

Elasmobranchs as bioindicators? A comparative study on ingestion of plastics in the Nordic region

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Abstract

Plastic pollution has become a major threat to many marine ecosystems, and there is a need for an improved understanding of its impact on marine organisms. Studies have mainly focused on lower trophic levels, including species occurring in the north, since they can be highly vulnerable to plastic pollutants. However, no studies have yet focused on elasmobranchs which belong to the highest trophic levels, in Nordic waters, and how they can provide important data for the monitoring of plastic pollutants. Elasmobranchs from other regions have been described as good bioindicators due to their propensity to bioaccumulation and biomagnification. This study aimed at quantifying and characterizing the ingested macroplastic particles above 1 mm in the stomachs of three elasmobranch species: one shark, the spurdog (*Squalus acanthias*) and two skate species the starry ray (*Amblyraja radiata*) and the Arctic skate (*Amblyraja hyperborea*) from the North Sea, the Norwegian Sea and the Barents Sea. Stomachs were digested with 10% KOH and the remaining particles were size-selectively filtered. Analysis of 229 stomach samples revealed only one plastic particle. This is in stark contrast to what has been previously reported for elasmobranchs in southern waters. Published models predicted a low likelihood for macroplastics in polar waters, nonetheless the expectation for this study was to find a substantially higher abundance of plastic particles in the target species than observed. For 27 individuals the spiral valves were also analyzed for comparison, but no plastics were found. Considering the rising amount of plastics released into the environment, it is very likely that especially top elasmobranch predators, especially in the Arctic, will be affected in the near future. We therefore highly advise further ecological and environmental studies on elasmobranchs, their role in regional food webs and the impact of pollutants on them and the entire connected ecosystems.

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1. Introduction

The use of plastics has steadily increased since the 1940's and has become the norm in modern living (Andrady & Neal, 2009). Since then, the production and usage of plastic products has grown exponentially, thus also leading to exponentially increasing rates of discards. This also caused the ubiquitous increase of plastic debris in marine ecosystems on a global scale (Andrady, 2017; Farady, 2019). Approximately 359 million metric tons of plastics were produced worldwide and *ca.* 62 million metric tons in Europe during 2018. In the same year, the collected amount of post-consumer plastic in Europe was *ca.* 30 million metric tons, which leaves *ca.* 33 million metric tons of plastic produced in Europe unaccounted for (Plasticseurope, 2019). It is likely that a significant proportion of the unaccounted fraction is the cause of increasing plastic pollution in *e.g.* the soil (Chae & An, 2018) and the world's oceans (Browne, 2015; Plasticseurope, 2019), and, accordingly, represents a major threat for terrestrial and marine ecosystems. In particular, plastic pollution in the oceans has become an increasingly important topic due to the adverse effects of plastic debris on the environment and the organisms therein (Barnes *et al.*, 2009; Browne, 2015; Avio *et al.*, 2017; Welden & Lusher, 2017; Chae & An, 2018; Farady, 2019). The World Economic Forum in 2016 projected that the plastic accumulated in the oceans will weigh out fish by 2050 (MacArthur *et al.*, 2016). Such estimates are based on the assumption that currently 75% of the overall waste in the ocean is plastic (*e.g.* granules, fibers, pellets and powders), and that as much as 5 million metric tons enter the oceans annually (Thompson, 2017).

Plastic is a synthetic polymer which is used for multiple purposes. Various chemical additives change the properties of plastic, *e.g.* giving it high hydrophobicity, an increased durability, change its color, create antifouling measures, give the plastic a higher thermal stability and self-healing polymers to name only a few methods to modify plastic materials (Almeida *et al.*, 2007; Mizrahi Dagan & Naveh, 2019; Pan *et al.*, 2019; Swarna *et al.*, 2019; Chen *et al.*, 2020; Li *et al.*, 2020). Plastic is also cost-efficient to produce, and thus benefits humanity in many ways like packaging of goods and food, building materials and clothes (Andrady & Neal, 2009). Plastic pollution comes in different size classes usually referred to as nano – (< 1µm; NP) , micro – (2 µm–5 mm; MP) and macroplastics (> 5 mm; MaP) (Germanov *et al.*, 2018). Within the < 5 mm categories there are two further distinctions namely primary or secondary plastics. Primary plastics are intentionally manufactured to be smaller than 5 mm, while secondary plastics are larger items broken down to the micro- and nanoplastic level through *e.g.*, strong winds, tides, UV light (photodegradation) or currents (Welden & Lusher, 2017). The most common plastic types that are found in the environment are polyethylene (PE), polypropylene(PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyamide (PA),

polystyrene (PS) and polyvinyl alcohol (PVA) (Avio *et al.*, 2017). Given the versatility of plastic products, plastic production is very unlikely to be reduced or even cease in the coming years. However, growing public concerns are currently sustaining research on the effects of plastics on ecosystems, and there are increasing efforts in developing alternatives that may at some point substitute plastic products (Song *et al.*, 2009; GESAMP, 2016; Haider *et al.*, 2019). In the context of pollution it is also important to note that there are already significant losses of plastics within the chain of production, which further contribute up to *ca.* 167,000 metric tons/year (in the EU) to the total amount of plastic that ends up in the environment (Hann *et al.*, 2018; Galafassi *et al.*, 2019).

1.1 Plastic in the marine environment

The first scientific study on plastics in the marine environment screened the epipelagic zone (surface waters) of the Sargasso Sea (Carpenter & Smith, 1972). The study reported an average of 3500 plastic particles in a 1300 km north to south transect, with a mean amount of one particle in 280 m² and plastic particle size of about 0.25 – 0.5 cm (*i.e.* macroplastic > 5 mm). Since then, other studies have revealed that roughly 70% – 90% of the plastic debris in the oceans is land–originated, while only an estimated 10% – 25% is directly disposed into marine environments (Morgana *et al.*, 2018). For the latter, fisheries, the shipping sector and marine aquaculture are held responsible for fishing equipment either deliberately or accidentally discarded into the oceans (Bråte *et al.*, 2017). A considerable part of the lost fishing equipment consists of floating fishnets (also referred to as “ghost fishing”), which are of particular concern not only as plastic debris but also because organisms can get entangled and die of starvation or inflicted wounds. However, ingested plastic bags and textile filaments that are major land–originated pollutants in the oceans can cause similar sublethal to lethal effects on marine organisms as well (Laist, 1997; Jacobsen *et al.*, 2010; Barreiros & Raykov, 2014; Stelfox *et al.*, 2016).

Another issue of concern is the slow degradation of plastics, which has led to an accumulation in the environment. Five forms of plastic degradation are usually distinguished: (1) biodegradation (degradation through organisms; usually microbes), (2) photodegradation (degradation process through light; UV light is a great contributor here), (3) thermooxidative degradation (oxidative breakdown of the material at moderate temperatures), (4) thermal degradation (usually not a natural degradation process) and (5) hydrolysis (reaction with water) (Andrady, 2011). Given the slow degradation, great amounts of plastics will accumulate in the marine environment, and will stay for a long time in the water column where they follow ocean currents and gyres (Lebreton *et al.*, 2012). Thus, plastic debris can be globally distributed and even be transported to remote beaches and/or

sink to the bottom of the ocean (Lusher *et al.*, 2015). Plastics can be ingested by marine organisms essentially anywhere, which is particularly likely if the particles resemble food (Murray & Cowie, 2011; Ryan, 2019).

1.2 The effects of plastics on marine life

It has been extensively documented that plastics in its various forms can cause exterior damage to marine organisms *e.g.* by entanglement, cutting into the body surface, or smothering (Stelfox *et al.*, 2016; Bernardini *et al.*, 2018). Examples include entanglements of the loggerhead sea turtle (*Caretta caretta*), the shortfin mako (*Isurus oxyrinchus*) and the commercially important lobster (*Nephrops norvegicus*) in larger plastic debris such as fishing gear (Murray & Cowie, 2011; Wegner & Cartamil, 2012; Barreiros & Raykov, 2014). However, plastics–caused internal damage has also received increasing attention in recent years (Alomar & Deudero, 2017; Fossi *et al.*, 2017; Bernardini *et al.*, 2018; Germanov *et al.*, 2018). Plastics have been reported internally in marine birds, *e.g.*, brown pelican (*Pelecanus occidentalis*), northern fulmar (*Fulmarus glacialis*), Brandt’s cormorant (*Phalacrocorax penicillatus*), fish, *e.g.*, blue shark (*Prionace glauca*), and marine mammals, *e.g.*, northern fur seal (*Callorhinus ursinus*), humpback whale (*Megaptera novaeangliae*), sea otter (*Enhydra lutris*) and sperm whale (*Physeter macrocephalus*) (Moore *et al.*, 2009; van Franeker *et al.*, 2011; Bernardini *et al.*, 2018; IJsseldijk *et al.*, 2018). A recent compilation (litterbase.awi.de/interaction_detail) count as many as 2248 species to be affected (both internally and externally) through direct plastic encounter (Tekman *et al.*, 2019). Most plastics are neutral buoyant plastic particles that have floated in the water column for extended periods of time (Barnes *et al.*, 2009).

There are various ways how organisms can take in plastics; the major three are:

1. *Plastic ingestion of prey items*: Ingestion of plastics, especially macroplastics, can cause harm in the digestive system but may also interfere with hunger and satiety levels. This may reduce the overall energy capacity of the affected individuals and cause diminished growth rates and failure of the endocrine and immune systems (Alexiadou *et al.*, 2019; Mancina *et al.*, 2020). Furthermore, the reproductive success may be affected, which can lead to slowly decreasing fish stocks (Cliff *et al.*, 2002; Rummel *et al.*, 2016; Bernardini *et al.*, 2018). However, some species manage to excrete plastic particles, and, thus, reduce some of the negative long–term effects of plastic ingestion. Examples of marine vertebrates are the genus of fur seals (*Arctocephalus*) (Eriksson & Burton, 2003) and the loggerhead sea turtle (Hoarau *et al.*, 2014) that are able to excrete plastics. But for many taxa the necessary data if and how plastics are excreted are not available (Ryan, 2019).

2. *Ingestion through gills*: Plastic particles can also be sucked in through the gills of fish, which is a major threat because their ion and energy allocation depend on a functioning breathing apparatus (Sussarellu *et al.*, 2016; Bråte *et al.*, 2017). However, there are only few detailed studies on plastic ingestion through gills (Browne *et al.*, 2008; Brennecke *et al.*, 2015). One report of plastic ingestion through the gills and partially clogging them was a beached whale shark (*Rhincodon typus*) in the Philippines (Abreo *et al.*, 2019). Another study examined the effects of microplastics on gills in the shore crab (*Carcinus maenas*), and observed that there was only a small negative change in oxygen consumption and ion regulation, which had no significant effects on the gill function and thus no adverse effects on the overall energy allocation (Watts *et al.*, 2016). The adverse effects of ingestion through gills have not been well studied and remain under discussion with some species being severely affected while others do not seem to be substantially harmed.

3. *Absorption of plastics-associated chemicals*: Plastic polymers are often mixed with potentially toxic additives (*e.g.* dyes, flame-retardants and softeners, POPs (persistent organic pollutants *e.g.* DDE (dichlorodiphenyldichloroethylene) and PCBs (polychlorinated biphenyls) (Teuten *et al.*, 2007, 2009). Ingested plastic particles become subject to digestive processes and may release the toxic chemicals components that then, in turn, may bind to other hydrophobic structures and compounds (Voparil & Mayer, 2000; Bakir *et al.*, 2014). High concentrations of POPs can cause endocrine disruption, teratogenicity (a reaction with a chemical agent that disrupts embryo or fetus development) and can also be harmful to the kidney and liver (Muirhead *et al.*, 2006; Yogui & Sericano, 2009). Even though chemicals can accumulate in plastics, there is no certainty that the chemical transfer from plastic particles to organic surface will occur (Koelmans *et al.*, 2015). In some cases the bioaccumulating process was reversed and had a beneficial effect on the organism (Teuten *et al.*, 2007; Gouin *et al.*, 2011; Chua *et al.*, 2014).

1.3 Plastic ingestion in marine predators

Marine predators ingesting plastics have been more frequently reported in recent years (predator species ingesting plastics n = 701; predator species being entangled n = 354 Kühn & van Franeker, 2020). Predators are in high trophic levels which makes them more susceptible to biomagnification (*i.e.* the process by which a compound (a pollutant) increases its concentration in the tissue of an organism as it moves along the food chain). This is true especially for cetaceans, sharks and sea birds (Santana *et al.*, 2017). In addition, plastics bioaccumulation (the net accumulation of a contaminant from all sources including water and diet) plays a considerable role in long lived marine predators,

because those plastic particles can remain in the organism's body for a long time (Romeo *et al.*, 2015). Several species (*e.g.* cetaceans and seabirds) have therefore been used as "bioindicator" (*i.e.* a "detector" revealing the existence of complex conditions resulting from a group of biotic and/or abiotic factors; they can range from an intracellular level to communities or ecosystems; (Bellan, 2008)) or "sentinel" species to assess the health of an ecosystem (Zacharias & Roff, 2001; Fossi *et al.*, 2018). An example of this is the Indo-Pacific humpback dolphin (*Sousa chinensis*), that presented a high concentration of microplastics in adult and juvenile animals alike (Zhu *et al.*, 2019).

Elasmobranchs (sharks, rays and skates), have also been suggested as sentinel species because of the high exposure to environmental toxins due to their longevity (Marcovecchio *et al.*, 1991; Vas, 1991) and because they play a vital role in regulatory processes within a food web (Coll *et al.*, 2013; Barría *et al.*, 2015; Navia *et al.*, 2017). Several studies found plastics ingestion in a number of shark species, in the Mediterranean Sea, the Indian Ocean and in the southwest Atlantic (Laist, 1997; Cliff *et al.*, 2002; Alves *et al.*, 2016; Bernardini *et al.*, 2018). Among those was the blue shark (*Prionace glauca*) in the Atlantic Ocean and in the Mediterranean Sea for which ingestion of plastics has been reported (Alves *et al.*, 2016; Fossi *et al.*, 2017; Bernardini *et al.*, 2018). Some shark species have been reported to ingest and incorporate chemicals stemming from plastics (Alves *et al.*, 2016; Bergami *et al.*, 2017; Bråte *et al.*, 2017; Powell *et al.*, 2018), but only few studies have addressed the possible effects of such chemicals on elasmobranchs (Alves *et al.*, 2016; Fossi *et al.*, 2017; Bernardini *et al.*, 2018).

So far, only a few studies have addressed the plastics ingestion of marine predators in the Nordic region (in this thesis defined as: North Sea, Norwegian Sea and Barents Sea as defined by IHO, 1953)) and the Greenland Sea (Provencher *et al.*, 2014; Bråte *et al.*, 2016; Avery-Gomm *et al.*, 2018; Smith, 2018; Moore *et al.*, 2020). Hitherto none have focused explicitly on elasmobranchs, though several studies have mentioned the occurrence of plastic and other anthropogenic materials in stomachs of elasmobranchs (Leclerc *et al.*, 2012; Nielsen *et al.*, 2014; Amélineau *et al.*, 2016; Nielsen *et al.*, 2019). The Greenland shark (*Somniosus microcephalus*) and the lesser spotted dogfish (*Scyliorhinus canicula*) are, as of right now, the only Nordic elasmobranch species where macroplastic particles (fishing gear, garbage) have been reported in the scientific literature (Leclerc *et al.*, 2012; Nielsen *et al.*, 2014; Smith, 2018; Nielsen *et al.*, 2019). In all cases the authors have pointed out the lack of studies on the topic and have suggested further detailed research on how these plastic particles influence elasmobranchs behavior and physiology since in Nordic waters the occurrence and threat of plastic pollution has become more evident (Andrady, 2011; Bergmann *et al.*, 2016; Bergmann, *et al.*, 2017a; Bergmann, *et al.*, 2017b; Cózar *et al.*, 2017; Welden & Lusher, 2017; Buhl-Mortensen & Buhl-Mortensen, 2018; Kanhai *et al.*, 2018; Morgana *et al.*, 2018).

1.4 The Nordic region as a focal point of marine plastic aggregation

There are three definitions for the boundaries of the Arctic and its waters. For this thesis the climate boundaries definition was chosen as the 10°C July isotherm (*i.e.* that the region has an average temperature of 10°C in July, which includes the Barents Sea and the northern parts of the Norwegian Sea) (Murray *et al.*, 1998). Generally, Arctic waters have been a focus of marine pollution monitoring for many years (AMAP, 1997; Lusher *et al.*, 2015; Tekman *et al.*, 2020). Changes in the Arctic can have a severe impact on other ecosystems. Those changes are due to the variation in the Thermohaline Circulation (THC) and freshwater melts that release methane which contribute to the amount of greenhouse gases in the atmosphere, to name only a few examples (Morison *et al.*, 2000; Kuhlbrodt *et al.*, 2007). Reports of plastics sightings on the water surface and the beaches (Bergmann *et al.*, 2017a) as well as in the water column and benthic habitat have increased in the Arctic to a high extent over the last 12 years (Bergmann *et al.*, 2017b; Morgana *et al.*, 2018; Tekman *et al.*, 2020). It was confirmed that microplastic particles had reached the sediments of the Fram strait near the Long-Term Ecological Research HAUSGARTEN observatory west of Svalbard (Bergmann *et al.*, 2017b). Many vessels (commercial, touristic and scientific) report floating macroplastic particles (> 5mm) on the surface. Also, during helicopter surveys of the Barents Sea and Fram Strait plastic particle pollution in Arctic waters has been detected (Bergmann *et al.*, 2016). This has led to the conclusion that all kinds of plastics can readily accumulate in the Arctic (Cózar *et al.*, 2017). Once entered the Arctic those particles become trapped through the THC and gyres systems (*e.g.* the Greenland gyre; see Figure 1). Some modelling approaches suggest an accumulation zone for debris within the Arctic Polar Circle in *ca.* 100 years and onward (van Sebille *et al.*, 2012).

The Northeast Atlantic has been described as the single, dominant high-accumulation zone for floating plastic debris, since this is where the THC converges and flow directly into the Barents Sea and northward towards the Arctic Ocean (Lebreton *et al.*, 2012; van Sebille *et al.*, 2012; Cózar *et al.*, 2017). Another indicator for the long-distance travel of plastic is the similar typology (*i.e.* same types of plastics in different environments) of plastics which was found in other gyre systems (*e.g.* in the subtropics) and the Mediterranean Sea (Cózar *et al.*, 2017). More urbanized regions close to the shore have been suspected of having a high influence with regard to plastic pollution (Nollkaemper, 1994). For the Norwegian Sea, it is first of all the more densely populated areas along the Norwegian coastline that contribute plastic pollutants through land runoffs (Bråte *et al.*, 2016). Aquatic sources (like aquaculture) contribute as well but not as much in comparison (Strand *et al.*, 2015). An increased amount of microplastic pollutants was found in *Mytilus spp.* which was used as a sentinel species to

help clarify the status of microplastic pollution in Norwegian waters. With the highest concentrations of plastic detected in the northernmost parts of Norway like the Barents Sea, the Hardangerfjord and the Oslofjord the level of microplastic pollution is a serious challenge for remote places and urban regions alike (Bråte *et al.*, 2018). Registration of composition and amount of beach litter has been the classical indicator for macroplastics around the Norwegian Sea. Macroplastics in marine organisms have been reported in fish (*e.g.* Atlantic cod (*Gadus morhua*)) but remain to this point rare (Bråte *et al.*, 2016). Those findings have potential use in further management efforts but since there are no clear unified standards on how to assess plastic pollution, comparisons between studies remain difficult (Strand *et al.*, 2015). Norway has urged its population through recycling and other environmental campaigns to reduce plastic waste (*e.g.* beach clean-up projects which some of those go back as far as 1979, Fishing for Litter (2018); Save the North Sea (2001–2002), *etc.*) (Hals *et al.*, 2011). Nevertheless, the level of plastic pollution is still high due to local or long-range transport (as described above in the Arctic waters), oil and gas exploration, fisheries, shipping and tourism. Those transport mechanisms create difficulties for identifying plastic origins, since a large portion of plastic debris is already worn down when it arrives in the Nordic region (Jeftic *et al.*, 2009; Kanhai *et al.*, 2018).

The North Sea has been identified as one of the major sources for transportation of plastic particles towards the north due to its direct influence of the THC which transports waters, into the Arctic (Strand *et al.*, 2015). A residual of this northward transition mixes with the waters of the Skagerrak, Kattegat and the Baltic Sea (see Figure 1). These in turn form shelf currents along the Danish and Norwegian coast. Collectively they form a local circulation that functions similar to the gyre systems near Greenland and in the subtropics which potentially accumulate plastic particles (Strand *et al.*, 2015). This kind of accumulation has been documented for particulate suspended matter which suggest that marine litter (including plastic) can follow suit (Eisma & Kalf, 1987; de Haas *et al.*, 2002). Estimates describe that about 10% of all marine litter end up in the Skagerrak region even though it makes up only 2% of the North Sea coastline (UNEP Regional Seas *et al.*, 2005). The impact of plastics on North Sea organisms has been evaluated mainly through the stomach content of sentinel species like the northern fulmar (van Franeker *et al.*, 2011; OSPAR, 2014). For the North Sea and Skagerrak nearly a decade (2002–2011) of sampling northern fulmar stomachs was conducted. The results were that 95% of the 2002–2007 samples had plastic ingested (averagely 35 pieces weighing 0.31 g). High spatial coverage data of this species has led to use the northern fulmar as Ecological Quality Objectives (EcoQOs) for the Northeast Atlantic set by the OSPAR (OSPAR, 2009; van Franeker *et al.*, 2011). Those results are above the threshold set by the EcoQOs (0.1 g of plastic in organisms). For now,

there have been little focus on marine submerged species, but plastic ingestion has been found in Atlantic cod (*Gadus morhua*) (Strand *et al.*, 2015). Thus, the perpetual plastic contamination in the North Sea and Skagerrak, due to the entrapment of marine pollution in those waters, can have severe consequences for all marine organisms living in these environments.

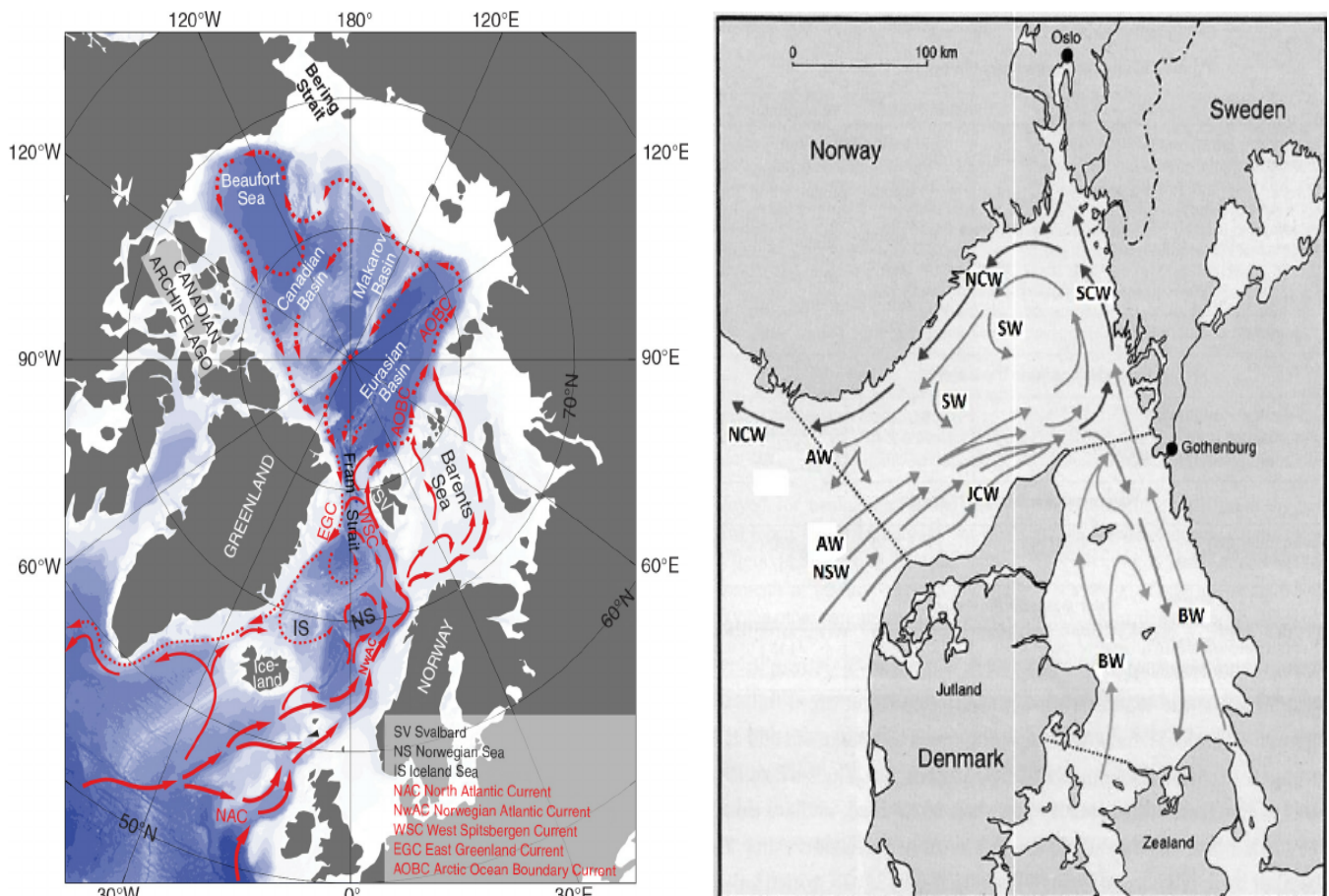


Figure 1. Current systems in the Arctic and Barents Sea (left; (Findlay *et al.*, 2015)) and the North Sea (right; BW = Baltic Water, SCW = Skagerrak Coastal Water, SW = Skagerrak Water, NCW = Norwegian Coastal Water, JCW = Jutland Coastal Water, NSW = North Sea Water, AW = Atlantic Water; after (Force, 1993))

1.5 Objectives

The overall objective of this thesis was to characterize and evaluate plastics ingestion in two elasmobranch species, the spiny dogfish (*Squalus acanthias*) and the starry ray (*Amblyraja radiata*) in different areas within the Nordic region. This choice of model species and study area will address critical knowledge gaps in our understanding of plastic ingestion of elasmobranchs in the Nordic region.

Elasmobranchs (cartilaginous fish) are particularly vulnerable to anthropogenic stress due to certain life history traits such as slow maturation, low recruitment and long longevity (Dulvy *et al.*, 2014), compared to most teleosts (bony fish). In addition, many species have a regulatory trophic position in

the ecosystem which makes them an important contributor inside the ecosystem. Plastics have been shown to be hazardous for sharks and skates alike and can thus diminish those respective roles in an ecosystem (Mancia *et al.*, 2020). The two elasmobranch species chosen for this study differ in feeding strategies (opportunistic and benthic) and habitat preferences. This allows for a comparison of the effects of feedings strategies on plastics ingestion and its level, and hence severity of potential impact. In addition to the two main study species, another skate species within the same genus (Arctic skate; *Amblyraja hyperborea*) was used for comparison between the two skate species in the high Arctic environment as a few samples became available that allowed for this small-scale comparison.

Within this framework, the following four sub-goals were formulated:

1. to identify the type and amount of ingested macroplastics (for this thesis defined as larger than 1mm),
2. to assess potential differences between areas within the Nordic region, and
3. to relate the level of ingested plastics with life history traits (*e.g.* size, sex, maturity) and dietary content (quantity and quality)
4. to compare findings between the two related skate species within the same area

2. Material and Methods

2.1 Study species

The three elasmobranch species studied here share some common characteristics including their functional similarities in spiral valve and stomach, which are important for this comparative study. Nevertheless, there are also large differences in the general anatomy (see Figs. 2 and 3), feeding strategies and habitat preferences of these species (Hureau *et al.*, 1984; Avsar, 2001). *S. acanthias* for example is an opportunistic feeder while *A. radiata* and *A. hyperborea* are benthic to demersal feeders. Other differences include the modes of giving birth, *i.e.* *S. acanthias* being ovoviviparous (giving birth to live offspring while egg capsule remains inside the mother's body) while the two skate species are oviparous (egg laying) (Breder & Rosen, 1966; Compagno, 1984; Hureau *et al.*, 1984; Ellis & Keable, 2008).

2.1.1 The spiny dogfish (*Squalus acanthias*)

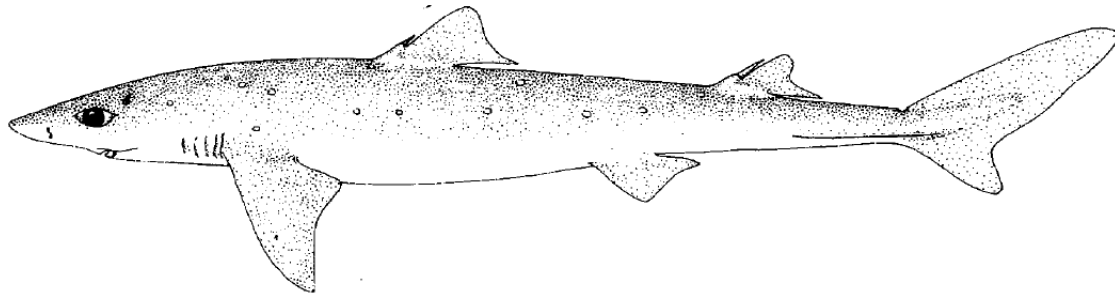


Figure 2. The overall habitus of *S. acanthias* (© Compagno, 1984)

Distribution, habitat use and population status:

The spiny dogfish is a benthopelagic coastal species with an oceanodromous life cycle, *i.e.* all life history events, and migratory behavior take place in saltwater (Riede, 2004). It exhibits a broad vertical distribution (0 – 1500 m) in the water column but is found mostly between 50 – 400 m depth (Cox & Francis, 1997; Mecklenburg *et al.*, 2018). The Northeast Atlantic population is thought to be a single stock that undergoes large scale seasonal movements even to a transatlantic extent (Aasen, 1964; Hjertenes, 1980; Gauld & MacDonald, 1982; Templeman, 1984; Vince, 1991). Most studies concerning the distribution of the species in the Northeast Atlantic are based on mark and recapture approaches of individuals in offshore areas (Aasen, 1964; Hjertenes, 1980; Vince, 1991; Gauld & MacDonald, 1982). *S. acanthias* favors temperatures around 7°C – 15°C, and it has been suggested that migratory behavior is dependent on temperature gradients (Compagno, 1984). In the Northeast Atlantic the population of spiny dogfish has declined by *ca.* 80% due to high commercial fishing activity in the beginning of the 20th century (Vince, 1991; Ellis *et al.*, 2008; Ellis *et al.*, 2015). However, in Norwegian waters the stock seems to be recovering as well (Albert *et al.*, 2019)

General Behavior, diet and life history:

S. acanthias is a schooling shark and is found segregated by sex and also by size (Smith *et al.*, 2008). This species is highly maneuverable, flexible (*i.e.* tight turning radius and a high turning rate) and agile (*i.e.* ability of fish to reorient itself fast (Webb, 1994)) (Domenici, 2001, 2004; Aleyev, 2012). The diet of the spiny dogfish in the eastern Atlantic waters consists mostly of crustaceans and fish, *e.g.* hermit crab (*Paragus spp.*), mud shrimp (*Callinassa spp.*), Norway lobster (*Nephrops norvegicus*) Atlantic mackerels (*Scomber scombrus*), whiting (*Merlangius merlangus*), and Norway pout (*Trisopterus esmarkii*) (Ellis *et al.*, 1996). *S. acanthias* tends to prey upon small size classes of fish when they are young. As the spiny dogfish grows the prey items size increases as well (Bowman *et al.*, 1984).

In the Norwegian Sea and the North Sea, the average size of *S. acanthias* ranges from 50–120 cm total length (TL) for females and 40–95 cm TL for males (Albert *et al.*, 2019 and references therein). Furthermore, there are also sexual differences for the age span of the spiny dogfish, with females reaching a higher maximum age than male individuals in the Norwegian waters (Albert *et al.*, 2019). A maximum age of 40 years for females and of 35 years for males was suggested (Nammack *et al.*, 1985). In the Norwegian waters and the North Sea, the age of 50 % maturity for females has been estimated to an average age of 9.5 years and an average TL of 77.8 cm while for males the average age was around 5 years and the average TL 60 cm (Albert *et al.*, 2019). In general terms, spiny dogfish are long-lived, have a low fecundity, and mature late (Ketchen, 1972; Cortés, 2000; Bubley *et al.*, 2012).

2.1.2 The thorny skate (*Amblyraja radiata*) and the Arctic skate (*Amblyraja hyperborea*)

The two main species of the genus *Amblyraja* found in the Northeast Atlantic (Horton *et al.*, 2020) are: *A. radiata* and *A. hyperborea* (Artsdatabanken, a database for biodiversity in Norway <https://www.artsdatabanken.no/>).

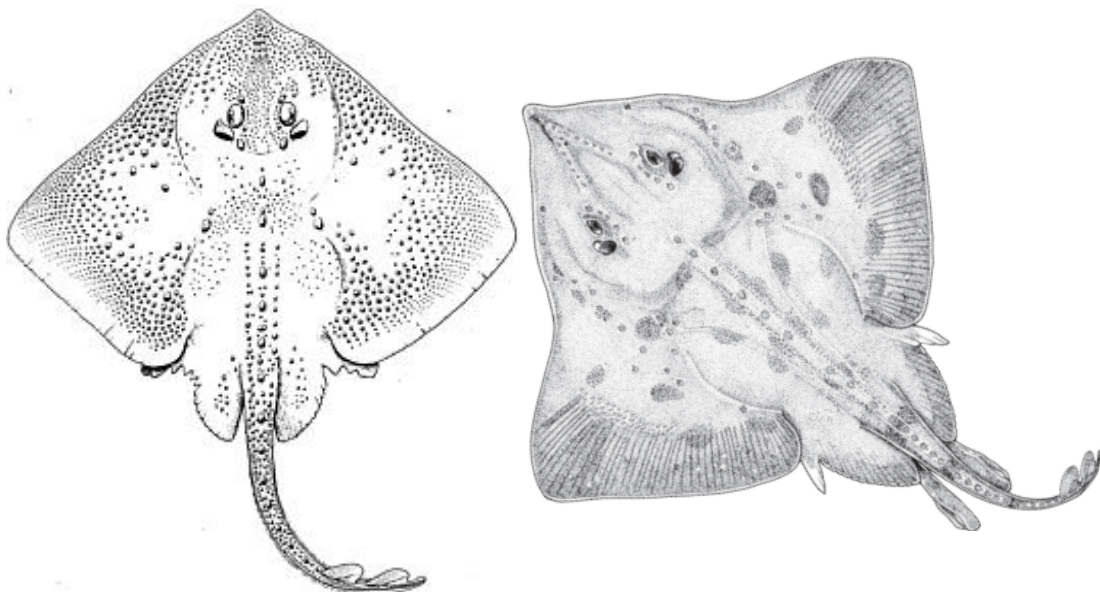


Figure 3. The overall habitus of *A. radiata* (left) and *A. hyperborea* (right) (© wikicommons and Ebert, 2014)

Distribution and habitat use:

A. radiata and *A. hyperborea* are distributed in the Northeast Atlantic throughout Iceland, Greenland–, Barents–, North Sea, Svalbard, down to the English channels and the western Baltic. In the western parts of the Atlantic they occur in Canada, Greenland, Hudson Bay, and South Carolina (USA). *A. radiata* has been reported even off the coast of Cape Town, South Africa (fishbase.se). *A. hyperborea* on the other hand is found in much colder waters of the Arctic basin (Bigelow &

Schroeder, 1953; Compagno, 1984). *A. radiata* is eurytherm (*i.e.* tolerates high temperature variation) within a temperature range from -1 to -14°C (Packer *et al.*, 2003). *A. hyperborea*, however, prefers a temperature range from -1 to -4°C , and is found in the deeper parts of the Arctic Ocean (92 – 2925 m; usually around 300 – 1500 m) (Hureau *et al.*, 1984; Mecklenburg *et al.*, 2018). *A. radiata* is usually found in offshore waters and is eurybathic (*i.e.* capable of living on the bottom in both deep and shallow water) at a depth ranging from 5 – 1540 m (usually living at 25 – 440 m) (Coad & Reist, 2018; Last *et al.*, 2016). Both species are benthic on either soft or hard seafloor. Their whole life cycles are oceanodromous (Riede, 2004).

Life history:

The maximum total length (TL) reported for *A. radiata* is 111 cm, and maturity is usually reached at 84 cm TL (NEFSC, 2000). For the Barents Sea the TL ranges are 15 – 66 cm for males and 17 – 68 cm for females. Mature fish are predominantly in the size range of 46 – 50 cm (Dolgov, 2005). For *A. hyperborea* the TL range in the Barents Sea ranges from 31 to 81 cm for males and from 28 to 85 cm for females (Dolgov, 2005).

Both species are slow-growing (Carlson & Goldman, 2006). It has been observed that TL and length at maturity vary widely and decrease with increased latitude. The general age at maturity was estimated to be 10.7 ± 0.7 years for females and 14.7 ± 1.4 years for males (Richardson, 2017 and references therein). A maximum age of at least 28 years was estimated (Templeman, 1984). The feeding habits of both species are similar. Both are at early stages of their life cycle typical benthos-feeders. Later on, they become more demersal feeders. This becomes evident from their diet of fishes and various decapods. The larger *A. radiata* grows the more it preys upon larger fish and crustaceans, while smaller food items (Gammaridea, Euphausiidae and Polychaeta) decrease (Dolgov, 2005). The *A. hyperborea* diet consists mostly of fish (*ca.* 90%) with little amounts of shrimp or fisheries waste (less than 10%) (Dolgov, 2005). The following species are prey items for small sized *A. radiata* and *A. hyperborea* (individuals < 40 cm): polychaetes and decapods which are the major prey items, followed by amphipods and euphausiids. Mysids contribute little to the diet (Packer *et al.*, 2003). Among the fish prey items are: Macrouridae, Myctophidae and *Sebastes sp.* (Packer *et al.*, 2003; González *et al.*, 2006).

2.2 Sampling

A total of 229 stomachs from the two target species were sampled from the years 2016 – 2019 at 77 locations across the Nordic region, which varied by species due to distribution and sample availability differences. In addition, 27 spiral valves of *A. radiata* and *A. hyperborea* were examined for a small-

scale comparison between stomachs and spiral valves with respect to plastic particles. This was motivated by a recent study showing high concentrations of (micro) plastic particles in spiral valves of the porbeagle shark (Maes *et al.*, 2020). This was however not part of the initial experimental design.

2.2.1 Experimental design

The study–design was set up to cover a wide spatial distribution for each given species, as well as include different length, weight, maturity classes and both sexes. This ensured a balanced experimental design and allowed for studying if any of those factors affect the ingestion of plastic particles. The samples were selected and grouped into three main areas, namely the North Sea, Norwegian Sea and Barents Sea. *S. acanthias* has a wide distribution along the North– and Norwegian Sea and was thus selected to be the model species for those regions. *A. radiata* has an even wider latitudinal distribution and extends northwards into the Barents Sea. Both species occur and had samples from the North Sea making it possible to compare for species–species differences within the same area. In addition, spatial differences in plastic ingestion could be compared between North–, Norwegian–, and Barents Sea within species, where possible. The samples were chosen as to include a few empty stomachs as well, besides mostly full ones, to examine if satiety levels have an impact on plastic ingestion.

From each area, 45 samples were selected, 15 individuals per “small”, “middle” and “large” stomachs. Stomach size was here used as a proxy for specimen size as not all life history data was available at the time. In addition, an equal sex ratio was attempted where possible. As the study progressed, additional samples of *S. acanthias* (n = 15) and *A. radiata* (n = 15) were added to increase sample size (resulting in a higher chance of finding any plastic particles).

2.2.2 Sampling locations

All 229 samples were collected in the Northeast Atlantic from the North Sea (52 locations) northwards to the Norwegian Sea (13 locations) and the Barents Sea (14 locations) covering the species’ large latitudinal spans. The samples were obtained mostly during scientific surveys implemented by the Institute of Marine Research (IMR). The surveys relevant for this thesis were: Reketokt (07.01.–28.01.2017 and 09.01.–25.01.2019), Vintertokt (24.01.–24.02.2019) and EggaNor survey (02.09.–17.09.2019). In 2019, 115 individuals were caught (Reketokt n = 67; EggaNor n = 17, Vintertokt n = 31), and in 2017, 45 individuals only during Reketokt (n = 45). The rest of the samples were obtained opportunistically through commercial fisheries (Fiskemottak) between 2016 and 2018 (n = 59; Table 1). The sampling area covered three climate zones (polar, boreal and temperate

oceanic), which have distinct characteristics. The polar region is characterized by extreme cold winters (ca. 6 months of subzero temperatures) and cold summers (Barents Sea). The boreal region exhibits cold winters and short cool summers. Precipitation is increasing towards the coastal regions usually during the warmer summer months (Norwegian Sea). The oceanic temperate zone has a smaller temperature variation because the North Sea acts as a buffer which renders summers warm and winters mild. Precipitation is high and occurs as rain throughout the year (Schneider *et al.*, 2013). Sampling areas and locations varied among the three studied species (*e.g.* *S. acanthias* occurs in the North Sea and has its northern distribution limit up to the Norwegian Sea, whereas *A. radiata* occurs in all three areas, *i.e.* can additionally be found in the Barents Sea; see Figure 4). *A. hyperborea* was only used for the Barents Sea comparison. For an overview of locations, time of the year and species see Table 1.

The North Sea:

The North Sea has a surface area of 575,300 km² and a volume of 43,294 m³ which makes it relatively small and extremely shallow in comparison to other oceans of the world (Lee, 1980). The close proximity of the Baltic and the Norwegian Sea significantly influences its salinity and temperature. A substantial fraction (49%) of the *A. radiata* and *S. acanthias* samples were taken from the North Sea during the Reketokt 2017 and 2019. The “Reketokt” survey is an annual shrimp survey in the North Sea and the Skagerrak using bottom trawling. The number of sampling locations for *S. acanthias* was 18 and 28 for *A. radiata*. There were 45 *S. acanthias* samples obtained from 18 locations during the 2017 Reketokt, and 59 sampled at 15 locations from commercial fisheries as landings. For *A. radiata* 67 samples were collected from 28 stations during the 2019 Reketokt.

The Norwegian Sea:

The Norwegian Sea is located to the west of Norway and includes the Norwegian and Lofoten Basin. It covers an area of 1,100,000 km² and reaches a maximum depth of over 3700 m. It is separated by the Fram Strait in the north from the rest of the Arctic Ocean, the Greenland–Scotland Ridge in the south and blocked west and east from Iceland and Norway respectively (Drinkwater *et al.*, 2013). For *S. acanthias* 16 samples in nine locations were obtained from the Norwegian Sea via commercial fishery landings in the years 2016 and 2018 (15.10.16 and 15.03.– 20.04.18; 9 locations in total). Additionally, three samples from two locations were secured for *A. hyperborea* during the EggaNor survey in September 2019. The “EggaNor” survey is a biennial Norwegian Sea continental slope deep–sea fish survey in autumn using bottom trawling

The Barents Sea:

This sea is located north of the Norwegian Sea and has an area of 1,400,000 km² with 230 m average depth. At 81° latitude it is cold enough that sea ice can form, but this is influenced through the warm water of the Atlantic Ocean current that moves northward into the Arctic, thus affecting the thickness and duration of the ice cover (Zeeberg & Forman, 2001; Drinkwater *et al.*, 2013). Here, during the Vintertokt (24.01. – 24.02.2019) 12 locations with 31 individuals of *A. radiata* were obtained. The “Vintertokt” is an annual NOR–RUS demersal fish survey in winter in the Barents Sea using bottom trawling. In addition, 7 individuals of *A. hyperborea* from two locations caught during the EggaNor survey (2019) were included.

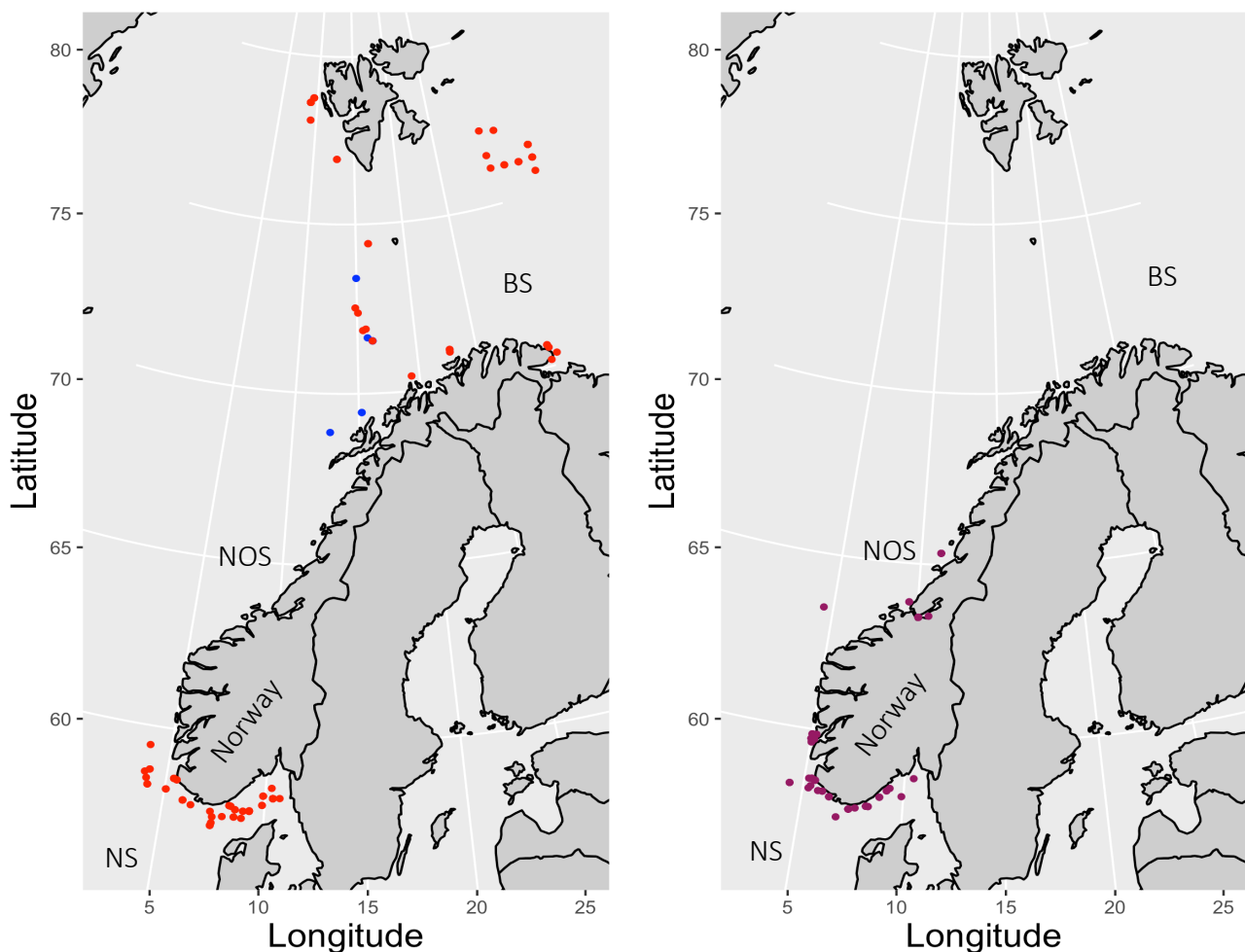


Figure 4. Overview map of the three sampled species; Left: spatial distribution of *A. radiata* (red) and *A. hyperborea* (blue) distribution, Right: spatial distribution of *S. acanthias* (dark pink); NS = North Sea, NOS = Norwegian Sea, BS = Barents

Table 1. Summary table of *S. acanthias*, *A. radiata* and *A. hyperborea* samples with, Species, Area, Sample source, Year, Time in the Year, No. of locations, No. of individuals, Females and Males

Species	Area*	Sample source	Year	Month	No.** of locations	No.** of Individuals	Females	Males
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<i>S. acanthias</i>	North Sea	Reketokt	2017	January	18	45	6	39
<i>S. acanthias</i>	North Sea	Landings	2017	October	3	21	19	2
<i>S. acanthias</i>	North Sea	Landings	2017	November	3	22	12	10
<i>S. acanthias</i>	Norw. Sea	Landings	2016	October	1	4	3	1
<i>S. acanthias</i>	Norw. Sea	Landings	2018	March	2	3	2	1
<i>S. acanthias</i>	Norw. Sea	Landings	2018	April	6	9	7	2
	TOTAL		3		33	104	49	55
<i>A. radiata</i>	North Sea	Reketokt	2019	January	28	67	35	32
<i>A. radiata</i>	Norw. Sea	EggaNor, Vintertokt	2019	February, September	20	33	18	15
<i>A. radiata</i>	Barents Sea	EggaNor, Vintertokt	2019	February, September	5	15	7	8
	TOTAL		1		42	115	60	55
<i>A. hyperborea</i>	Norw. Sea	EggaNor	2019	September	4	10	3	7
	TOTAL		1		4	10	3	7

*Norw. Sea = Norwegian Sea;

**No. = Number

2.2.3 Sampling protocol

I took part in the Reketokt 2019 (North Sea) as a student researcher for two weeks and sampled 49 *A. radiata* as my own samples. I additionally processed the 10 *A. hyperborea* individuals from the Vintertokt 2019 and EggaNor survey 2019. Those were sampled at a wet lab on land (Tromsø, Norway). All other individuals had been previously processed and samples were taken by scientists at the IMR.

All specimens were measured (length, weight) and sexed. Through a maturity scale specific to ovoviviparous (*S. acanthias*) and oviparous (*A. radiata* and *A. hyperborea*) elasmobranchs the maturity stage of the individuals was determined (see Appendix Tables 1 – 4). The stomachs were extracted by cutting as close as possible on the front end of the digestive system and in front of the spiral valve at the back end, thus carefully avoiding the spill of prey items. The sampled stomachs were then placed into plastic bags and frozen until further processing. Additionally, tissue and spine/vertebrate samples were also taken and stored in either ethanol or frozen. Individuals that were not immediately

processed during the sampling cruises were kept frozen until later lab dissection. Specimens that stemmed from commercial fisheries were frozen whole and sent to IMR facilities where they were appropriately processed as mentioned above. For 10 *A. hyperborea* and 17 *A. radiata* samples the spiral valve was also extracted and analyzed for plastics content.

2.3 Stomach processing

2.3.1 Diet assessment

For assessing the stomach content of the three species a stepwise protocol was used that is briefly described as follows: stomachs were cut open to assess the content by visual inspection. Any identified items were sorted into three general categories (*i.e.* shrimp-like, fish-like, other content) and transferred onto a glass Petri dish. Subsequently, the sorting was confirmed under a stereomicroscope at 10x/21B x 1.0 ranging from 0.63 to 5.0 x10 (6.3–50 times magnification). The wet weights (WW; Mettler Toledo PB 403 – S) for the prey items and the empty stomachs were recorded separately. The prey categories were intentionally kept broad for higher time efficiency, as the diet analysis was not the main aim of this thesis. After sorting the prey items, they were rinsed back into a glass beaker with the original stomach.

2.3.2 Pilot study

In the beginning of the project a small-scale pilot study on six stomach samples was conducted with the aim of testing published methods and optimizing the technical procedures for the IMR lab facilities. The published digestion approach suggested to use a heating cabinet with a rotating mechanism to dissolve the organic material faster at 60°C for 24h with 300 rpm agitation (Dehaut *et al.*, 2016; Kühn *et al.*, 2017). Since such a machine was not available the first trial was to assess the dissolving efficiency of a heating cabinet alone at 60°C for 24h. Another trial was done with a rotating shaker (KS501 digital) at *ca.* 110 rpm at room temperature (19–21°C) under a fume hood. In both instances the time of dissolving was noted and compared to find the optimal way of digesting the stomachs and their contents. The remaining solution after the stomach digestion was filtered through two metal mesh sieves (Test Sieve, BODY 316L Mesh S-Steel /RF S/N; Body 200mm x 50mm, 5mm and 1mm mesh size; Retsch GmbH, Figure 5). These metal sieves were used to ensure that no plastic particles would break off from the equipment and contaminate the samples. Additionally, it provided an optimal filter to hold the range of plastic particles relevant for this thesis (> 1mm).



Figure 5. 5mm metal filter stacked on 1mm filter with a glass beaker beneath

2.3.3 Digestion protocol

The entire stomachs were submerged with at least 100 ml of a 10% potassium hydroxide solution following the digestion protocol of Karami *et al.* (2017) and Kühn *et al.* (2017) for the downstream analyses of plastic components. The mixture was filled in a glass beaker (beaker size depended on stomach size) covered with an aluminum foil lid and incubated at room temperature on a shaker (KS501 digital) at *ca.* 110 rpm (based on the results from the preliminary study described above; Figure 6). The time of incubation was adapted according to the stomach's size and content in order to make sure that all organic matter was fully digested. After digestion of the stomach content, the remaining solution was particle filtered by stacking a 5mm and 1mm metal mesh sieve (Test Sieve, BODY 316L Mesh S–Steel /RF S/N; Body 200mm x 50mm, 5mm and 1mm mesh size; Retsch GmbH) on top of each other. The analysis of all remaining plastics and potentially also organic particles was performed under a stereomicroscope (Leica MZ75; 6.3–50 times magnification). Identified plastic particles were sorted into three categories (thread like, sheet like, color) and then transferred into a paper bag for later analysis (ID, plastic category, date).



Figure 6. Upper row: Example of stomach content (left to right) crustacean, mixed (crustacean and fish), single fish; Bottom row: experimental setup of the digestion.

2.3.4 Positive control

In order to ensure the definite preservation and recognition of plastic particles if present, clear plastic parts were mixed with two stomach samples before the digestion step to confirm that plastic particles > 1 mm could be detected and retrieved. These positive controls (samples 201899961 individual 17 and 201899967 individual 1) were otherwise processed the same as all other samples.

2.4 Data exploration and manipulation

All data were explored and manipulated through the R statistical software version 3.5.1 (R Core Team, 2018) with RStudio version 1.1.463. Data Exploration was done according to the suggested approach by Highland Statistics (Zuur *et al.*, 2010) checking for outliers between y and x variables, zero inflated values and collinearity between y and x values using R in Rstudio. Plotting and mapping of the graphs was done with the ggplot2, tidyverse, lattice, maps, mapdata, grid, marmap and dplyr packages (Sarkar, 2008; Pante & Simon-Bouhet, 2013; Wickham, 2016; Becker *et al.*, 2018; Becker & Brownrigg,

2018; R Core Team, 2018; Wickham *et al.*, 2019, 2020). The rest of the analysis is described in the respective parts below.

The raw data consisted of 229 observations with 26 variables. The data was then filtered to select the relevant variables for further analysis. For the life history analysis and plotting of the maps ten variables (sex, individual weight, length, maturity stadium, depth, weight of fish content, weight of shrimp content, weight of others content, latitude and longitude) were used. Because of very small individuals, assumingly embryos, five *A. radiata* individuals were excluded from the weight and length analysis (< 0.021 kg; < 15 cm TL). They were originally included in the whole data set, in order to confirm the assumption that embryos will not have ingest any macroplastic particles yet. The weight of those samples was recorded during a survey, which resulted in potentially unreliable weight measurements, as the weather conditions at sea prevent such precise fine-scale measurements.

2.4.1 Life history data analysis

First the data were explored for outliers through the boxplot and Cleveland dotplots resulting in the exclusion mentioned above. Then the data was checked for Y and X relationships of all ten variables to detect eventual patterns (ggplot2 package). Length measurements were converted from mm to cm and individual weight from grams (g) in kilograms (kg). Maturity scales were converted into integers (*i.e.* real numbers) to work in RStudio. Then sex and maturity stages were changed into categorical values by using the factor function in R.

Only the individual weight, length, sex and maturity stadium data were used for all three species, since those were the only data that were available for all species (see Appendix Table 5). “Fish content” (*i.e.* the amount of fish found in the stomachs) was examined for *S. acanthias*, since “shrimp content” (*i.e.* the amount of shrimp found in the stomachs) and “others content” had insufficient data to be analyzed. Fish content of *S. acanthias* was plotted against length and weight in using the ggplot2 package. For *A. radiata* and *A. hyperborea* the fish content and the shrimp content were analyzed through plotting against length and weight. All diet related data was plotted against sex through the boxplot function (ggplot2 package) to determine which of the sexes ingested the most of each content category for each species. Through the summary and sd functions the average and standard deviation of each species were determined for length, weight, depth, weight of fish content, weight of shrimp content and weight of others content. Maturity was plotted using pie charts (ggplot2 package) to evaluate the proportion of mature and immature individuals in each maturity stadium for each species.

Some of the maturity stadiums for *A. radiata* were not available. Those maturity stadiums were estimated by length comparisons according to previous survey data (IMR length data of *A. radiata* Reketokt 1990 – 2019) (see Appendix Table 5). This was done in R using the filter function of the ggplot2, tidverse and dplyr package.

2.4.2 Mapping spatial distribution

At first a map of Norway was generated with the map_data("world","Norway") function of the ggplot2 package. Then the latitude and longitude values were extracted for each species separately. The ocean boundaries were divided into North Sea, Norwegian Sea and Barents Sea (including four sampling station to the west coast of Svalbard) using the International Hydrology Organization definition (IHO, 1953). The maps were then plotted with the ggplot2 function using geom_polygon and coord_map (projection = stereographic) functions as additional layers. This served as a base for all other mapping. Then the latitude and longitude of the different species locations were plotted on top of that base using the geom_point function. The proportion of sexes for each ocean was determined using the subset function to separate the individuals either into North Sea, Norwegian Sea or Barents Sea. This was done by latitude.

3. Results

Pilot study and positive controls: Incubating stomachs in a heating cabinet for 24h at 60°C in 10% KOH solution resulted in a partial digestion. On the other hand, samples that were set up on a rotating table at room temperature (19 – 21°C) in 10% KOH solution (under a fume hood; ca. 110 rpm) were completely digested. This latter approach was therefore used for all the other samples with the modification that the time of incubations was adjusted according to stomach size. It was demonstrated through the positive controls that plastic particles (> 1 mm) can be retrieved with 100% accuracy through the metal filter sieve set up.

3.1 *Squalus acanthias*

3.1.1 Life history traits

The 104 *S. acanthias* samples consisted of 55 males and 49 females. This equated to a sex ratio of 1:1.12. The length of the individuals ranged from 25 – 101 cm with a mean value of 71 cm (SD ± 17.8 cm). Male length ranged from 25 – 84 cm with a mean of 67 cm (SD ± 17.1 cm), and female length ranged from 25 – 101 cm with a mean value of 75.6 cm (SD ± 17.7 cm).

The weight of all *S. acanthias* samples ranged from 0.048 – 4.8 kg with a mean value of 1.7 kg (SD \pm 959.4 g). Males ranged from 0.060 – 2.1 kg with a mean value of 1.3 kg (SD \pm 592.3 g). For the females the weight range was 0.048 – 4.8 kg with a mean of 2.2 kg (SD \pm 1.1 kg). Females had the highest weight at 4.8 kg among all the samples. The majority of all samples (80 of 104 individuals, *i.e.* 77%) were in the weight range from 1 – 3 kg. The weight and length relationship of all samples is shown in Figure 7.

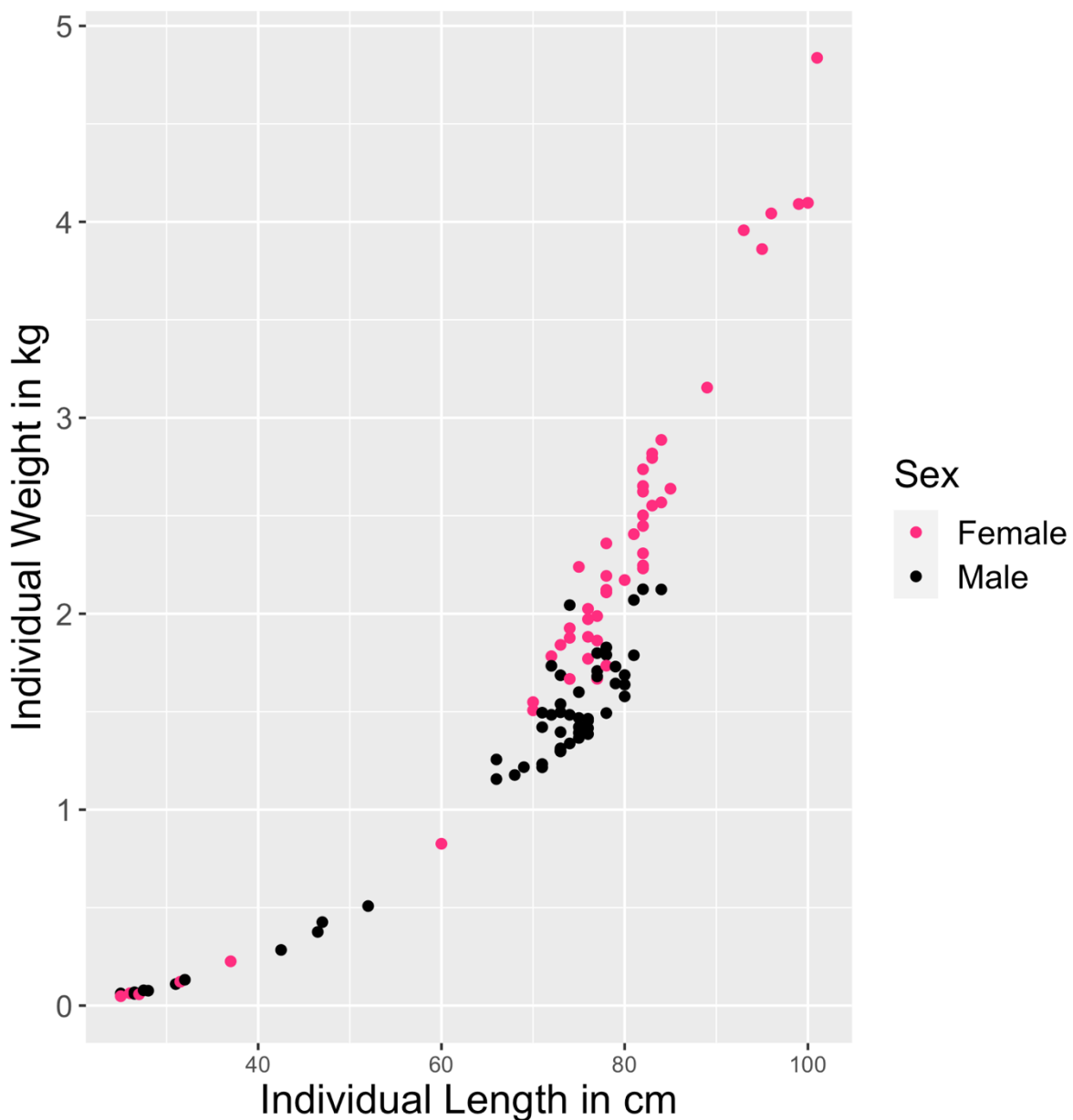


Figure 7. Weight and length relationship of *S. acanthias* sorted by sex (pink = female, black = male)

S. acanthias individuals were sampled at 19 different depths ranging from 0 – 500 m. The mean depth was 224.7 m (SD \pm 108.8 m). The samples from the North Sea (88 total) were collected from 57.641 – 59.860 latitude. There were 51 males and 37 females resulted in a sex ratio of 1:0.73. The samples of

the Norwegian Sea (16 individuals) were collected from 63.466 – 65.336 latitude. There were 4 males and 12 females resulting in a sex ratio of 1:3.

The maturity stadium of the female samples was assigned on a scale ranging from 1 – 6 (see Appendix Table 1). Most female samples were in the “Developing” (24.5%), “Early Pregnancy” (30.6%) and “Mid Pregnancy” (20.4%) maturity stadiums (Figure 8). The maturity proportion of the females in the North Sea was 54.1% while in the Norwegian Sea it was 75%. The highest maturity stadium among the male samples was “Active” and the lowest “Immature” (maturity stadium 1 – 4; see Appendix Table 2). Most male samples were in the “Capable to reproduce” (50.9%) and “Active” (29.1%) maturity stadiums (see Figure 8). The maturity proportion of the males in the North Sea was 76.5% while in the Norwegian Sea it was 100%.

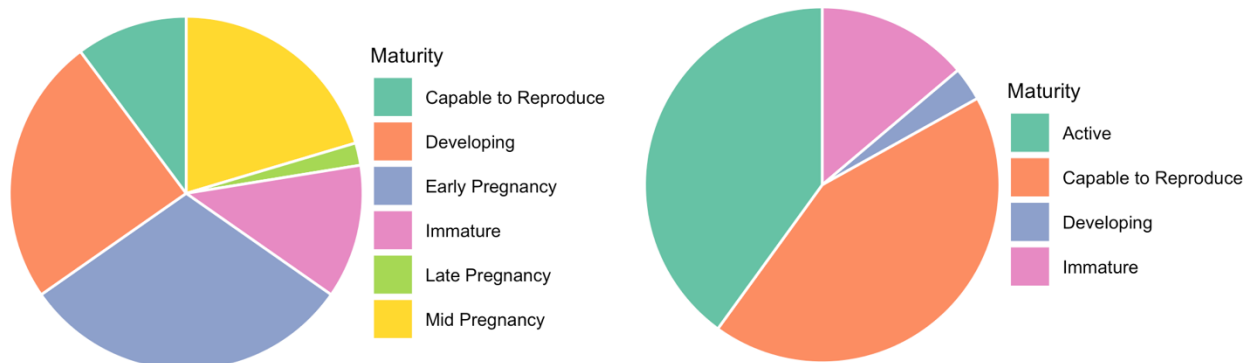


Figure 8. Left: Different maturity stadiums among all female samples (“Immature” n = 6; “Developing” n = 12; “Capable to Reproduce” n = 5; “Early Pregnancy” n = 15; “Mid Pregnancy” n = 10; “Late Pregnancy” = 1); Right: Different maturity stadiums among all male samples (“Immature” n = 9; “Developing” n = 2; “Capable to Reproduce” n = 28; “Active” n = 16)

3.1.2 Diet analysis

Of the 104 individuals, 47 (45.2%) ingested prey items and 57 (54.8%) had an empty stomach. Out of the 47 that ingested prey items, 43 individuals ingested fish (91.5%). Of the 43 individuals that ingested fish, 35% were males (n= 15) and 65% were females (n= 28) (see Figure 9). It could be generally reported that the amount of fish ingested outweighed the amount of any other category (fish content: 1.9 kg, shrimp content: 20 g and others: 14 g). The average amount of ingested fish was 44.7 g (SD ± 50.8 g). Among those that ingested fish, two individuals also ingested other prey items in a different prey category. One ingested shrimp and the second did ingest prey items in the “others” category. Three individuals ingested shrimp (1.6 – 6.9 g). Two of those also ingested “others” (total 7.7 g). Only one individual ingested prey in the “others” category while ingesting nothing else (0.2 g). All of the individuals that ingested anything else besides fish weighed above 1.5 kg and had a size range of 74 – 100 cm. For the detailed individual list refer to Appendix Table 5.

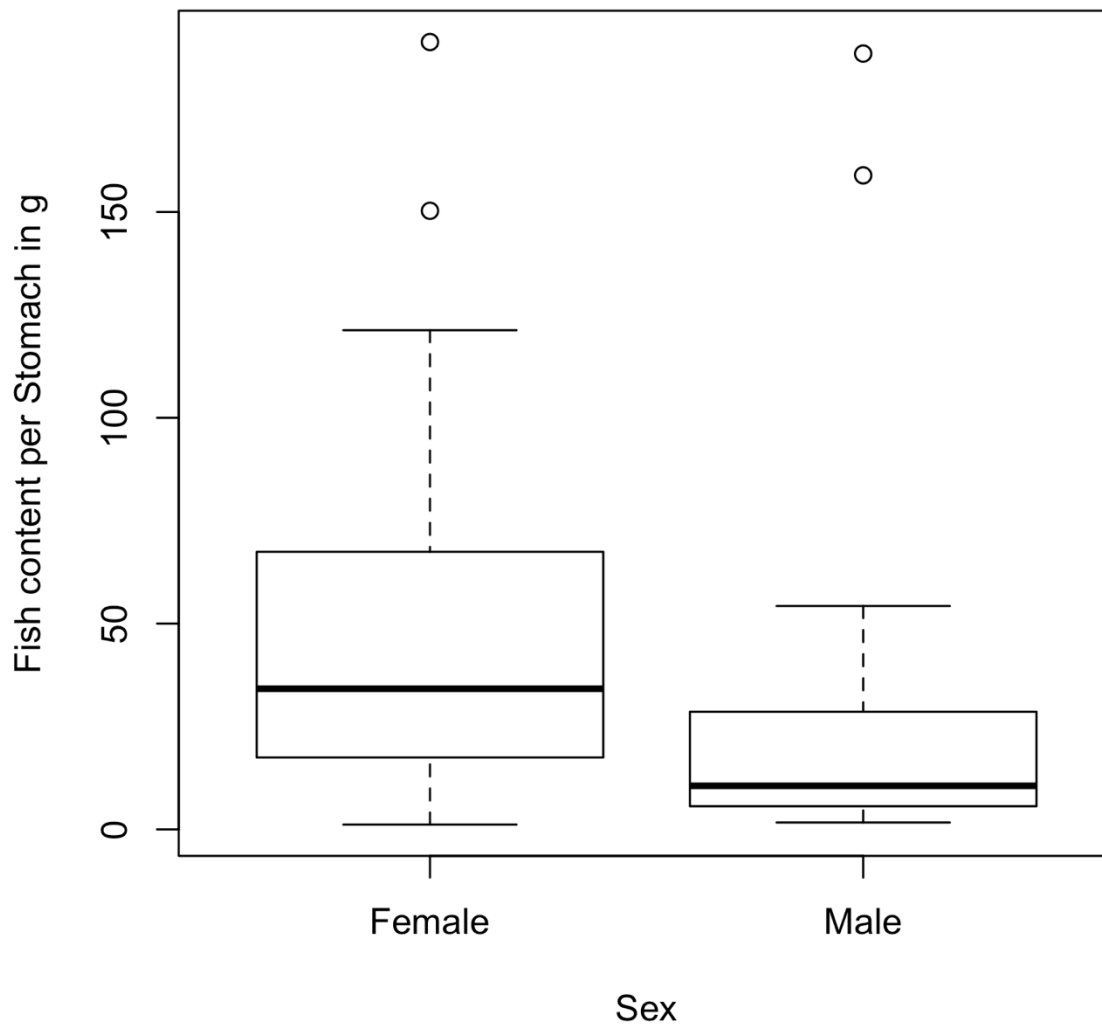


Figure 9. The fish content (in gram) per sampled stomach for each sex; 28 female and 15 male individuals

3.2 *Amblyraja radiata*

3.2.1 Life history traits

The 115 *A. radiata* samples consisted of 58 males and 57 females. This equated to a sex ratio for all samples of 1:0.99. The length of the individuals ranged from 15.5 – 63 cm with a mean value of 40.9 cm (SD \pm 13.4 cm). Male length ranged from 15.5 – 63 cm with a mean of 41 cm (SD \pm 13.9 cm), and female length ranged from 16 – 63 cm with a mean value of 40.1 cm (SD \pm 13.9 cm).

The weight of all the *A. radiata* samples ranged from 0.024 – 2.1 kg with a mean value of 0.761 kg (SD \pm 0.588 kg). Males ranged from 0.024 – 2.1 kg with a mean value of 0.808 kg and (SD \pm 0.615 kg). For

the females the weight range was 0.033 – 2 kg with a mean of 0.713 kg (SD \pm 0.561 kg). The weight and length relationship of all samples is shown in Figure 10.

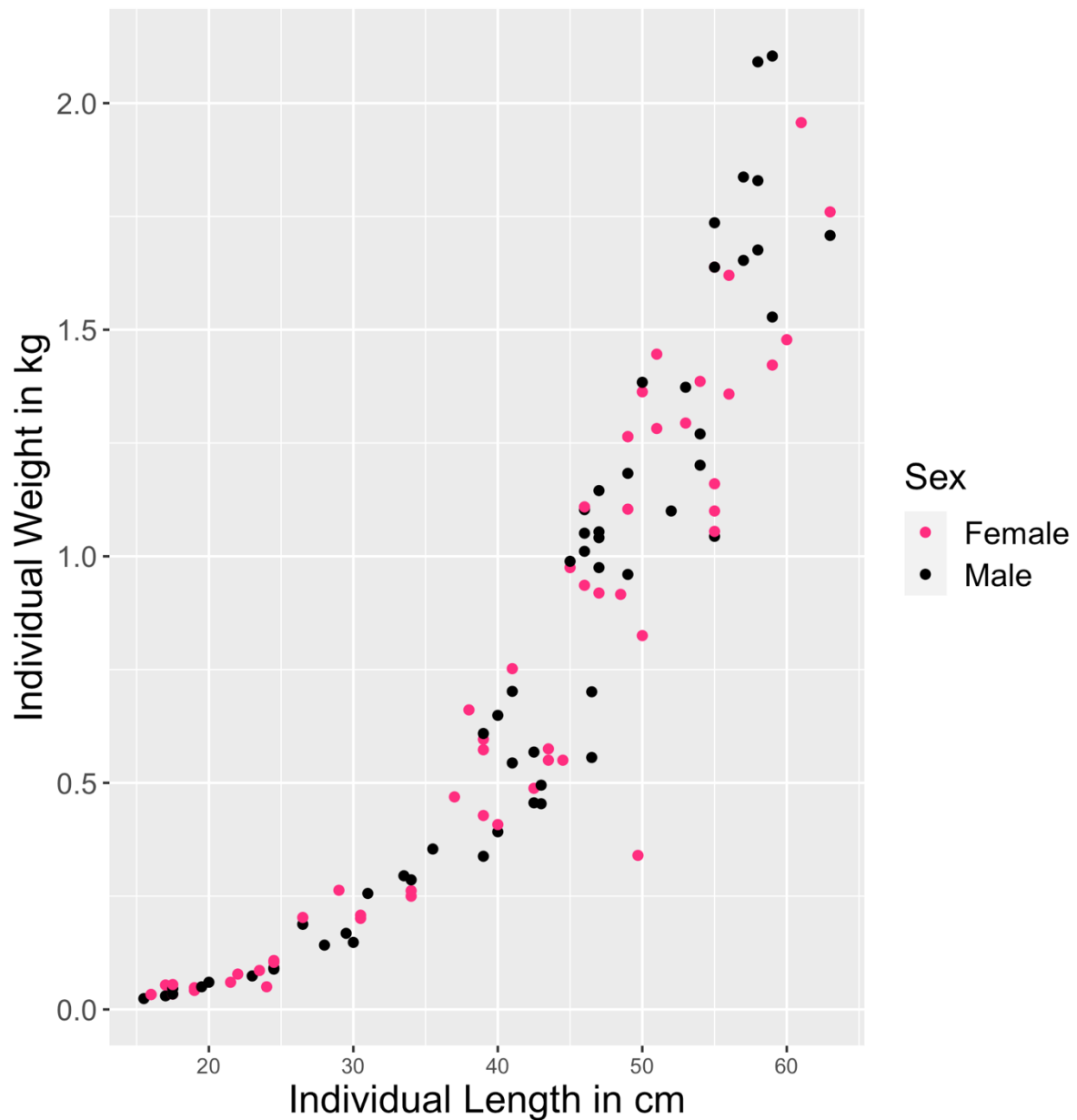


Figure 10. Weight and length relationship of all *A. radiata* samples

A. radiata individuals were sampled at 54 different depths ranging from 0 – 900 m. The mean depth was 335.8 m (SD \pm 139.2 m). The samples from the North Sea (67 total) were collected from 57.4415 – 59.4594 latitude. There were 34 males and 33 females which resulted in a sex ratio of 1:0.97. The Norwegian Sea samples (33) were collected from 70.1877 – 76.4767 latitude. There were 16 males and 17 females which resulted in a sex ratio of 1:0.94. The samples from the Barents Sea (15 total) were collected from 76.9358 – 78.7422 latitude. There were 8 males and 7 females which resulted in a sex ratio of 1:0.88.

The maturity stadium of the female samples was assigned on a scale ranging from 1 – 4a (see Appendix Table 3). Most female samples were in the “Immature” (57.9%) and “Developing” (17.5%), maturity stadiums (see Figure 11). The maturity proportion of the females was 21.2% in the North Sea, 100% in the Norwegian Sea and 23.5% in the Barents Sea. The highest maturity stadium among the male samples was “Regenerating” and the lowest “Immature” (maturity stadium 1 – 4a; see Appendix Table 4). Most male samples were in the “Immature” (46.6%), “Developing” (22.4%) and “Capable to reproduce” (22.4%) maturity stadiums (see Figure 11). The maturity proportion of the males was 11.8% in the North Sea, 62.5% in the Norwegian Sea and 50% in the Barents Sea.

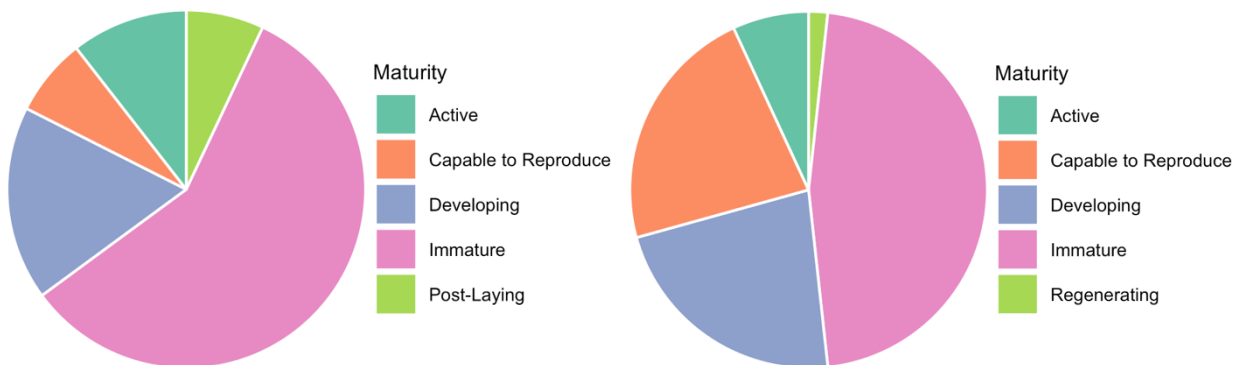


Figure 11. Left: Different maturity stadiums among all female samples (“Immature” n = 33; “Developing” n = 10; “Capable to Reproduce” n = 4, “Active” n = 6, “Post-Laying” n = 4); Right : Different maturity stadiums among all male samples (“Immature” n = 27; “Developing” n = 13; “Capable to Reproduce” n = 13, “Active” n = 4, “Regenerating” n = 1)

3.2.2 Diet analysis

Of the 115 individuals 43 (37.4%) ingested prey items and 72 (62.6%) had an empty stomach. Out of the 43 that ingested prey items, 24 individuals ingested fish (55.8%). Of the 24 individuals that ingested fish, 16.3% were males (n=7) and 83.7% were females (n = 36) (see Figure 12). The average amount of ingested fish was 10.7 g (SD ± 20 g). Among those that ingested fish, 16 individuals also ingested prey in the shrimp category. Among those 16 individuals the average amount of shrimp ingestion was 9 g (SD ± 7.3 g). There were 19 of the 43 individuals which ingested shrimp without ingesting anything else (44.2%). The average amount of shrimp ingested by the 19 individuals was 6.4 g (SD ± 7.8 g). It could be generally reported that the amount of fish and shrimp ingested differed between 10.13 g and was nearly equal in amount (fish: 256.2 g, shrimp: 266.3 g and others: 0 g). There were no ingested prey items in the “Others” category. For the detailed individual list refer to Appendix Table 5.

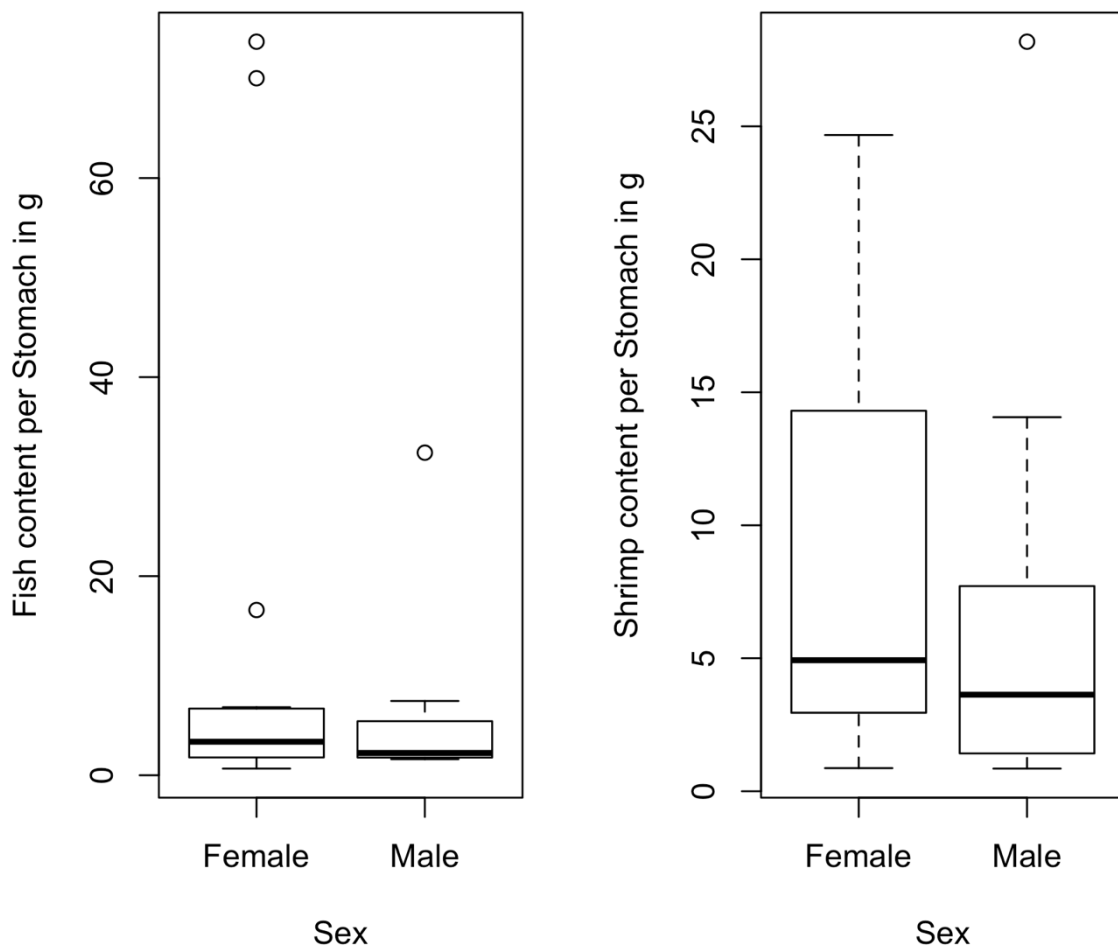


Figure 12. The fish and shrimp content (in gram) per sampled stomach for each sex; fish content: 17 females and 7 males individuals, shrimp content: 20 females and 16 males

3.3 *Amblyraja hyperborea*

3.3.1 Life history traits

The 10 *A. hyperborea* samples consisted of 7 males and 3 females. This equated to a sex ratio for all samples of 1:1.8. The length of the individuals ranged from 18 – 78 cm with a mean value of 62.5 cm (SD ± 17.6 cm). Males length ranged from 52 – 78 cm with a mean of 66.8 cm (SD ± 8.8 cm) and female length ranged from 18 – 77 cm with a mean value of 52.3 cm (SD ± 30.6 cm).

The weight distribution of the *A. hyperborea* samples ranged from 0.044 – 4 kg with a mean value of 2.6 kg (SD \pm 1.2 kg). Males ranged 1.6 – 3.6 kg with a mean value of 2.8 kg (SD \pm 0.750 kg). For the females the weight range was 0.044 – 4 kg with a mean of 2.2 kg (SD \pm 1.9 kg). The weight and length relationship of all the samples is shown in Figure 13.

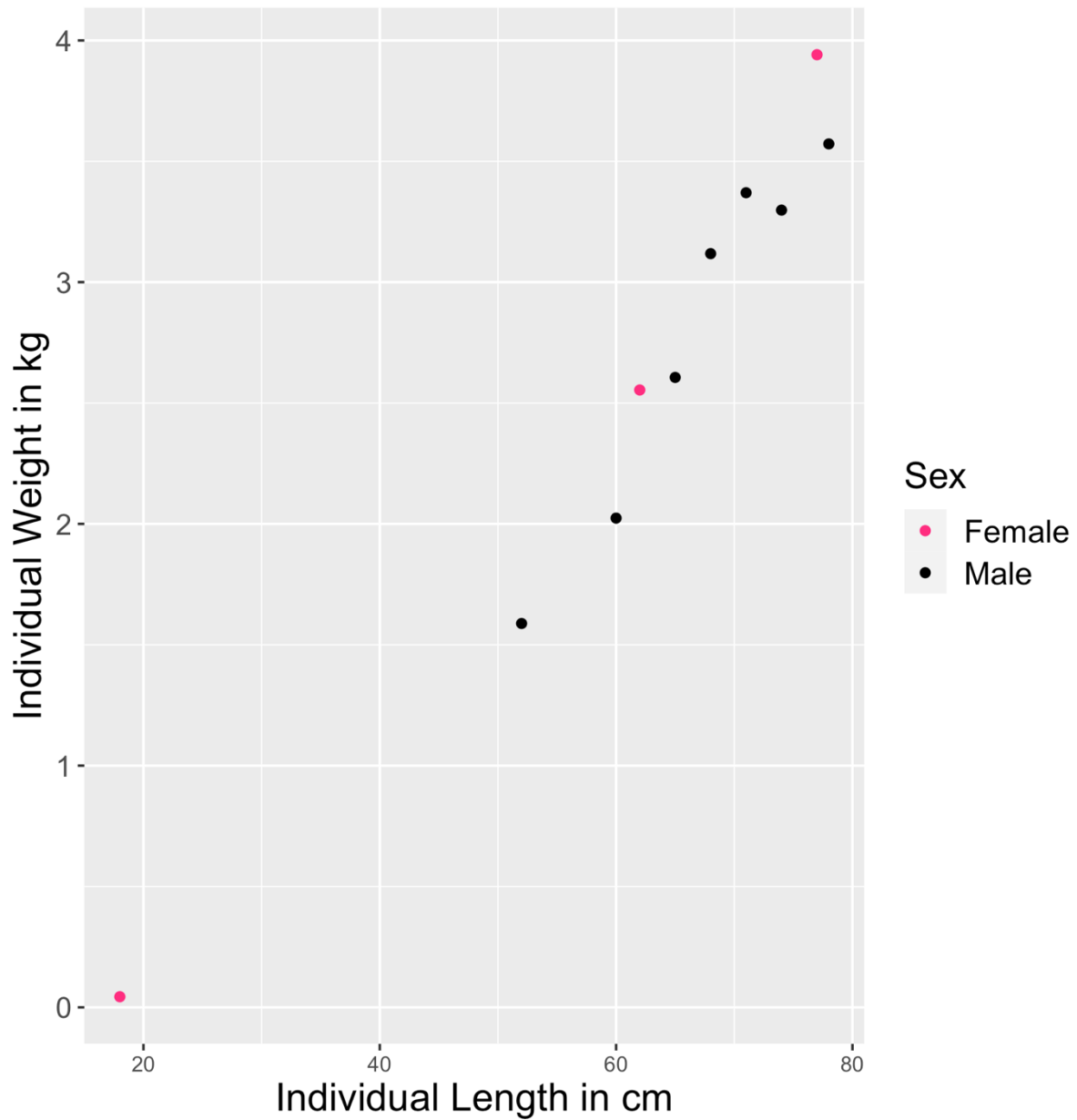


Figure 13. Weight and length relationship of *A. hyperborea*

A. hyperborea individuals were found at 4 different depths ranging from 700 – 900 m. The mean depth was 335.8 m (SD \pm 42.1 m).

All 10 samples were collected in the Norwegian Sea ranging from 68.8667 – 73.3995 latitude.

The maturity stadium of the female samples was assigned on a scale ranging from 1 – 3b (see Appendix Table 3). The female samples were in the “Immature” (33.3%), “Developing” (33.3%) and “Active” (33.3%) maturity stadiums (see Figure 14). The maturity proportion of the females in the Norwegian Sea was (33.3%). The highest maturity stadium among the male samples was “Active” and the lowest “Immature” (maturity stadium 1 – 3b; see Appendix Table 4). Most male samples were in the “Capable to reproduce” (57.1%) maturity stadium (see Figure 14). The maturity distribution of the males in the Norwegian Sea was 71.4%.

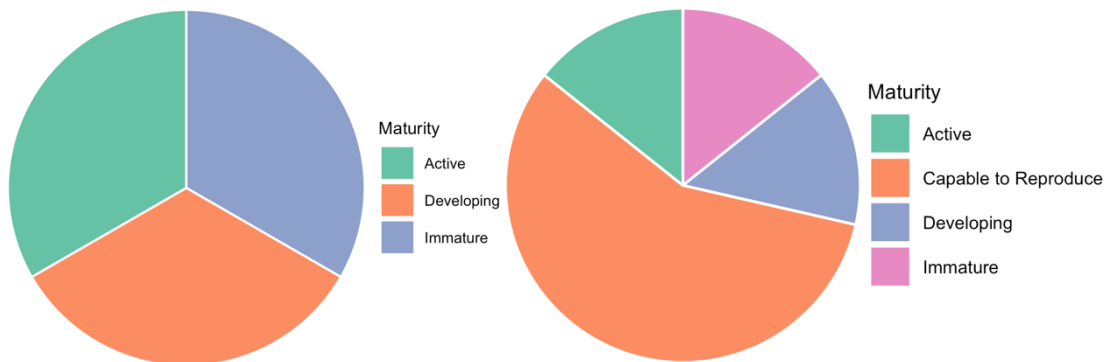


Figure 14. Left: Different maturity stadiums among all female samples (“Immature” n = 1; “Developing” n = 1; “Active” n = 1) ; Right: Different maturity stadiums among all male samples (“Immature” n = 1; “Developing” n = 1; “Capable to Reproduce” n = 4, “Active” n = 1)

3.3.3 Diet analysis

Of the ten individuals five (50%) ingested prey items and five (50%) had no prey items. Of the five individuals that ingested prey items one was female (10%) and four were males (40%) (see Figure 15). Out of the five that ingested prey items four individuals ingested fish (80%). The mean fish ingestion was 86.7 g (SD ± 88.4 g). All the individuals that ingested fish were male. One of the five that ingested fish ingested also shrimp (19.2 g). There was one individual that ingested shrimp (10 g). It can be generally reported that the amount of fish outweighed the shrimp ingestion (fish: 346.8 g, shrimp: 29.1 g and others: 0 g). For the detailed individual list refer to Appendix Table 5.

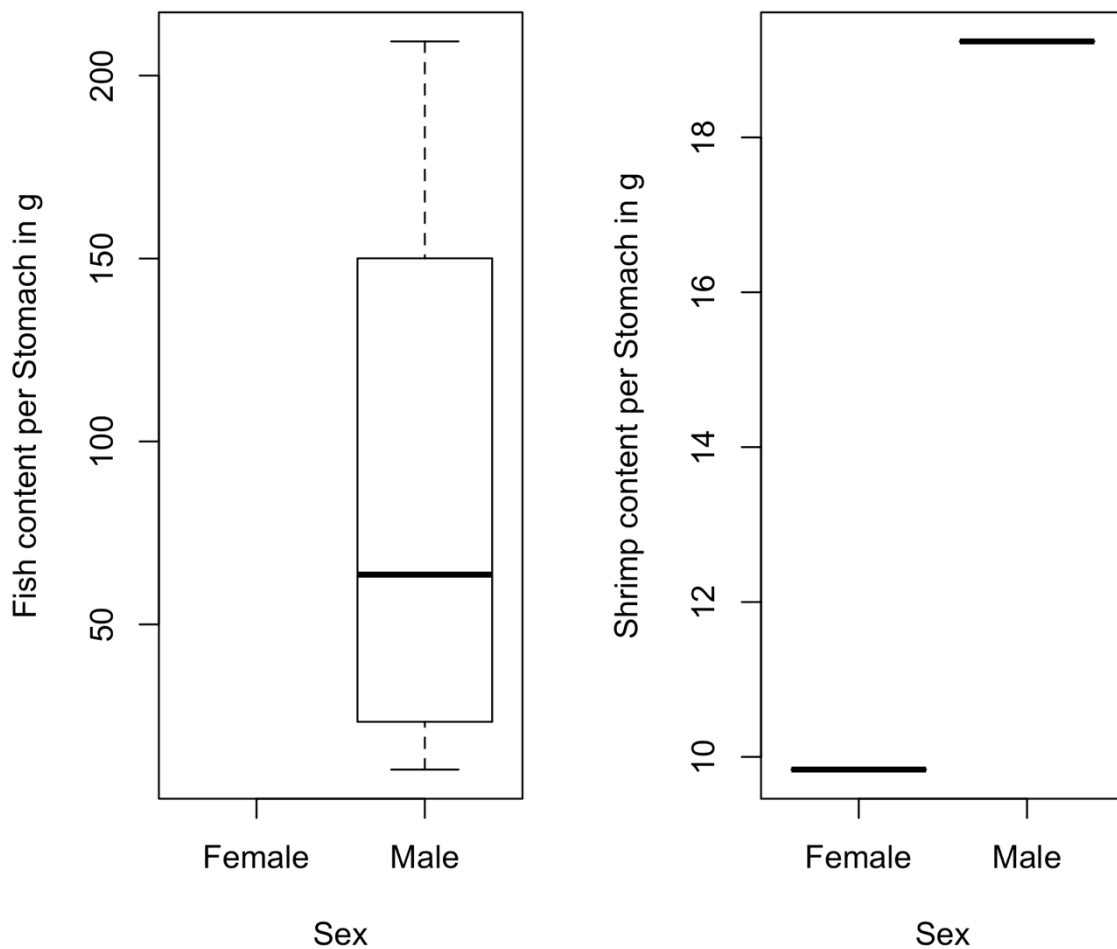


Figure 15. The fish content (in gram) per sampled stomach for each sex; 4 female and 1 male individuals

3.4 Plastic content in species stomachs

Out of 229 individuals, 226 (97.1%) had no plastic particles ingested. There were three (2.9%) individuals that ingested plastic particles, but only one particle was in the target range of this thesis (> 1 mm; 0.44%). The only “target plastic particle” was a black threadlike plastic particle of 6.5 cm length (see Figure 16) found in a female *S. acanthias* at 65.336 latitude. The other, smaller than the target, plastic particles were found in two individuals of *A. hyperborea* containing four particles in total. Three of these particles were blue, threadlike and found in a male individual at 71,5427 latitude (Norwegian Sea) (see Figure 16). The fourth particle was found in another male found at 71,6377 latitude (Norwegian Sea) with one threadlike, brown particle (for all three individuals refer to Appendix Table 5 for more detailed information).

All three individuals that ingested plastics were north of the boundaries of the North Sea, over 50 cm long and weighed more than 1.5 kg. The three individual’s maturity stadiums ranged from

“immature” to “capable to reproduce”. The plastic particles were found in two empty and one full stomach. All analyzed spiral valves were free of plastic contamination.

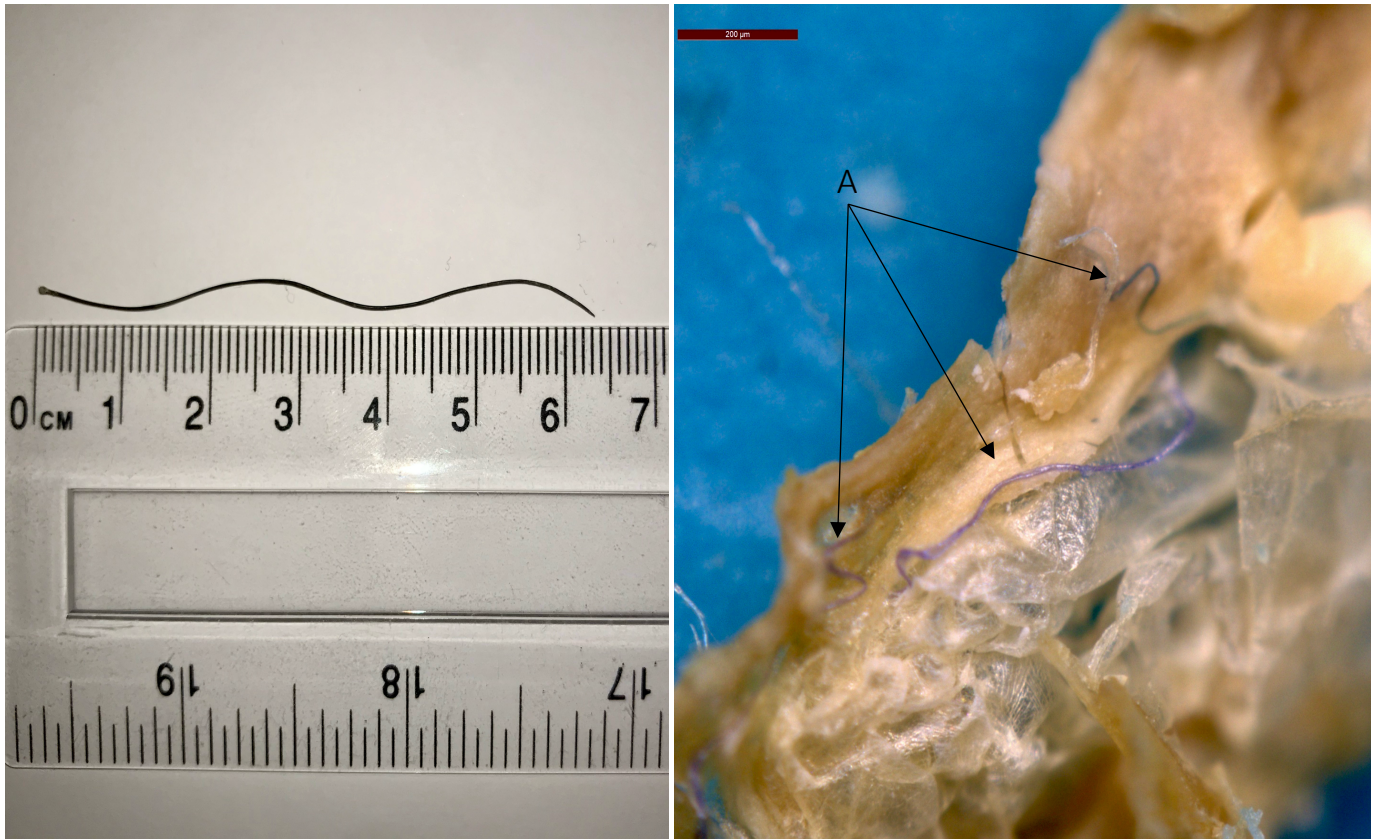


Figure 16. Left the black plastic particle (ca. 6.5 cm long) from the female *S. acanthias*; right A) the three plastic particles in one of the males of *A. hyperborea* (< 1mm); it was not possible to take a picture of the brown particle due to loss of the particle

4. Discussion

This study offered first insights of plastic ingestion in three elasmobranch species *S. acanthias*, *A. hyperborea* and *A. radiata* in the North Sea, the Norwegian Sea and the Barents Sea. Out of the 229 samples only three individuals (1.31%) contained plastics. However, only one of the particles can be classified as a macroplastic particle, whereby the others were smaller than one mm which was the lower limit set for macroplastics in this thesis. The three plastic particles found had a threadlike or filamentous appearance. Filaments and threadlike particles are common particles to find among the ocean's debris. Many stem from fisheries (e.g. filaments from nets) and households but also through the wear and tear of abiotic and biotic factors filamentous particles can emerge (Lang, 1990; Good *et al.*, 2010; Napper & Thompson, 2016). The plastic particles were not chemically characterized in the course of this thesis, but according to Andrady, (2015) the most common plastics particle components are PE, PP, PS, PVC, nylon and PET oceanwide. These components are a likely match for

the particles found in this project as well since those components originate usually from packaging and fishing gear. Such components of plastics were also found in other studies that investigated the ingestion of plastics in elasmobranchs (Cliff *et al.*, 2002; Fossi *et al.*, 2014; Bråte *et al.*, 2016; Alomar & Deudero, 2017; Bernardini *et al.*, 2018; Valente *et al.*, 2019; Maes *et al.*, 2020).

Plastic particles were expected to occur more frequently in the stomachs of larger individuals and in general in higher amounts as demonstrated in other elasmobranch species around the world (Alves *et al.*, 2016; Bernardini *et al.*, 2018; Abreo *et al.*, 2019) including the Greenland shark (*Somniosus microcephalus*) occurring in the Arctic Ocean (Leclerc *et al.*, 2012; Nielsen *et al.*, 2014, 2019). This low ingestion rate detected for the samples in this study suggests that macroplastics are probably a limited threat to elasmobranchs in Nordic waters. All three species in this study are mesopredators (*i.e.* medium sized predators) with opportunistic feeding strategies. As typical for elasmobranchs, they are also long-lived and late maturing (Dulvy *et al.*, 2014). The threat of various pollutants might be different for other elasmobranch species that live mostly at the surface or in the pelagic zone (*i.e.* water column in the open water) or exhibit different feeding strategies. The impact of plastics ingestion for filter feeding species for example will have different risks where plastics of all sizes can accumulate since filter feeding elasmobranchs are quite large compared to the species in this thesis and can directly ingest large plastics while feeding (*e.g.* the basking shark which can be 10 m long, and also occurs in Norwegian waters; Sims, 2008). All kinds of plastics can occur in such a large elasmobranch which increases the general risk of being negatively influenced by plastics (as described in the Introduction). The actively on prey items, hunting elasmobranchs might not be as negatively influenced since they differentiate between certain prey items being able to “just try” plastics before they are ingested. This was observed around the island of Hawaii where different plastic items have been reported that had clear bite marks of predatory fish (including sharks) suggesting a trial and error approach when it comes to prey items that are unknown (Carson, 2013).

According to the literature, as many as 72.9% of all aquatic life encounter macroplastics (> 5 mm) followed by microplastics (23.7%) and other plastic types (3.4%) (litterbase.awi.de; Tekman *et al.*, 2019). Such high estimates of plastic encounters challenge the results of this project that macroplastics at least are low in abundance in the stomachs of the targeted elasmobranch species. The low number detected here suggests that maybe a different size range (below 1 mm) like microplastics should be considered. For microplastics ingestion there are only a few studies that focus on other elasmobranch mesopredators *e.g.* the small spotted catshark (*Scyliorhinus canicula*), the blackmouth catshark (*Galeus melastomus*) and the velvet belly lanternshark (*Etmopterus spinax*)

(Smith, 2018; Valente *et al.*, 2019). For *A. radiata* and *A. hyperborea* there are no studies that could shed light on how those species are affected by microplastics. The author of this thesis suggests that the two skate species might be more vulnerable in ingesting microplastics than *S. acanthias*, since microplastics can accumulate and remain on the bottom of the ocean where benthic species like skates can easily ingest such particles. Microplastics ingestion might be through sediment (Lusher *et al.*, 2015; Bergmann *et al.*, 2017a) or through prey items that already ingested microplastics (bioaccumulation). The latter is most likely also true for *S. acanthias*. The position in the water column may also be an important parameter. *S. acanthias* occurs usually in the demersal water zone (*i.e.* fish that live and feed on or near the bottom of the ocean) but can move vertically into the pelagic zone (*i.e.* all of the ocean other than the sea floor or near the coast) and the water surface (Cox & Francis, 1997). The retention time for plastics in the pelagic zone is lower than at the bottom, because those particles are likely to sink due to organisms growing on those particles, adhere to phytoplankton or aggregate with other free-floating particles (*e.g.* marine snow) (Woodall *et al.*, 2014; Ryan, 2015).

Additionally, oceanic processes can play a role in continually moving and degrading particles downward to the ocean floor *e.g.* dense shelf water cascading, cycles of freezing and thawing and coastal storms (Ivanov *et al.*, 2004; Sanchez-Vidal *et al.*, 2012; Cózar *et al.*, 2017). This may be seen as a further indicator that skates might be more subject to microplastics ingestion than *S. acanthias*, because *S. acanthias* is less exposed. Also, the prey items that *S. acanthias* feeds on are mostly organisms that are not necessarily associated with the benthic fauna (*e.g.* Ctenophora, pelagic fish and squids; (Ellis *et al.*, 1996; Stehlik, 2007)), further reducing the risk of possible microplastics ingestion. Additionally, elasmobranchs rely to a high degree on their electromagnetic senses (ampullae of Lorenzini) for hunting purposes (Murray, 1974). Since plastic particles are poor conductors (Shrivastava, 2018) the detection of those particles through an electromagnetic sensor is very difficult if not impossible, *i.e.* that plastics don't get ingested on the basis of being actively hunted for which adds to the suggestion that elasmobranchs ingest plastic particles on an accidental basis (Valente *et al.*, 2019).

4.1 Evaluating various factors affecting the level of macroplastic ingestion

It was originally attempted to evaluate if certain factors like the investigated species, area and diet would have an effect on the type and level of macroplastics found in the stomachs of the analyzed individuals. However, due to the extremely low rate of macroplastics found across all 229 individuals, *i.e.* less than 1%, this type of comparison would be meaningless. Instead, the author only discusses the theoretical considerations for such comparisons in short below.

Differences in the uptake of macroplastic particles between the studied shark and skate species were considered with respect to the preferred habitats and feeding habits of both species. In short, skates, including both *Amblyraja* species investigated here, are feeding almost exclusively on the bottom of the ocean, whereby *S. acanthias* is feeding on or close-to the bottom and is also capable of extensive vertical migrations (Stehlik, 2007). This means this shark can spend a large amount of time disassociated from the benthic habitat where plastic accumulations are expected. Hence, the hypothesis was to find more macroplastic particles in *A. radiata* from the North Sea, compared to *S. acanthias* from the same area for example. Such comparison was however not possible based on the extremely low level of detected macroplastics. However, a study about plastics ingestion in elasmobranchs in the Ionian Sea on *S. acanthias* (n = 10) and two other skate species (thornback ray (*Raja clavata*; n = 2) and longnosed skate (*Raja oxyrinchus*; n = 10)) also found no evidence for ingestion of any plastics particles in either of the species, sharks and skates alike (Anastasopoulou *et al.*, 2013). In the same study, the blackmouth catshark (*Galeus melastomus*; n = 741) had the highest percentage (3.2 %) of ingested macroplastics, which was also the species with the highest number of analyzed samples (Anastasopoulou *et al.*, 2013). *G. melastomus* is also present further north, for example in the North Sea. It would therefore be interesting to compare this species spatially and to examine the macroplastics found in the North Sea environment. The number of *S. acanthias* from the Ionian Sea studied by Anastasopoulou *et al.*, (2013) was relatively low (10 specimens). These results could either imply that (1) the sample size was too low given the fact that the highest rate of microplastic ingestion in a species was 3.2%, which would mean 0.32 macroplastic particles present in the 10 samples in total which of course is not possible, or (2) that *S. acanthias* is able to avoid macroplastic particles or ingests those very rarely. Further research is certainly necessary to understand these complex relationships between different elasmobranch species and plastics ingestion.

A comparison by sex, size and maturity stadium was also originally planned as for example *S. acanthias* females grow larger and become older than males (Stehlik, 2007; Albert *et al.*, 2019) leading to a longer time for plastics to accumulate, hence potentially increasing the probability of finding it in the analyzed samples (Smith, 2018). As many species are undergoing dietary shifts associated with different life stages, it was hypothesized that a change in diet composition in *S. acanthias* for example from mainly shrimp to mainly fish, might cause a change in the pattern of ingested macroplastics, either with respect to the type or level of ingested macroplastics, or both. As this was not possible to investigate, further considerations with respect to diet are focusing on

between-species differences and their potential implications instead. In an empty stomach, the one plastic particle was found in *S. acanthias* (1/104, corresponding to about 1%). The retention rate of prey items inside the stomach is here an important measure for comparison. Unfortunately, this has not been studied extensively and, there is no study involving the three species studied here, thus far. One study of the lesser spotted dogfish (*Scyliorhinus canicula*) found that it takes *ca.* 250 hours (*ca.* 10 days) for a stomach to completely evacuate (under laboratory conditions) (Sims *et al.*, 1996). This would give gastric acids time to digest and destroy bigger particles into smaller ones (Karami *et al.*, 2017). In the study of Sims *et al.*, (1996) this time varied with the amount of prey ingested. Smaller prey items had a lower stomach retention rate than bigger once (Bush & Holland, 2002). The same study also pointed out that ingestion commenced after *ca.* 50% of the prey items were digested. The authors suggested that these findings are only preliminary since physiological factors in nature have different effects and cause different results of retention rate and feeding behavior than experiments in the laboratory. But sharks are found to be generally infrequent feeders (Leigh *et al.*, 2017 and references therein) thus suggesting that plastics might stay longer in the intestines and can cause serious damage when associated with *e.g.* plastics associated toxins or heavy metals (Marcovecchio *et al.*, 1991; Vas, 1991; Bergami *et al.*, 2017). This could mean that due to the lower number of plastic particles in the Nordic waters compared to lower latitudes, elasmobranchs, including *A. radiata*, *A. hyperborea* and *S. acanthias*, may ingest plastics less frequently. But potential toxins like POPs might leach into the tissue of the species during the process causing sublethal damage without plastics being present.

4.2 Macroplastics in other parts of the digestive tract

No macroplastics were found in the additionally investigated spiral valves of *A. radiata* and *A. hyperborea*. These samples were added late in the study, due to a recently published study examining the content of spiral valves in the porbeagle (*Lamna nasus*), a pelagic shark also found in the North Sea and the Norwegian Sea (Maes *et al.*, 2020). In that study which focused on microplastics in the Celtic Sea (Northeast Atlantic) however, substantial amounts of microplastics (< 1mm) were estimated, being as high as *ca.* 6000 particles per individual spiral valve. The authors suggested that since the spiral valve is not subject to regurgitation it could be a good indicator of how top predators are affected through microplastics accumulation. They also suggest the porbeagle as a potential bioindicator of microplastics, because of the species position as a top predator in the food web and its wide range of prey organisms (*e.g.* pelagic fish, gastropods, crabs, squid (Francis *et al.*, 2009)) that are ingested by this type of large, pelagic and highly migratory shark. *S. acanthias* and *L. nasus* have both been shown to have wide spatial distribution ranging across the Atlantic Ocean (Templeman, 1984;

Francis *et al.*, 2009) and it could therefore be hypothesized that similar findings could be revealed in the spiny dogfish. In how far large amounts of microplastics also infer large amounts of macroplastics, has not been tested yet, but would be very interesting.

4.3 Elasmobranchs as bioindicators for macroplastics

Bioindicators have been used as proxies in order to assess the health of an ecosystem for many decades (Zacharias & Roff, 2001). As for now only seabirds have been used to assess the amount of macroplastic debris in the Nordic waters (OSPAR, 2008; Strand *et al.*, 2015) though other organisms like blue mussels (*Mytilus edulis*), Greenland sharks, the Norway lobster (*Nephros norvegicus*) and sperm whales show plastics ingestion of different extent and size classes (Jacobsen *et al.*, 2010; Murray & Cowie, 2011; Nielsen *et al.*, 2014; Bråte *et al.*, 2017; IJsseldijk *et al.*, 2018; Nielsen *et al.*, 2019). Elasmobranchs have previously been suggested as bioindicators for certain environmental pollutants such as toxic heavy metals (Alves *et al.*, 2016; Torres *et al.*, 2014, 2017; Bezerra *et al.*, 2019) and microplastics (< 5 mm) (Fossi *et al.*, 2014; Bernardini *et al.*, 2018; Maes *et al.*, 2020). The studies above suggest mostly sharks (*P. glauca*, *L. nasus* and *C. maximus*) as suitable species for bioindicators. The main reason sharks are considered as potential bioindicators for plastics are that the species mentioned above exhibit slow growth and longevity (enabling the assessment of an ecosystem over extended periods), being on the top of the food chain (possibly accounting for bioaccumulation and biomagnification; few natural predators if any as apex predator), sole reliance on marine resources (*i.e.* all life history events and food intake happen in the ocean) and a wide spatial distribution (possibly assessing plastics pollution across local regions as well as whole seas or oceans). Those traits are also exhibited by *S. acanthias* which would qualify this species to be a bioindicator for plastics. The review of Bezerra *et al.*, (2019) is hitherto the only paper suggesting skates and rays as bioindicators for heavy metal pollution. *A. radiata* and *A. hyperborea* qualify as bioindicators, similar to sharks, as well, but they lack the wide spatial migratory behavior that *e.g.* *S. acanthias* exhibits. However, since *A. radiata* is found in all Nordic waters comparisons across specific habitats could be possible and it could therefore be a useful bioindicator species for monitoring programs along the Norwegian coastline.

The fact that only one macroplastic particle was found in the studied species in this study may challenge the suitability of the species as bioindicators for macroplastics. The question is hard to answer from only this study, but there are other shark species that also could be considered like *G. melastomus* (which already showed a higher rate of plastic ingestion elsewhere, see Anastasopoulou *et al.*, (2013)), *E. spinax* (also a mesopredator) or even *L. nasus*, *C. maximus* and *S. microcephalus* being apex predators with very different feeding strategies. But for several of these species there are

other problems associated with their use as bioindicators, *e.g.*, that *S. microcephalus*, *C. maximus* and *L. nasus* are near threatened (Kyne *et al.*, 2017), endangered (Rigby *et al.*, 2019) and critically endangered (Stevens *et al.*, 2006), respectively. For *G. melastomus* and *E. spinax* the usage as a bioindicator may be possible, but before looking at other species as bioindicators it may be more promising to extend the size range for the plastic particles to be studied to include also microplastics.

4.4 Critical assessment of the method and experimental design

It was demonstrated through the preliminary study and positive control that macroplastics, as defined in this thesis, can be recovered and visually assessed by identifying organic and inorganic particles through a stereomicroscope. KOH as a digestion agent proved to be reliable in dissolving stomachs and the content therein as recommended by Dehaut *et al.*, (2016), Kühn *et al.*, (2017) and Hurley *et al.*, (2018). Shaking the samples with 10% KOH on a rotation device, proved to be effective and a more relevant factor than temperature. But increased temperature up to 50°C in combination with shaking could speed up the digestion process of bigger samples (Dehaut *et al.*, 2016) and should be considered for large scale studies. In this study some stomachs took almost three days to fully digest, depending on the stomach's size. Further, increasing the concentration of the KOH would result in a faster digestion, but may also harm the integrity of the plastic particles. Thus, the protocol used in this thesis proved appropriate to extract plastics larger than 1 mm. The author is confident that if plastic particles would have been present in the stomachs within the targeted size range, it would have been detected, as demonstrated with the positive control.

A standardized protocol for plastics extraction would be needed to compare results among studies, but this is difficult since for example many studies (including this one) use their own definition of "plastic category", *i.e.* the cutoff in particle size that suits their research question, sampling program and facilities best. For a study on smaller plastic particles (*i.e.* < 1 mm) the digestion protocol used in this study could also be used, with a few modifications: (1) the storage of the samples in plastic bags is not advisable since abrasions of the plastic bag could contaminate the samples. The use of metal cases would avoid this problem, (2) as extracting microplastics has been proven to be a delicate procedure since the contamination of the sample is likely to happen, precautionary actions are needed. Those include *e.g.* wearing a wool/natural fiber lab coat without plastics and nitrile glove was advised (Collard *et al.*, 2015), and (3) a blank sample of the surrounding should be taken which then serves as a control of how much plastics is around the actual set up of the experiment (Brâte *et al.*, 2017; Karami *et al.*, 2017; Hurley *et al.*, 2018).

It has been observed that elasmobranch species, including the species in this project, show instances of regurgitating prey items once caught through trawling (Nammack, 1982; González *et al.*, 2006) and as a mechanism to exclude prey items that are too big to fit into the stomach (Leigh *et al.*, 2017; Maes *et al.*, 2020). Trawling was the primarily used method on surveys to catch specimens. This could be one reason why plastic particles were not found as often, because they regurgitated their stomach content before they were landed.

The 229 samples that were analyzed in this study with a plastic retrieval rate of less than 1% overall which however only equates to one sample for one for the studied species, namely *S. acanthias*. As only one individual out of 104 contained one macroplastic particle, increasing the sample size even three-fold, would have not resulted in the possibility to conduct a more sophisticated analysis of the factors driving macroplastic ingestion, which was one of the main goals of this thesis. As no macroplastic particles were found in the two skate species here, discussions about increased sample sizes would be speculative at best. However, in the samples of *A. hyperborea* plastic particles below 1 mm were found but only because they were associated with bigger particles of organic matter which were not fully digested at the time and the plastics were hence easy to detect and separate early in the digestion process. As mentioned above, a more comprehensive study should therefore foremost focus on increasing the range of the particles sizes to also include microplastics for one model elasmobranch species and then, as a next step increase sample sizes to estimate a reliable rate of plastic ingestion to compare that across different areas and life history traits.

4.5 Conclusion and future outlook

The main finding of this study was that the two main species (*A. radiata* and *S. acanthias*) were not heavily contaminated with ingested macroplastics (particles > 1 mm). The data for *A. hyperborea* were too limited for such a conclusion, even though no macroplastics were found in the stomachs of that species either. This suggests that the main two mesopredatory elasmobranch species studied in this thesis, are not highly impacted by plastics larger than 1 mm in Norwegian waters. The method of isolating plastics above 1 mm proved to be adequate to reliably extract plastics if present. At first glance, this is a very positive result considering the massive pressure of climate change, ocean acidification and human pollutants affecting the oceans. In future studies, the size category of targeted plastics should most likely be reconsidered. Although at first glance a lower boundary of 1 mm seems reasonable, a major fraction of plastics in elasmobranch stomachs may already be smaller if one assumes that with increasing latitude the particle size of average plastics may decrease. Furthermore, the toxicity of plastic particles should also be addressed since plastic particles leech

toxins that may have lethal to sublethal effects on individuals that ingest those. This was not within the scope of this thesis but would add valuable information of what and how much of a given toxin is in an area (*i.e.* actually using elasmobranchs as a bioindicator). That said other body parts, like gills, liver, spiral valve or muscles should be further investigated as well because those have possible effects on locomotion, detoxification and the overall immune system of the individual. With more information on the effects of plastics on organisms in general available, policy makers can make informed decisions on *e.g.* which additives should or can be used for producing plastics. This could lead to alleviation of toxic loads in an ecosystem and an improved overall health of species. There are only timid attempts from governments to implement regulations addressing the plastics problem, which will enable plastics to remain in the environment for the foreseeable future. It is thus crucial to understand the effects of plastics on marine life besides stopping an increase in plastics pollution. How long-lived and vulnerable species like elasmobranch species will react and what the overarching consequences are, will still need to be determined, even though possible explanations have come forth in recent years.

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Literature

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Appendix

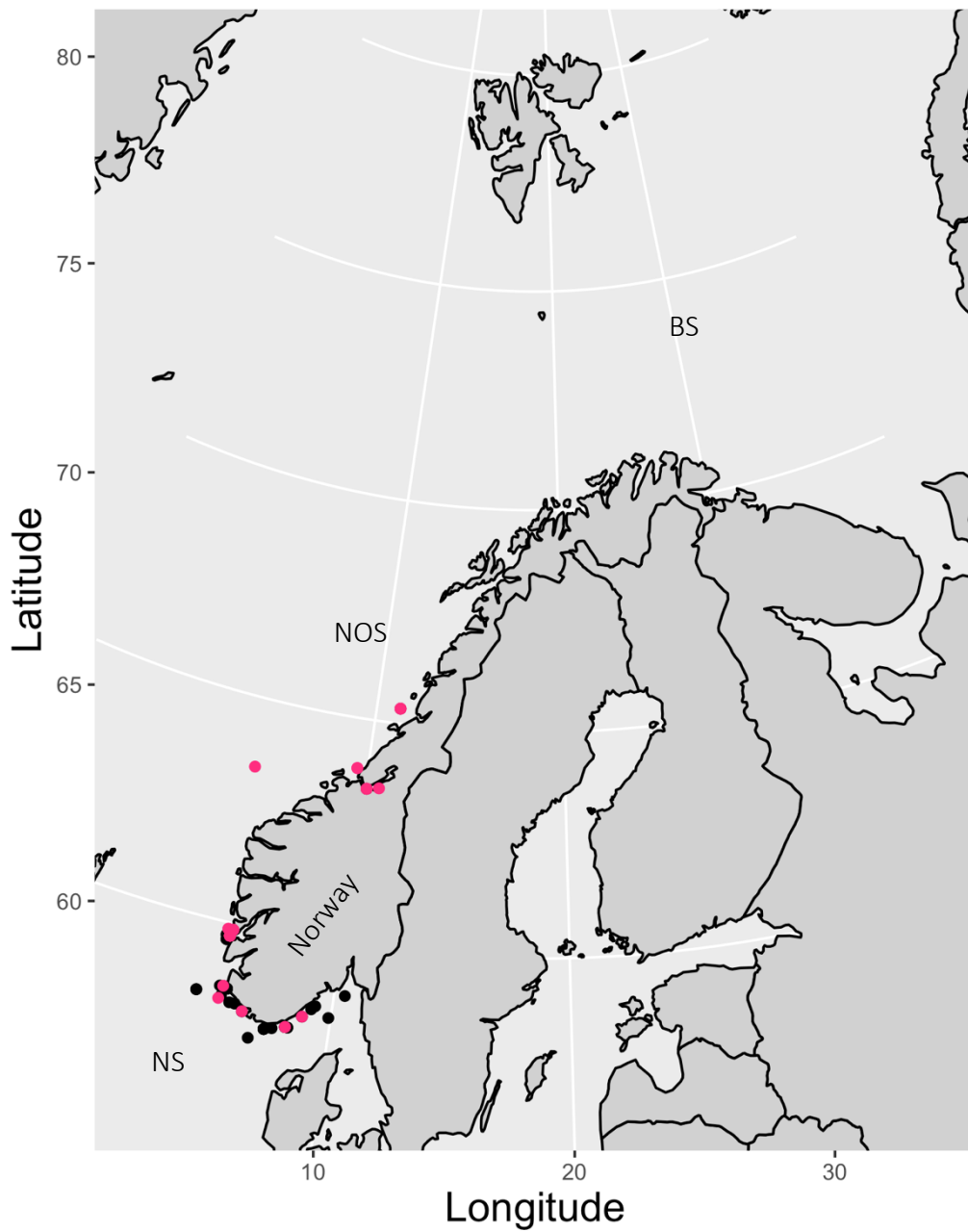


Figure 1. Spatial sex distribution of all the *S. acanthias* samples. Black dots = male; pink dots = female; NS = North Sea, NOS = Norwegian Sea, BS = Barents Sea

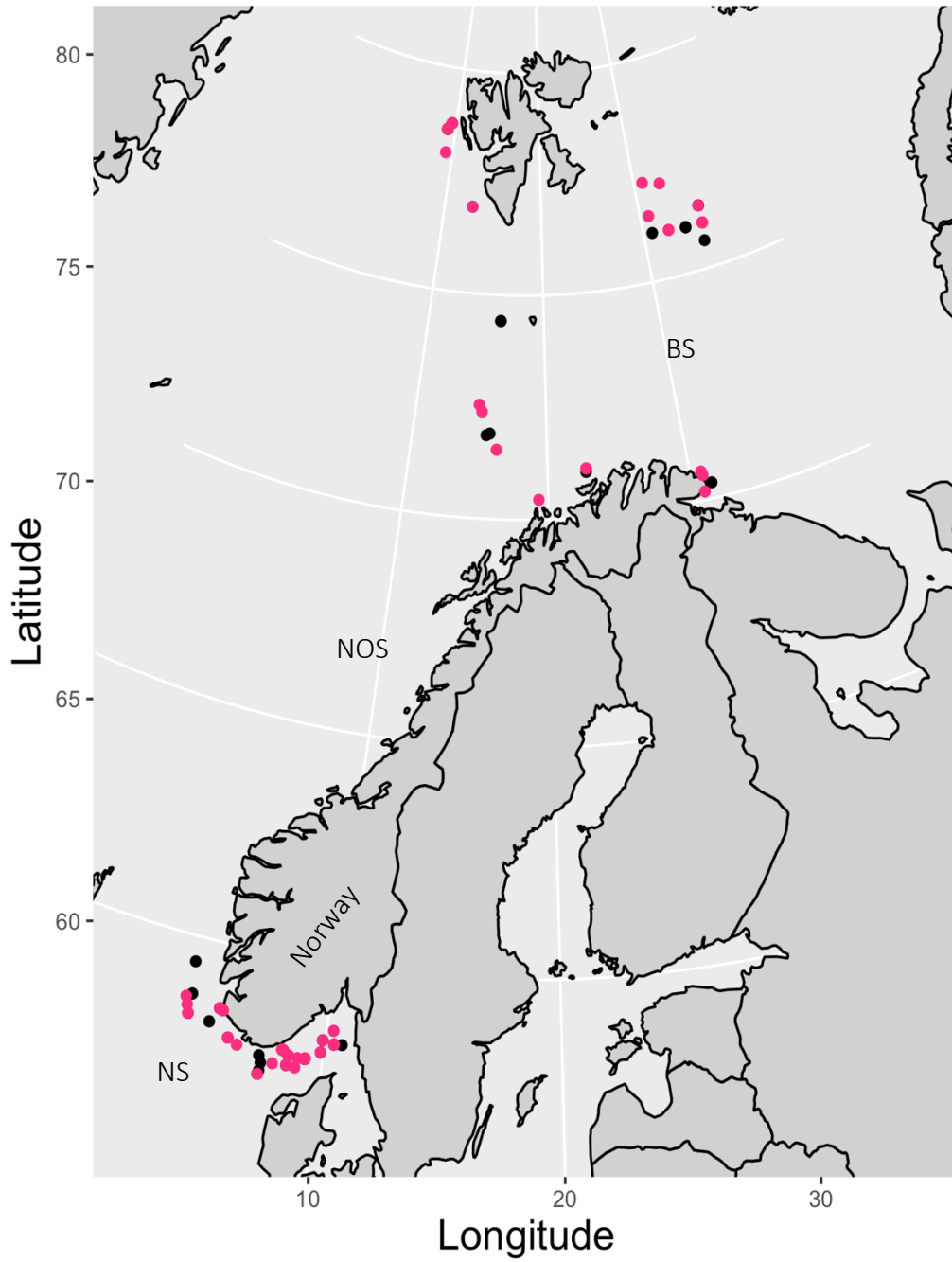


Figure 2. Spatial sex distribution of all the *A. radiata* samples; black dots = males, pink = females; NS = North Sea, NOS = Norwegian Sea, BS = Barents Sea

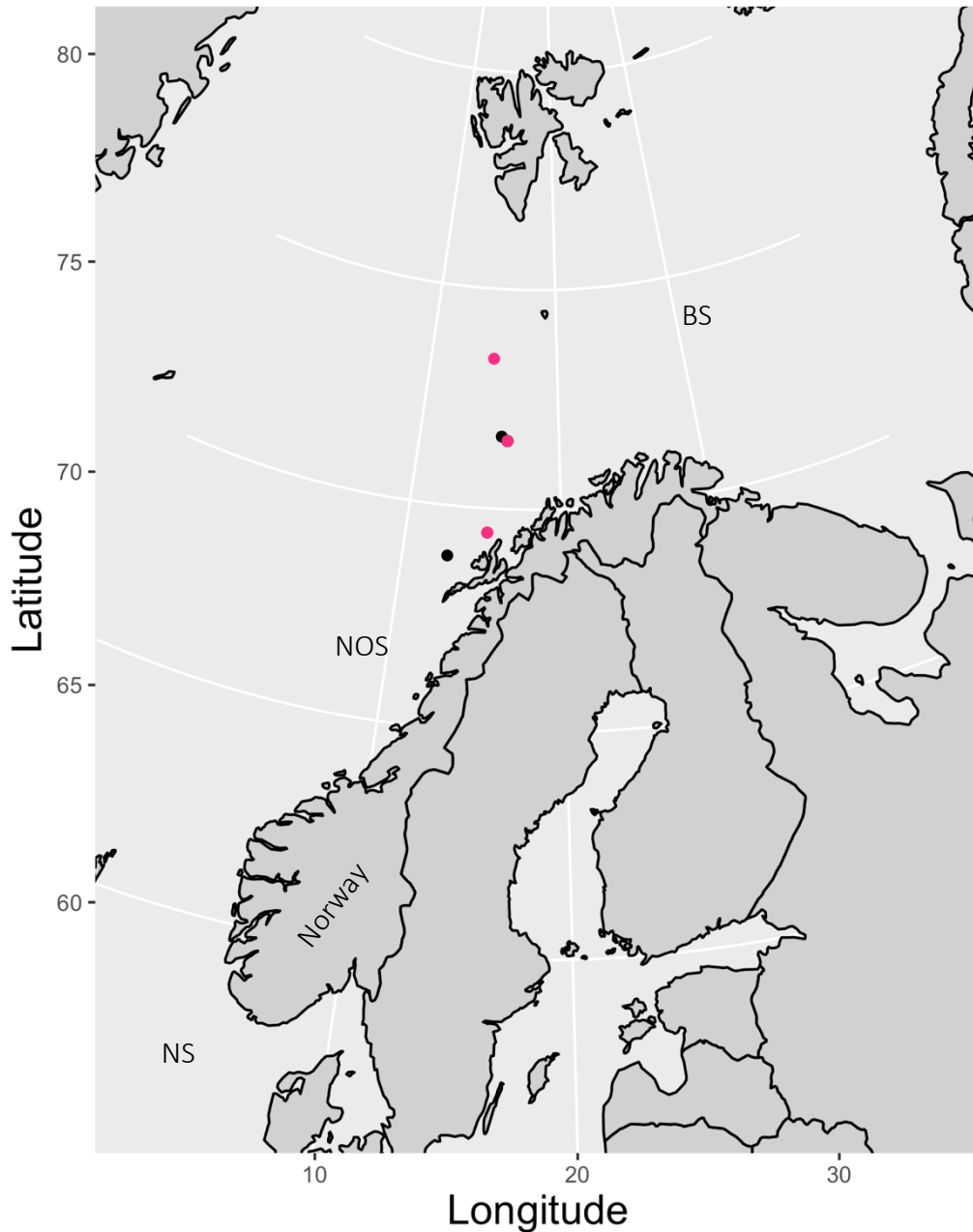


Figure 3. Spatial sex distribution of *A. hyperborea*; black dots = male, pink dots = females; NS = North Sea, NOS = Norwegian Sea, BS = Barents Sea

Table 1. Maturity stadiums of ovoviparous female elasmobranchs (in this thesis *S. acanthias*)

Ovoviparous elasmobranchs - females			
MATURITY	STADIUM	DESCRIPTION	NOTES
IMMATURE	1. IMMATURE	Ovaries: small and whitish; undistinguishable ovarian follicles. Oviducal gland: often not visible. In some	

		<p>species a thickening of the oviducts where the gland will develop may be visible</p> <p>Uteri: thread-like and narrow.</p>	
IMMATURE	2. DEVELOPING	<p>Ovaries: follicles of different stadiums of development. Some small and medium sized yolked follicles may be present.</p> <p>Oviducal gland: distinguishable and developing</p> <p>Uteri: enlarging</p>	
MATURE	3. CAPABLE to REPRODUCE	<p>Ovaries: presence of large yolked follicles ready to be ovulated.</p> <p>Oviducal glands: fully developed</p> <p>Uteri: fully developed.</p>	
MATERNAL	4a. EARLY PREGNANCY	<p>Ovaries: different sized follicles are present according to stadiums of ovulation.</p> <p>Oviducal glands: fully developed (possibly regressing)</p> <p>Uteri: well filled and rounded with yolk content (usually candle shape).</p> <p>Embryos cannot be observed.</p>	<p>Yolk could be found in oviducts and/or oviducal gland (ovulation in process). Atretic follicles may be present in the ovaries (species specific).</p>
MATERNAL	4b. MID PREGNANCY	<p>Ovaries: small to medium, possibly yolked follicles (active ovaries) or small, unyolked and/or atretic follicles (inactive ovaries).</p> <p>Oviducal glands: fully developed (possibly regressing)</p>	<p>In the different reproductive strategies follicles may begin to enlarge (synchronous)</p>

		<p>Uteri: well filled and rounded.</p> <p>Embryos are always visible, small and with a relatively large yolk sac.</p>	<p>or ovaries may remain inactive (asynchronous).</p>
MATERNAL	4c. LATE PREGNANCY	<p>Ovaries: medium to large yolked follicles (active ovaries) or small, unyolked follicles and/or atretic follicles (inactive ovaries)</p> <p>Oviducal glands: fully developed</p> <p>Uteri: embryos fully formed, yolk sacs reduced or absent.</p>	<p>In the different reproductive strategies follicles enlarged (synchronous) or ovaries may remain inactive (asynchronous).</p>
MATERNAL	5. POST-PARTUM	<p>Ovaries: Similar to stadium 4c.</p> <p>Oviducal glands: Similar to stadium 4c.</p> <p>Uteri: enlarged and flaccid (likely to have just given birth).</p>	<p>In the different reproductive strategies follicles enlarged (synchronous) or ovaries may remain inactive (asynchronous).</p>

Table 2. Maturity stadiums of ovoviviparous male elasmobranchs (in this thesis *S. acanthias*)

Ovoviviparous elasmobranchs - Males			
MATURITY	STADIUM	DESCRIPTION	NOTES
IMMATURE	1. IMMATURE	<p>Testes: small and undeveloped.</p> <p>Ducts: straight and thread-like.</p> <p>Claspers: flexible, non-calcified and usually shorter than pelvic fins.</p>	
IMMATURE	2. DEVELOPING	<p>Testes: developing according to species-specific characteristics (usually with segments^{2,1} or</p>	2.1 mainly found in sharks

		lobules ^{2.2} visible) but not occupying the whole surface. Ducts: developing and beginning to coil. Claspers: flexible, partially calcified and as long as or longer than pelvic fins.	2.2 mainly found in batoids
MATURE	3a. CAPABLE to REPRODUCE	Testes: fully developed according to species-specific characteristics (segments ^{3a.1} or lobules ^{3a.2} completed). Ducts: tightly coiled and filled with sperm. Seminal vesicles (when present) are developed. Claspers: rigid, fully calcified and longer than pelvic fins.	3a.1. mainly found in sharks 3a.2. mainly found in batoids
MATURE	3b. ACTIVE	Testes: similar to stadium 3a. Ducts: sperm flowing out of the cloaca on pressure. Seminal vesicles (when present) can be full ^{3b.1} or empty ^{3b.2} . Claspers: fully formed; however, with clasper gland dilated, sometimes swollen and/or reddish. Sperm may be present in clasper groove or gland.	Depending on the reproduction strategy: 3b.1. Full seminal vesicles 3b.2. Empty seminal vesicles
MATURE	4. SPENT (regressing and regenerating)	Testes: shrunken and flaccid. Ducts: empty and flaccid. Seminal vesicles (when present) empty. Claspers: fully formed.	

Table 3. Maturity stadiums of oviparous female elasmobranchs (in this thesis *A. radiata* and *A. hyperborea*)

Oviparous elasmobranchs - Females			
MATURITY	STADIUM	DESCRIPTION	NOTES

IMMATURE	1. IMMATURE	<p>Ovaries: small and whitish. Undistinguishable or very small ovarian follicles.</p> <p>Oviducal gland: often not visible. In some species a thickening of the uteri where the gland will develop may be visible.</p> <p>Uteri: thread-like and narrow.</p>	<p>Ovaries: in some sharks very small follicles are visible.</p>
IMMATURE	2. DEVELOPING	<p>Ovaries: Unyolked follicles and some small and medium yolked ones may be present.</p> <p>Oviducal gland: distinguishable and developing mostly in skates.</p> <p>Uteri: enlarging</p>	<p>Unyolked follicles: the group adopted the term unyolked follicles to distinguish from the yolked ones according to development degree that could be observed in this stadium.</p> <p>Oviducal gland: distinguishable and developing mostly in skates.</p>
MATURE	3a. CAPABLE TO REPRODUCE	<p>Ovaries: presence of large yolked follicles ready to be ovulated.</p> <p>Oviducal glands: fully developed.</p> <p>Uteri: fully developed and turgid.</p>	<p>Uteri: are turgid differently from the stadium 4a.</p>
MATURE	3b. ACTIVE	<p>Ovaries and Oviducal glands: similar to stadium 3a</p> <p>Uteri: presence of egg capsules</p>	

MATURE	4a. POST-LAYING	<p>Ovaries: flaccid with follicles (unfolked and folked) of different sizes. POFs and atretic follicles visible.</p> <p>Oviducal glands: fully developed but may be reduced in size mostly in skates.</p> <p>Uteri: enlarged, flaccid and vascularized.</p>	<p>Ovaries: flaccid with unfolked and folked follicles of different sizes, according to species.</p> <p>Oviducal glands: in some skate species could be reduced.</p> <p>Uteri: the uteri appearance could be also vascularized.</p>
MATURE	4b. REGENERATING	<p>Ovaries: large with small and medium sized folked follicles. Pre-ovulatory follicles absent.</p> <p>Oviducal glands: fully developed but may be reduced in size mostly in skates.</p> <p>Uteri: enlarged but not flaccid.</p>	<p>Oviducal glands: in some skate species could be reduced.</p> <p>Uteri: in stadium 4b uteri are not flaccid.</p>

Table 4. Maturity stadiums of oviparous male elasmobranchs (in this thesis *A. radiata* and *A. hyperborea*)

Oviparous elasmobranchs - Males			
MATURITY	STADIUM	DESCRIPTION	NOTES
IMMATURE	1. IMMATURE	<p>Testes: small and undeveloped (in skates, sometimes with visible lobules).</p> <p>Ducts: straight and thread-like.</p> <p>Claspers: flexible, non-calcified and shorter than pelvic fins.</p>	

IMMATURE	2. DEVELOPING	<p>Testes: developing (in skates, lobules clearly visible but not fully developed).</p> <p>Ducts: developing and beginning to coil.</p> <p>Claspers: flexible, partially calcified and usually or as long as or longer than pelvic fins. In some sharks do not pass the pelvic fins.</p> <p>Alar thorns: could be present at primordial stadium (single row) in some skate species.</p>	<p>Thorns: in several skates, primordial alar thorns are visible due to a sexual dimorphism. The detection of the thorns could be useful to distinguish the stadium 2 from the stadium 1</p>
MATURE	3a. CAPABLE TO REPRODUCE	<p>Testes: fully developed according to species-specific characteristics. Sperm does not flow on pressure.</p> <p>Ducts: tightly coiled and filled with sperm.</p> <p>Seminal vesicle (when present) are developed.</p> <p>Claspers: rigid, fully calcified and longer than pelvic fins (in some sharks they may only be as long as the pelvic fins).</p> <p>Alar and/or malar thorns: could be present in some skate species.</p>	<p>Testes: they could appear fully developed with different structure (e.g. in skates with lobules)</p> <p>Thorns: in several skates, alar and/or malar thorns are visible due to a sexual dimorphism. The detection of these thorns could be useful to distinguish the stadium 3a</p>

			from the stadium 2
MATURE	3b. ACTIVE	<p>Testes: similar to stadium 3a.</p> <p>Ducts: sperm observed flowing out of the cloaca on pressure.</p> <p>Seminal vesicle (when present) can be full or empty</p> <p>Claspers: fully calcified sometimes with glans dilated, swollen and reddish. Sperm flows on pressure and it may be present in groove or glans.</p> <p>Alar and/or malar thorns: could be present in some skate species.</p>	<p>Seminal vesicle: depending on reproductive strategy.</p> <p>Thorns: in several skates, alar and/or malar thorns are visible due to a sexual dimorphism.</p>
MATURE	4. SPENT (regressing and regenerating)	<p>Testes shrunken and flaccid. On pressure sperm does not flow.</p> <p>Sperm ducts and seminal vesicle empty and flaccid.</p> <p>Claspers: fully formed.</p> <p>Alar and/or malar thorns: could be present in some skate species.</p>	<p>According to the working group, the term SPENT in males indicates the reproductive suspension (including regressing and regenerating phases)</p> <p>Seminal vesicle and thorns: similar to stadium 3b</p>

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
Catch Date	Sample source	Species	Ind. ID	Lat.	Lon.	Depth in m	Sex	Total w. in g	Length in mm	Ma. St.	Stomach w. in g	Valve w. in g	Fish w. in g	Shrimp w. in g	Others w. in g	Plastics Color	Plastics Shape	Plastics w. in g	# particles
07.01.-28.01.17	Reketokt	SQA	201722081_8	57.896	7.3665	383.85	2	508	520	2	28.6	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722082_1	57.945	7.6812	295.74	2	426	470	2	17.9	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_10	58.023	8.2063	234.95	2	78	275	1	5.2	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_2	58.023	8.2063	234.95	2	284	425	1	-	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_3	58.023	8.2063	234.95	1	226	370	1	4.9	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_4	58.023	8.2063	234.95	2	76	280	1	4.9	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_7	58.023	8.2063	234.95	2	68	265	1	4.2	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_8	58.023	8.2063	234.95	2	60	265	1	3.6	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722085_9	58.023	8.2063	234.95	1	58	270	1	5.1	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722086_1	58.02	8.3102	387.46	2	1396	730	3	32.9	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722086_2	58.02	8.3102	387.46	2	1680	770	3	43.6	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722116_1	58.893	10.4573	177.05	2	1300	730	3	50.7	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722125_1	58.576	9.2961	275.58	2	1484	740	3	43.3	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722126_1	58.369	9.9046	488.95	2	1730	790	3	101.5	-	-	-	-	-	-	-	-
07.01.-28.01.17	Reketokt	SQA	201722129_1	58.498	9.1481	271.63	2	1495	710	3	42.0	-	-	-	-	-	-	-	-
25.10.17	Fiskemottak	SQA	201799070_2	59.86	5.0220	-	1	2109	780	2	132.4	-	96.9	-	-	-	-	-	-
25.10.17	Fiskemottak	SQA	201799070_3	59.86	5.0220	-	1	2193	780	5	159.3	-	54.9	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_13	59.86	5.2390	-	1	1549	700	2	70.6	-	43.2	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_2	59.86	5.2390	-	1	2638	850	2	99.9	-	191.2	-	-	-	-	-	-

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
Catch Date	Sample source	Species	Ind. ID	Lat.	Lon.	Depth in m	Sex	Total w. in g	Length in mm	Ma. St.	Stomach w. in g	Valve w. in g	Fish w. in g	Shrimp w. in g	Others w. in g	Plastics Color	Plastics Shape	Plastics w. in g	# particles
23.10.17	Fiskemottak	SQA	201799071_23	59.86	5.2390	-	1	1841	730	3	87.0	-	10.1	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_30	59.86	5.2390	-	2	1484	720	4	73.0	-	188.4	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_31	59.86	5.2390	-	1	2246	820	4	138.2	-	-	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_35	59.86	5.2390	-	1	2231	820	2	147.9	-	75.2	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_37	59.86	5.2390	-	1	2448	820	4	121.0	-	63.1	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_38	59.86	5.2390	-	1	1988	770	4	112.7	-	49.5	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_42	59.86	5.2390	-	1	1972	760	5	189.2	-	-	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_48	59.86	5.2390	-	1	2239	750	3	117.7	-	-	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_5	59.86	5.2390	-	1	2568	840	3	156.0	-	-	-	-	-	-	-	-
23.10.17	Fiskemottak	SQA	201799071_9	59.86	5.2390	-	1	3154	890	4	114.1	-	22.0	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_1	59.86	5.2390	-	1	1926	740	4	76.5	-	50.8	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_25	59.86	5.2390	-	1	1863	770	4	98.9	-	10.6	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_30	59.86	5.2390	-	1	2359	780	5	139.3	-	54.8	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_34	59.86	5.2390	-	1	1770	760	2	109.8	-	-	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_6	59.86	5.2390	-	1	1882	760	4	73.5	-	-	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_8	59.86	5.2390	-	2	2070	810	4	79.3	-	9.3	-	-	-	-	-	-
22.10.17	Fiskemottak	SQA	201799072_9	59.86	5.2390	-	1	2172	800	3	161.3	-	71.7	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_33	59.72	5.1600	100.00	1	2737	820	4	113.7	-	-	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_34	59.72	5.1600	100.00	1	2652	820	4	190.1	-	23.1	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_36	59.72	5.1600	100.00	1	2552	830	6	104.3	-	20.6	4.1	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_37	59.72	5.1600	100.00	1	2122	780	2	116.6	-	28.8	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_39	59.72	5.1600	100.00	1	2025	760	4	96.8	-	-	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_41	59.72	5.1600	100.00	1	2795	830	5	158.8	-	103.2	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_42	59.72	5.1600	100.00	1	2406	810	5	123.6	-	23.4	-	-	-	-	-	-
15.11.17	Fiskemottak	SQA	201799073_45	59.72	5.1600	100.00	1	1877	740	2	153.9	-	5.7	-	6.0	-	-	-	-

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
Catch Date	Sample source	Species	Ind. ID	Lat.	Lon.	Depth in m	Sex	Total w. in g	Length in mm	Ma. St.	Stomach w. in g	Valve w. in g	Fish w. in g	Shrimp w. in g	Others w. in g	Plastics Color	Plastics Shape	Plastics w. in g	# particles
15.11.17	Fiskemottak	SQA	201799073_46	59.72	5.1600	100.00	1	1507	700	2	306.4	–	150.2	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799073_48	59.72	5.1600	100.00	1	2887	840	5	93.7	–	1.1	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799073_50	59.72	5.1600	100.00	1	1783	720	2	65.7	–	27.0	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799073_51	59.72	5.1600	100.00	1	2818	830	5	150.8	–	2.0	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_16	59.731	5.0120	80.00	2	1256	660	4	87.2	–	158.8	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_29	59.731	5.0120	80.00	2	1497	730	3	48.9	–	4.1	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_31	59.731	5.0120	80.00	2	1217	690	4	47.7	–	2.9	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_36	59.731	5.0120	80.00	2	1686	730	4	74.5	–	1.6	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_55	59.731	5.0120	80.00	2	1734	720	4	114.3	–	54.2	–	–	–	–	–	–
15.11.17	Fiskemottak	SQA	201799074_57	59.731	5.0120	80.00	2	1708	770	4	79.3	–	5.4	–	–	–	–	–	–
22.11.17	Fiskemottak	SQA	201799075_13	59.633	5.0500	100.00	2	1177	680	4	57.2	–	–	–	–	–	–	–	–
22.11.17	Fiskemottak	SQA	201799075_36	59.633	5.0500	100.00	2	1600	750	4	71.3	–	–	–	–	–	–	–	–
22.11.17	Fiskemottak	SQA	201799075_40	59.633	5.0500	100.00	2	1156	660	4	53.3	–	–	–	–	–	–	–	–
22.11.17	Fiskemottak	SQA	201799075_6	59.633	5.0500	100.00	2	1421	710	4	77.5	–	31.5	–	–	–	–	–	–
10.04.18	Fiskemottak	SQA	201899953_10	63.514	10.6920	–	1	1735	780	4	62.3	–	1.2	–	–	–	–	–	–
11.04.18	Fiskemottak	SQA	201899956_1	63.89	9.4700	–	1	3957	930	4	99.3	–	39.4	–	–	–	–	–	–
11.04.18	Fiskemottak	SQA	201899956_3	63.89	9.4700	–	1	2502	820	4	86.3	–	–	6.8	3.6	–	–	–	–
13.04.18	Fiskemottak	SQA	201899957_2	63.466	10.0860	–	2	1687	800	4	39.3	–	7.1	–	–	–	–	–	–
06.04.18	Fiskemottak	SQA	201899959_5	63.466	10.0860	–	2	2125	820	4	67.0	–	25.6	–	–	–	–	–	–
20.04.18	Fiskemottak	SQA	201899961_17	63.466	4.4330	–	1	1667	740	3	–	–	–	–	0.2	–	–	–	–
03.04.18	Fiskemottak	SQA	201899966_2	63.466	10.0860	–	1	3861	950	5	136.3	–	14.2	–	–	–	–	–	–
03.04.18	Fiskemottak	SQA	201899966_3	63.466	10.0860	–	1	4097	1000	5	91.3	–	–	6.8	4.0	–	–	–	–
03.04.18	Fiskemottak	SQA	201899966_4	63.466	10.0860	–	1	4837	1010	4	132.5	–	–	–	–	–	–	–	–
21.03.18	Fiskemottak	SQA	201899967_1	63.466	10.0860	–	2	1799	770	4	–	–	19.6	–	–	–	–	–	–
15.03.18	Fiskemottak	SQA	201899968_5	63.466	10.0860	–	1	4043	960	4	100.5	–	22.2	–	–	–	–	–	–

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
Catch Date	Sample source	Species	Ind. ID	Lat.	Lon.	Depth in m	Sex	Total w. in g	Length in mm	Ma. St.	Stomach w. in g	Valve w. in g	Fish w. in g	Shrimp w. in g	Others w. in g	Plastics Color	Plastics Shape	Plastics w. in g	# particles
15.03.18	Fiskemottak	SQA	201899968_7	63.466	10.0860	–	1	4091	990	5	145.3	–	121.2	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955049_2	71.0902	22.7615	215.58	1	469	370	1	15.3	12.9	1.8	23.4	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955019_2	70.6362	30.7617	173.93	1	8	110	1	0.1	0.1	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955007_1	70.1877	30.7582	121.06	1	54	170	1	2.0	3.2	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955048_1	71.0132	22.7467	222.81	2	256	310	1	9.3	4.6	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955080_1	70.4433	19.3477	176.53	1	428	390	1	12.1	11.5	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973038_1	72.5255	14.8477	602.76	1	340	497	1	27.1	15.6	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973030_1	71.8558	15.4917	825.66	2	649	400	1	23.5	16.8	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973022_1	71.5427	16.2972	729.25	1	33	160	1	21.8	19.1	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973032_1	71.8960	15.7505	562.86	2	1054	470	2	23.3	17.3	–	0.9	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955012_1	70.5535	30.8407	93.68	1	596	390	2	16.1	14.0	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955009_1	70.3505	31.3425	184.8	2	1183	490	3	23.0	24.2	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973061_2	74.4185	16.2678	414.21	2	2091	580	4	54.0	39.7	–	3.0	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973061_1	74.4185	16.2678	414.21	2	1653	570	4	34.0	23.5	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955019_1	70.6362	30.7617	173.93	1	1294	530	4	28.3	29.2	–	–	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973092_1	78.0719	9.2500	736.78	1	975	450	5	43.6	40.8	4.4	15.5	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201955049_1	71.0902	22.7615	215.58	1	1104	490	5	21.8	23.7	–	19.1	–	–	–	–	–
02.09.-17.09.19	Egga-Nord	ARA	201973037_1	72.3755	15.0833	637.44	1	936	460	5	33.3	24.1	–	20.2	–	–	–	–	–

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
Catch Date	Sample source	Species	Ind. ID	Lat.	Lon.	Depth in m	Sex	Total w. in g	Length in mm	Ma. St.	Stomach w. in g	Valve w. in g	Fish w. in g	Shrimp w. in g	Others w. in g	Plastics Color	Plastics Shape	Plastics w. in g	# particles
24.01.-24.02.19	Vintertokt	ARA	201970318_2	76.0648	32.4805	316.70	1	263	290	1	8.8	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970378_3	78.7422	9.4870	270.04	2	12	110	1	3.3	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970325_1	76.0863	30.8620	329.55	2	989	450	1	25.9	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970377_7	78.5997	9.0590	560.18	2	702	410	1	21.0	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970315_1	76.0238	34.1458	266.69	2	1011	460	2	26.6	–	1.6	6.2	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970318_1	76.0648	32.4805	316.70	1	1957	610	2	50.4	–	1.7	3.8	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970308_1	76.0163	35.8180	247.23	1	1446	510	2	38.8	–	2.1	24.6	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970377_2	78.5997	9.0590	560.18	2	609	390	2	19.2	–	2.2	9.1	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970321_1	77.1353	32.6638	182.10	1	1363	500	2	44.4	–	3.3	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970365_2	76.9358	12.8923	261.41	2	975	470	2	19.1	–	3.4	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970324_1	76.4767	30.8363	286.70	1	1109	460	2	35.4	–	6.8	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970310_1	76.4112	35.9277	275.12	2	1638	550	2	30.4	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970315_2	76.0238	34.1458	266.69	2	1384	500	3	32.4	–	1.6	14.0	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970310_2	76.4112	35.9277	275.12	1	1264	490	3	26.7	–	16.6	8.5	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970305_1	75.6172	35.5497	166.54	2	1837	570	3	38.1	–	32.4	4.1	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970310_4	76.4112	35.9277	275.12	2	1676	580	3	27.7	–	–	1.1	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970377_4	78.5997	9.0590	560.18	2	1041	470	3	31.0	–	–	1.6	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970315_4	76.0238	34.1458	266.69	2	2104	590	3	43.5	–	–	9.9	–	–	–	–	–

Catch Data		Species ID & Spatial Information				Depth, Life History Traits and Intestines Weight							Dietary and Plastics Information						
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24.01.-24.02.19	Vintertokt	ARA	201970315_3	76.0238	34.1458	266.69	2	1736	550	3	26.2	–	–	28.1	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970310_5	76.4112	35.9277	275.12	2	1373	530	3	20.4	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970310_3	76.4112	35.9277	275.12	2	1829	580	3	26.2	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970378_2	78.7422	9.4870	270.04	1	919	470	4	24.0	–	1.3	2.9	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970377_1	78.5997	9.0590	560.18	2	1145	470	4	39.5	–	7.4	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970365_1	76.9358	12.8923	261.41	1	1282	510	4	22.3	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970378_1	78.7422	9.4870	270.04	2	1051	460	4	25.2	–	–	–	–	–	–	–	–
24.01.-24.02.19	Vintertokt	ARA	201970377_3	78.5997	9.0590	560.18	2	1103	460	5	61.2	–	–	3.9	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973010_3	69.451	15.2258	754.59	1	3941	770	3b	120.5	81.2	–	9.8	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973022_1	71.543	16.2972	729.25	1	2554	620	2	95.8	58.9	–	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973049_1	73.4	14.9793	830.91	1	44	180	1	3.6	2.7	–	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973008_1	68.867	12.8115	721.22	2	–	700	3b	96.6	79.5	–	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973010_1	69.451	15.2258	754.59	2	3298	740	2	132.4	91.8	–	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973022_3	71.543	16.2972	729.25	2	3118	680	3a	232.9	115.8	90.7	19.2	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973022_4	71.543	16.2972	729.25	2	3572	780	3a	287.3	109.4	209.3	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973022_8	71.543	16.2972	729.25	2	2606	650	3a	119.8	85.3	–	–	–	blue	tl	0.002*	3
02.09.-17.09.19	EggaNord	AHY	201973022_9	71.543	16.2972	729.25	2	2024	600	3a	96.1	42.1	10.3	–	–	–	–	–	–
02.09.-17.09.19	EggaNord	AHY	201973026_1	71.638	15.8715	830.91	2	1588	520	1	59.9	51.1	36.4	–	–	brown	tl	0.001*	1

