pel)[3] <- "BS_pel"

names(Pel)[4] <- "AR_pel"


names(Ben)[2] <- "AH_ben"

names(Ben)[3] <- "BS_ben"

names(Ben)[4] <- "AR_ben"


Pel <- melt(Pel,id.vars="Species")
```

```
Ben <- melt(Ben,id.vars="Species")


pel2 <- Pel

ben2 <- Ben


df <- rbind(pel2, ben2)


positions <- c("BS_pel","BS_ben","AH_pel","AH_ben","AR_pel","AR_ben")


df %>%
  mutate(name = fct_relevel(variable,
           "AH_pel", "AH_ben", "BS_pel",
           "BS_ben", "AR_pel", "AR_ben" )) %>%
  ggplot( aes(x=name, y=value, fill=variable)) +
    geom_bar(stat="identity")+
    xlab("Skates")+ylab("Relative abundance")+ggtitle("(Eriksen et al., 2020)")+
      scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))+
  scale_x_discrete(limits = positions)
```

Pelagic vs benthic diet, (Eriksen et al., 2020) %F

```{r}

#What is not certain

F3 <- subset(F2, Species = "Ammodytes marinus") #Benthopelagic

F3 <- subset(F3, Species != "Micromesistius poutassou") # Kolmule



#What is not pelagic

Pel <- F3

Pel <- subset(Pel, Species != "Arctinula greenlandica")

Pel <- subset(Pel, Species != "Brada inhabilis")

Pel <- subset(Pel, Species != "Chionoecetes opilio")

Pel <- subset(Pel, Species != "Crangon allmanni")

Pel <- subset(Pel, Species != "Crossaster papposus")

Pel <- subset(Pel, Species != "Cryptonatica affinis")

Pel <- subset(Pel, Species != "Gadus morhua")

Pel <- subset(Pel, Species != "Golfingia margaritacea")

Pel <- subset(Pel, Species != "Hippoglossoides platessoides")

Pel <- subset(Pel, Species != "Hippoglossus hippoglossus")

Pel <- subset(Pel, Species != "Hyas coarctatus")

Pel <- subset(Pel, Species != "Idotea pelagica")

Pel <- subset(Pel, Species != "Laona finmarchica")

Pel <- subset(Pel, Species != "Lebbeus polaris")
```

```
Pel <- subset(Pel, Species != "Leptagonus decagonus")

Pel <- subset(Pel, Species != "Leptoclinus maculatus")

Pel <- subset(Pel, Species != "Limacia clavigera")

Pel <- subset(Pel, Species != "Lumpenus lampretaeformis")

Pel <- subset(Pel, Species != "Lycodes pallidus")

Pel <- subset(Pel, Species != "Melanogrammus aeglefinus")

Pel <- subset(Pel, Species != "Microstomus kitt")

Pel <- subset(Pel, Species != "Ophiocten sericeum")

Pel <- subset(Pel, Species != "Ophiopholis aculeata")

Pel <- subset(Pel, Species != "Pagurus bernhardus")

Pel <- subset(Pel, Species != "Pagurus pubescens")

Pel <- subset(Pel, Species != "Pandalus borealis")

Pel <- subset(Pel, Species != "Pandalus montagui")

Pel <- subset(Pel, Species != "Pontophilus norvegicus")

Pel <- subset(Pel, Species != "Priapulus caudatus")

Pel <- subset(Pel, Species != "Sabinea sarsi")

Pel <- subset(Pel, Species != "Sabinea septemcarinata")

Pel <- subset(Pel, Species != "Saduria sabini")

Pel <- subset(Pel, Species != "Sclerocrangon ferox")

Pel <- subset(Pel, Species != "Stegocephalus inflatus")

Pel <- subset(Pel, Species != "Triglops murrayi")
```

```
Pel <- subset(Pel, Species != "Sebastes mentella")


#What is not Benthic

Ben <- F3

Ben <- subset(Ben, Species != "Boreogadus saida")

Ben <- subset(Ben, Species != "Boreomysis arctica")

Ben <- subset(Ben, Species != "Centropages humatus")

Ben <- subset(Ben, Species != "Claupea harengus")

Ben <- subset(Ben, Species != "Mallotus villosus")

Ben <- subset(Ben, Species != "Meganyctiphanes norvegica")

Ben <- subset(Ben, Species != "Themisto abyssorum")

Ben <- subset(Ben, Species != "Thysanoessa inermis")

Ben <- subset(Ben, Species != "Thysanoessa longicaudata")


names(Pel)[2] <- "AH_pel"

names(Pel)[3] <- "BS_pel"

names(Pel)[4] <- "AR_pel"


names(Ben)[2] <- "AH_ben"

names(Ben)[3] <- "BS_ben"

names(Ben)[4] <- "AR_ben"
```

```
Pel <- melt(Pel,id.vars="Species")

Ben <- melt(Ben,id.vars="Species")


pel2 <- Pel

ben2 <- Ben


df <- rbind(pel2, ben2)


positions <- c("BS_pel","BS_ben","AH_pel","AH_ben","AR_pel","AR_ben")


df %>%

  mutate(name = fct_relevel(variable,

           "AH_pel", "AH_ben", "BS_pel",

           "BS_ben", "AR_pel", "AR_ben" )) %>%

  ggplot( aes(x=name, y=value, fill=variable)) +

    geom_bar(stat="identity")+

    xlab("Skates")+ylab("% Frequency of occurrence")+ggtitle("(Eriksen et al., 2020)")+

      scale_fill_manual(values =
c("indianred1","limegreen","steelblue1","indianred1","limegreen","steelblue1"))+

  scale_x_discrete(limits = positions)
```

Diet of skates with empty stomachs

```{r}

# %R

df <- subset(TDPR, select = c(1:5,15,39,43,61,66))

df1 <- aggregate(df[c(-1,-2,-3,-4,-5)], df[c("best_identity","species_name")], sum)

df1 <- subset(df1, species_name != "")

df1 <- subset(df1, best_identity>0.98)

df1 <- subset(df1, select = c(2:7))

df1 <- melt(df1,id.vars="species_name")

df1 <- subset(df1, value>0)



positions <- c("BS018","BS045","AH037","AR006","AR010")



ggplot(df1,aes(variable,value,fill=species_name))+

  geom_bar(stat="identity")+

  xlab("Skates")+ylab("Relative abundance")+

  scale_x_discrete(limits = positions)



# %F

df <- subset(TDPF1, select = c(1:5,15,39,43,61,66))

df1 <- aggregate(df[c(-1,-2,-3,-4,-5)], df[c("best_identity","species_name")], sum)
```

```
df1 <- subset(df1, species_name != "")

df1 <- subset(df1, best_identity>0.98)

df1 <- subset(df1, select = c(2:7))

df1 <- melt(df1,id.vars="species_name")

df1 <- subset(df1, value>0)


ggplot(df1,aes(variable,value,fill=species_name))+

  geom_bar(stat="identity")+

  xlab("Skates")+ylab("% Frequency of occurrence")+

  scale_x_discrete(limits = positions)
```

Internal yolk sac reads?

```{r}
df <- subset(TDPR, select = c(1:5,66,36))

df <- aggregate(df[c(-1,-2,-3,-4,-5)], df[c("best_identity","species_name")], sum)

df <- subset(df, species_name != "")

df <- subset(df, best_identity>0.98)

df <- subset(df, select = -1)

df <- melt(df,id.vars="species_name")

df <- subset(df, value>0)
```

```
ggplot(df,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("Relative abundance")

df <- subset(TDPF1, select = c(1:5,66,36))
df <- aggregate(df[c(-1,-2,-3,-4,-5)], df[c("best_identity","species_name")], sum)
df <- subset(df, species_name != "")
df <- subset(df, best_identity>0.98)
df <- subset(df, select = -1)
df <- melt(df,id.vars="species_name")
df <- subset(df, value>0)

ggplot(df,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("% Frequency of occurrence")
```

Secondary prey, passive ingestion, and other strange results

```{r}
df <- subset(TDPF1,species_name != "")

df <- subset(df,phylum_name != "Annelida")
```

```
df <- subset(df,phylum_name != "Echinodermata")

df <- subset(df,phylum_name != "Mollusca")

df <- subset(df,phylum_name != "Nemertea")

df <- subset(df,phylum_name != "Priapulida")


df <- subset(df,class_name != "Malacostraca")

df <- subset(df,class_name != "Ostracoda")

df <- subset(df,class_name != "Actinopterygii")

df <- subset(df,class_name != "Ascidiacea")


df <- subset(df,species_name != "Zygomolgus dentatus")

df <- subset(df,species_name != "Chiridius gracilis")

df <- subset(df,species_name != "Gaetanus tenuispinus")

df <- subset(df,species_name != "Microcalanus pusillus")

df <- subset(df,species_name != "Pseudocalanus acuspes")

df <- subset(df,species_name != "Pseudocalanus minutus")

df <- subset(df,species_name != "Metridia longa")

df <- subset(df,species_name != "Scolecithricella minor")

df <- subset(df,species_name != "Oithona similis")

df <- subset(df,species_name != "Calanus hyperboreus")

df <- subset(df,species_name != "Pseudosagitta maxima")
```

```
df1 <- subset(df,phylum_name == "Arthropoda")

df2 <- subset(df,phylum_name == "Chaetognatha")

df3 <- subset(df,phylum_name == "Chordata")

df4 <- subset(df,phylum_name == "Cnidaria")


df1 <- subset(df1, select = -c(1:4))

df1 <- melt(df1,id.vars="species_name")

df1 <- subset(df1, value>0)


df2 <- subset(df2, select = -c(1:4))

df2 <- melt(df2,id.vars="species_name")

df2 <- subset(df2, value>0)


df3 <- subset(df3, select = -c(1:4))

df3 <- melt(df3,id.vars="species_name")

df3 <- subset(df3, value>0)


df4 <- subset(df4, select = -c(1:4))

df4 <- melt(df4,id.vars="species_name")

df4 <- subset(df4, value>0)
```

```
positions <- c(
"AL843","AL844","AL846","AL852","AL847","AL849","AL838","BS022","BS045","AL845","AL850","BS044",
"BS043","AL851","AL839","AL841","BS046","BS042","BS018","",
"AH039","AH029","AH030","AH028","AH036","AH038","AH049","AH050","AH040","AH032","AH037","AH041",
"AH031","AH035","AH033","AH034","",
"AR003","AR021","AR027","AR019","AR024","AR047","AR048","AR015","AR014","AR013","AR012","AR020",
"AR025","AR016","AR010","AR023","AR017","AR026","AR011","AR007","AR006","AR008","AR009","AR002",
"AR001","AR004","AR005")

ggplot(df1,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("% Frequency of Occurrence")+ggtitle("Arthropoda")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

ggplot(df2,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("% Frequency of Occurrence")+ggtitle("Chaetognatha")+
```

```
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

ggplot(df3,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("% Frequency of Occurrence")+ggtitle("Chordata")+
  theme(axis.text.x = element_text(angle = 90))

ggplot(df4,aes(variable,value,fill=species_name))+
  geom_bar(stat="identity")+
  xlab("Skates")+ylab("% Frequency of Occurrence")+ggtitle("Cnidaria")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = positions)

df <- subset(TDPF1, select = c(1:21))
df$order_name <- TDS$order_name
df$family_name <- TDS$family_name
df$genus_name <- TDS$genus_name

dfa <- subset(df,species_name == "Meganyctiphanes norvegica")
dfb <- subset(df,genus_name == "Sagitta")
```

```
dfc <- subset(df,species_name == "Boreogadus saida")

df <- rbind(dfa,dfb,dfc)

df <- subset(df,select = -c(1:4,22:24))

df <- melt(df,id.vars="species_name")

pos <-
c("AH039","AH029","AH030","AH028","AH036","AH038","AH049","AH050","AH040","
AH032","AH037","AH041","AH031","AH035","AH033","AH034")

names(df)[1] <- "species"

ggplot(df,aes(variable,value,fill=species))+
  geom_bar(stat="identity")+
  xlab("Arctic skate")+ylab("% Frequency of occurrence")+
  theme(axis.text.x = element_text(angle = 90))+
  scale_x_discrete(limits = pos)
```