UNIVERSITETET I NORDLAND

MASTEROPPGAVE

Diet composition of cod (*Gadus morhua*):

small-scale differences in a sub-arctic fjord

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Abstract

Saltstraumen MPA is characterized by a strong tidal current with an ecosystem of high diversity, consisting of high densities of sessile filter feeders, such as e.g. Actiniaria, Alcyonacea, Porifera and Holothuroidea. Saltstraumen also support high abundances of both demersal and pelagic fish species. However, information on the fauna of Saltstraumen and the associated fjord, Skjerstadfjorden is solely based on observations by divers and underwater photos, and no information is available about the trophic interaction in the ecosystem. In this study, the diet composition of Atlantic cod in Saltstraumen MPA is assessed in relation to size, and the small-scale differences in diet composition between Saltstraumen and Skjerstadfjorden is investigated. Samples were collected as a part of a citizen science project, where the public was encourage to hand in the stomachs of fish caught in Saltstraumen and Skjerstadfjorden, in summer 2014.

The present study showed small-scale spatial difference in cod diet between the Saltstraumen MPA and Skjerstadfjorden, both in general and between size classes. Across all size classes the diet in Saltstraumen was dominated by fish, crabs, sea cucumbers and brittle stars, whereas fish, crabs, bivalves, *Lithodes maja* and sea urchins, dominated the diet in Skjerstadfjorden. Mysids and Euphausids were also important in Skjerstadfjorden, whereas polychaetes were important in both locations. Cannibalism was found in both locations, but was twice as frequent in Skjerstadfjorden.

The high densities of sessile filter feeders observed in Saltstraumen, only partly contributed to the diet of cod, with only Holothuroidea and Porifera found in 17% and 2% (Oef) of the stomachs, respectively. Instead, the associated mobile fauna, such as crabs (Brachyura) and brittle stars, seems to supplement fish in the diet of cod, and pelagic or bentho-pelagic invertebrates seem to be of little importance in the diet. Especially the intermediate size class (40-69 cm) of cod in Saltstraumen fed on brittle stars, which were less important prey in Skjerstadfjorden. It remains unclear to what extent the high diversity of benthic invertebrates in Saltraumen is also reflected in the diet of cod, because of low numbers of stomach samples from Skjerstadfjorden. Nevertheless, my results indicate that differences in the benthos and fish fauna between Saltstraumen and Skjerstadfjorden also lead to differences in the diet of cod on relatively small spatial scales.

1 Introduction

Human impacts like e.g. overexploitation, habitat transformation and pollution has resulted in degradation and biodiversity loss in many marine ecosystems. (Lotze et al. 2006). In an effort to halt loss of biodiversity, it was decided at the tenth meeting of the Conference of the Parties to the Convention on Biological Diversity in 2010 to aim for 10% of coastal and marine areas to be designated as marine protected areas (MPAs) by 2020. This was "to ensure that by 2020 ecosystems are resilient and continue to provide essential services thereby securing the planet's variety of life, and contributing to human wellbeing and poverty eradication." (IISD 2010). As a response, the Norwegian government established MPAs in Norwegian coastal areas of importance. The purpose of MPAs in Norway is to preserve areas that contain threatened, rare and vulnerable nature types, and that have a special scientific value. One of the MPAs created was the MPA of Saltstraumen in Northern Norway, established in June 2013 (Fig. 1.1). Saltstraumen has a rare type of nature consisting of a strong tidal current with an ecosystem of high diversity.

The MPA of Saltstraumen aims to protect the sea floor, including algae and sessile animals, and to prevent constructions that are harmful to the marine environment (Miljøverndepartementet 2013). Knowledge about the species present in Saltstraumen is mostly based on underwater photos and observations from divers. (Reiss, unpubl. data). Species found in Saltstraumen include high abundances of sessile invertebrates and fish, and thus Saltstraumen is an important area for recreational fishing, with many tourist visiting the area each year. The few studies conducted include a study of the benthic communities in the adjacent fjords Skjerstadfjorden and Saltfjorden (Gaidukov, Master Thesis), and oceanographic studies (Eliassen et al. 2001, Skreslet 2002).

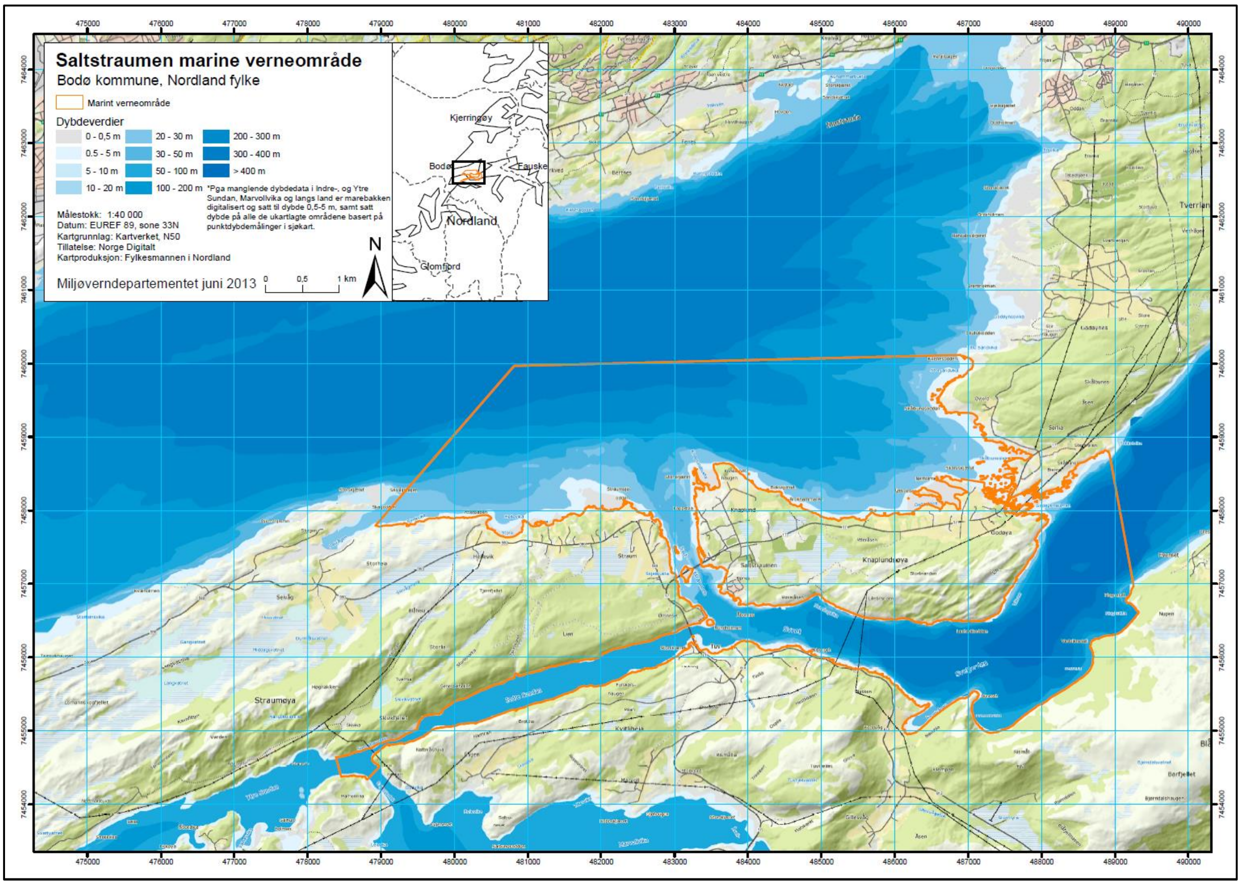

Figure 1-1 Map showing the protected area of Saltstraumen MPA (red line). (Miljøverndepartementet 2013)

Although Saltstraumen is known for high abundances of fish and invertebrates, no information is available about the trophic interaction in the ecosystem. Atlantic cod (*Gadus morhua*) is an omnivorous species and the diet usually reflects the available prey in the ecosystem (Svåsand et al. 2000). Cod is also the most abundant demersal fish species in Saltstraumen. Larvae and postlarvae feed on plankton, and juveniles (up to 25 cm length) mainly on small crustaceans, such as Euphausids and copepods, progressively replaced by medium and large decapods, like shrimps and crabs (Cohen 1990, Bromley et al. 1997, Arnott et al. 2000). An ontogenetic shift occurs from small invertebrates to fish with increasing size of cod and the diet of older individuals is dominated by fish. Slowly moving invertebrate prey like molluscs and echinoderms are generally not important (Svåsand et al. 2000). Furthermore, there are no seasonal changes in the proportion of benthic invertebrates in the cod diet. The consumption of fish, however, varies with season and Clupeids are important in many areas during summer and autumn (Cohen 1990). Diet composition also varies between locations, when comparing studies from Iceland (Jaworski et al. 2006), the southern Baltic sea (Pachur et al. 2013), the Celtic sea (Du Buit 1995), the Norwegian Skagerrak coast (Hop et al. 1992) and Balsfjord in northern Norway (Klemetsen 1982). A comparison of cod diet along the Norwegian coast revealed that the diet changed in accordance to the distributions and abundances of prey species. In southern and western Norway labrids and gobies are important, whilst Euphausids, capelin and herring were of high importance in northern Norway, north of Vestfjorden (Svåsand et al. 2000).

Therefore, in this study the diet composition of cod is investigated for an area inhabited by a benthic invertebrate community that differs remarkably from the adjacent regions in terms of diversity and abundance.

The main objectives are i) to assess the diet composition of Atlantic cod in Saltstraumen MPA in relation to size and ii) to investigate the small-scale differences in diet composition between Saltstraumen and Skjerstadfjorden. I hypothesise that a diet shift from invertebrate to non-invertebrate prey will occur later in the life cycle of demersal fish in an ecosystem with a high abundance of invertebrate prey such as Saltstraumen.

I used a citizen science approach to collect stomach samples in the two study sites, where the general public were involved in the sample collection. By collecting the stomachs of fish that already have been caught, the fish diet could be studied in a sustainable and efficient way and allowed at the same time to inform the local public about the project and the importance of ecosystem studies.

2 Materials and methods

2.1 Study area

The two studied areas, Saltstraumen and the inner part of Skjerstadfjorden, is a part of the tidally energetic fjord system Saltfjorden – Skjerstadfjorden, located in Northern Norway (Fig. 2.1). Skjerstadfjorden is a glacially carved fjord about 50 km long with a 530 m deep basin. The water exchange between the two fjords occurs through the narrow and shallow channels, Godøystraumen and Saltstraumen. Skjerstadfjorden is also connected to the open ocean by another channel, Sundstraumen. The majority of the 2,7 x 10^8 m^3 water exchanged between tides flows through Saltstraumen, which has a sill depth of 26 m and an outlet width of 255 m (at the surface)(Eliassen et al. 2001). The average velocity (at spring tide) in Saltstraumen is 6 m s^{-1}, but maximum velocity can reach or even exceed 10 m s^{-1} at some locations in the stream (Gjevik 2009). Bottom water in the deep basins of Skjerstadfjorden is exchanged several times each year due to the strong currents. It seems that anoxic conditions, often occurring in deep fjord basins (Holte et al. 2005), rarely take place and oxygen levels remain high throughout the year (Eliassen et al. 2001, Skreslet 2002).

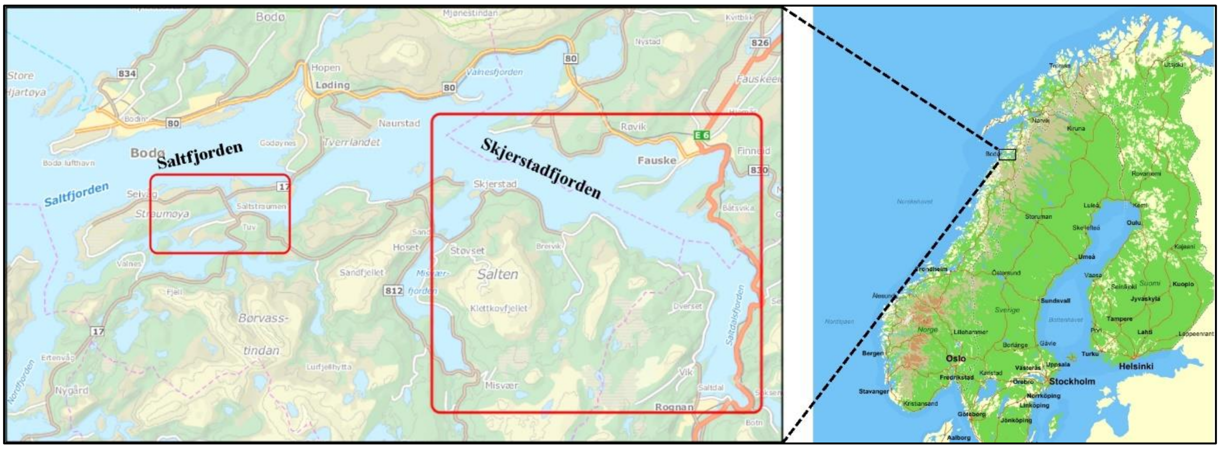

Figure 2-1 Location of the study areas in fjord system Saltfjorden-Skjerstadfjorden: Saltstraumen (small red box) and Skjerstadfjorden outside Fauske (big red box).

The strong currents likely transport large amounts of food plankton through Saltstraumen and provides food for high densities of sessile filter feeders and hyperbenthic predators, such as e.g. Actiniaria, Alcyonacea, Porifera and Holothuroidea. Saltstraumen also support high abundances of both demersal and pelagic fish species. Demersal species include Atlantic cod (*Gadus morhua)*, Atlantic halibut (*Hippoglossus hippoglossus*), wolf fish (*Anarchchas lupus*), angler fish (*Lophius piscatorius*), common ling (*Molva molva*) and haddock (*Melanogrammus aeglefinus*). Pelagic species include saithe (*Pollachius virens*), Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*). Information on the fauna of Saltstraumen and Skjerstadfjorden is solely based on observations by divers and underwater photos, whereas no information is available from systematic monitoring or research projects (Reiss, unpubl. data).

2.2 Sampling strategy– a citizen science approach

The sampling of cod stomachs in Saltstraumen and Skjerstadfjorden was done in summer 2014 (Fig. 2.1). In Skjerstadfjorden, samples were collected between 2 July and 15 September. The sampling area was expanded 3 August to include the head of the fjord. In Saltstraumen, samples were collected between 3 July and 10 August. In total 373 stomachs from Atlantic cod caught in the study areas were collected, 313 from Saltstraumen and 60 from Skjerstadfjorden (Fig. 2.2 and 2.3). Stomachs from haddock, wolf fish and halibut were also collected, but were not included in this study.

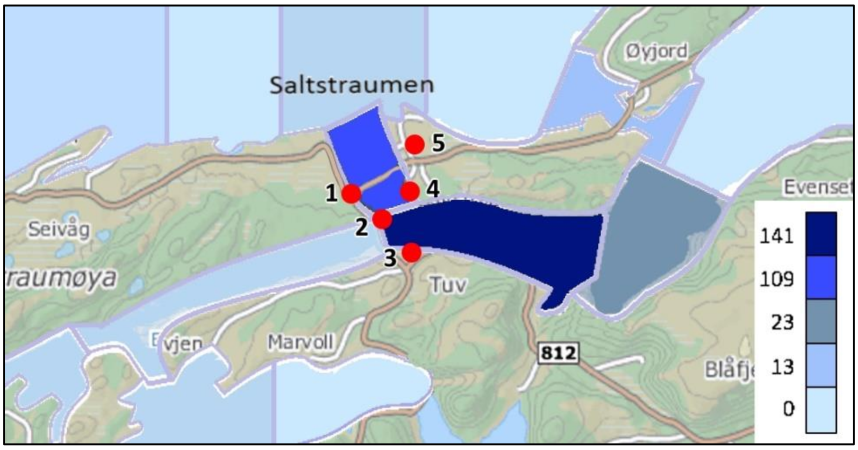

Figure 2-2 Heat map showing the number of cod caught in the different parts of Saltstraumen. Red dots mark the collecting stations at the west end of the Saltstraumen bridge (1), which was moved to Ørnneset (2) July 12th, Saltstraumen Brygge (3), Kafè Kjelen (4), Saltstraumen camping (5).

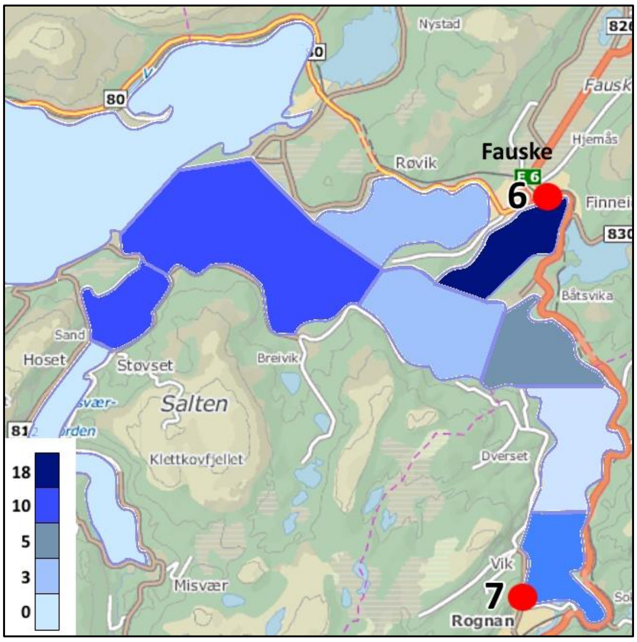

Figure 2-3 Heat map showing the number of cod caught in the different parts of Skjerstadfjorden. Red dots mark the collecting stations in the harbours of Fauske (6) and Rognan (7).

Samples were collected as a part of a citizen science project, where the public was encouraged to hand in the stomachs of fish caught in the study areas. Collecting the stomach of fish that already has been caught enabled us to study fish diet in a sustainable and efficient way. To recruit participants for the study, information was given on posters and leaflets (appendix 1 and 2), presented at four collecting stations in Saltstraumen and two in Skjerstadfjorden (Fig. 2.2 and 2.3), as well as in newspaper articles and by talking to anglers on site. At the collecting stations, anglers could hand in samples themselves, and got the necessary equipment comprising leaflets (in Norwegian, English and German), pencils, measuring tapes and zip-locked plastic bags for fish stomachs. On each bag, a form was attached where anglers had to fill in catch date and time, species, fish length, type of fishing gear, and to mark the catch site on a map (appendix 3). Samples were handed in by using marked freezers (stations 3, 5, 6 and 7) or provided cooling boxes (stations 1, 2 and 4), which were emptied several times a day. In Saltstraumen, stomachs were also collected by directly approaching anglers and taking samples from their fish. Samples were frozen as soon as possible (after maximum 5 h).

2.3 Sample processing

In the laboratory, 229 of the 372 stomachs (59 from Skjerstadfjorden and 170 from Saltstraumen) were analysed. The samples were carefully defrosted in a microwave. Large and very small stomachs were defrosted at room temperature to prevent overheating of the stomach content in the microwave. Food items were sorted and identified to lowest taxon possible, based on morphological characteristics (Lincoln 1979, Smaldon 1979, Ryland et al. 1995, Hartmann-Schröder 1996). All taxa were counted and weighed (g wet weight). The degree of digestion was assessed and each taxon was stored in 70% ethanol. Tissue samples for DNA barcoding analysis was taken from all prey fish and stored in 70% pure ethanol at -30 °C.

2.4 DNA barcoding

2.4.1 DNA extraction and PCR

DNA was extracted from a 2 mm cube of tissue (~10-15 mg) using DNeasy Blood & Tissue kit, cat. no. 69506 (Qiagen Norge, Oslo, Norway) (QIAGEN 2006). DNA was eluted using 100 µl AE buffer. There was one negative control in each run (7 in total). DNA concentration was measured, using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Samples with concentration higher than 160 ng/µl was diluted to 150 ng/µl.

PCR reactions were run on 96-well plates using Expand High Fidelity PCR system, cat. no. 11 732 650 001 (Roche Norge, Oslo, Norway) with total volume 25 µl (Roche 2005). A universal primer cocktail for DNA barcoding of fish, containing two forward and two reverse primers was used, as describe by Ivanova et al. (2007) (Table 2.1). To avoid enzymatic activity during setup, PCR reactions were prepared by combining two reaction mixes, as recommended by the manufacturer. PCRs were carried out in 25 µl volumes, containing primers (5 µM of each), deoxynucleotide mix (200 µM of each dNTP), Expand High Fidelity buffer with 15 mM $MgCl_2$, Expand High Fidelity enzyme mix (~1.0 U/rx), ~150 ng template and PCR-grade water.

Table 2.1 Primers used in PCR, two forward (F) and two reverse (R) primers. Each have a 17 (red) or 18 (blue) nucleotide sequence from the bacteriophage vector M13 at the 5' end, and a sequence specific to the barcode locus, *COI* (black).

Primer	Sequence (5' - 3')	Ratio	F/R
VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	1	F
FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	1	F
FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA	1	R
FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	1	R

The plate was sealed with plate sealing film, spun in a centrifuge for a couple of seconds and run in Veriti® 96-Well Thermal Cycler (Applied Biosystems, Foster City, USA) (thermal cycling conditions used, is presented in Table 2.2). Each plate had one negative and one positive control, as well as controls from DNA extractions.

Table 2.2 Thermal cycling conditions used for PCR and sequencing reactions.

	PCR	Sequencing reactions
Initial activation of enzyme:	94°C 2 min	96º C, 5 min
Denaturation:	95°C 30 sec	96 º C, 10 sec.
Annealing:	52°C 40 sec	50 º C, 5 sec.
Elongation:	72°C 1 min	60 º C, 4 min.
Number of cycles:	25	25
Terminal elongation:	72°C 7 min	-

PCR products were visualized on 1% agarose gels containing SYBR® Safe DNA Gel Stain. One Kb Plus DNA Ladder (Invitrogen by Life Technologies, Oslo, Norway) was used to determine the size of PCR products. Electrophoresis was run for 1.5 hours at 120 V in room temperature, using 0,5x TBE buffer. Excess primers and nucleotides were enzymatically removed from successful amplifications (with a single ~700bp band) as preparation for sequencing, using USB ExoSAP-IT PCR Product Cleanup (Affymetrix, Inc., Cleveland, USA) (Affymetrix 2014).

2.4.2 BigDye reaction and sequencing

Forward and reverse sequencing reactions were run for each of the successfully amplified samples on 96-welled plates using BigDye Terminator v3.1 Cycle Sequencing Kit, Catalogue number: 4337455 (Applied Biosystems), total volume 20 µl, with 7-2 µl PCR product and 0-5 µl of PCR-grade water, calculated for each sample to make the final template concentration between 5-20 ng/20 μl (Universitetssykehuset Nord-Norge 2010). The plate was sealed with plate sealing film, spun in a centrifuge for a couple of seconds and run in Veriti® 96-Well Thermal Cycler (Applied Biosystems) (thermal cycling program, used is presented in Table 2.2). Samples were then sent to the DNA sequencing lab at Universiteitsykehuset Nord-Norge for Sanger sequencing.

2.4.3 Species designation

Forward and reverse sequences for each specimen were combined using MEGA 6.06 (Tamura et al. 2013) and Chromas lite 2.1.1 (Technelysium 2007). Primer sequences were removed, leaving a maximum of 652 bp *COI* sequence for each specimen. Species designations were based on BLAST searching at NCBI, using the Megablast search option (Zhang et al. 2000) and at Barcode of Life Data Systems (Ratnasingham et al. 2007).

2.5 Statistical analysis

2.5.1 Univariate measurements

Frequency of occurrence (O_{ef}), relative abundance (by number; A_N and weigh; A_W), relative importance index (RI), total number and weight of prey per stomach and number of taxa vas used as univariate characteristics of the diet composition in the study areas. Total prey number and weight, and taxa number reported in this study refer to all samples in that particular location or size class within a location. The frequency of occurrence (O_{efi}) shows how common a prey item is in the diet (Houlihan et al. 2001). It is calculated by determining the percentage of stomachs in which prey item i occurs as follows:

$$O_{efi} = 100 * (N_i / N_{ef})$$

Where N_i is the number of stomachs that contains prey item i and N_{ef} is the number of stomachs in that location or size class within a location that contains food.

The relative abundance (A_i, by number; A_{Ni} and weigh; A_{Wi}) provides information on the contribution of each prey to the stomach content (Houlihan et al. 2001). It is calculated as the number of individuals or the weight of an individual prey item as a percentage of all prey items from that location or size class:

$$A_i = 100 * (\Sigma S_i / \Sigma S_t)$$

Where S_i is number or weight of prey item i in the stomachs and S_t is the total number or weight of all prey.

All three indices above give an indication of importance of taxa in the fish diet, but they will rank prey items differently depending on their contribution in terms of numbers (A_N), weight (A_W) or occurrence (O_{ef}). In contrast, the relative importance index (RI) combines all three indices and is therefore considered as a more general ranking index (Houlihan et al. 2001). It is calculated as the absolute importance index (AI) for each prey group as a percentage of the total absolute importance index as follows:

$$RI = 100 * (AI / \Sigma AI)$$

Where AI is the sum of percentage frequency of occurrence, percentage abundance by number and percentage abundance by weight:

$$AI = O_{efi} + A_{Ni} + A_{Wi}$$

Furthermore, correlation analysis of the relationship between predator length and prey number, weight, number of prey taxa, and prey length was conducted, using cor.test in R with the non-parametric method "kendal" Assumptions were tested with the Shapiro-Wilk test (for normality). Number of prey taxa, number of prey per stomach and total weight of stomach content was also plotted against 10 cm size classs of cod to show the distribution of variances (Crawley 2005). Plots and correlation analysis was performed using R version 3.1.2 (The R Foundation for Statistical Computing, 2014)

2.5.2 Multivariate analysis

The diet composition based on abundance of prey taxa were compared using multivariate cluster analysis on square root transformed data to reveal spatial difference in diet between locations. ANOSIM analysis (analysis of similarities) was used to test for significant differences in diet composition between locations and size classes and SIMPER analysis (Similarity Percentage analysis) was used to identify the contribution of different taxa to the dissimilarities. All multivariate analysis were performed using PRIMER version 6.1.6 (Clarke et al. 2001).

3 Results

3.1 General diet composition

In total, 3264 individuals from 109 taxa (71 on family level), were found in the 232 cod stomachs analysed. At the two locations, 2410 and 854 individuals from 95 and 52 taxa (71 and 39 at family level), were found in the 173 and 59 stomachs in Saltstraumen and Skjerstadfjorden, respectively. These were represented by 8 and 6 phyla (Saltstraumen, Skjerstadfjorden); Arthropoda (40, 16), Annelida (19, 10), Chordata (10, 9), Mollusca (14, 11), Echinodermata (9, 5), Cnidaria (1, 0), Nemertea (only in Saltstraumen) and Porifera (both locations). A list of taxa found in this study is presented in Table 3.1. Fish species identified by DNA Barcoding included *Gadus morhua, Trisopterus esmarkii, Lumpenus lampretaeformis, Ammodytes marinus, Triglops murrayi, Ciliata mustela and Hippoglossoides platessoides* (identity 0.99-1, E-value 0; Appendix 4). The further analysis were done on family or higher taxa (except ANOSIM, which was done both on species level and on higher taxa groups).

Table 3.1 List of taxa found in cod stomachs of Saltstraumen (S) and Skjerstadfjorden (F) (*=not possible to determine lower taxon, **=identified by DNA barcoding)

Phylum	Class	Family	Species	Location
Porifera*				S/F
Cnidaria	Anthozoa	Actiniaria*		S
Echinodermata	Asteroidea	Asteriidae	*Asterias rubens*	S
	Ophiuroidea	Ophiactidae	*Ophiopholis aculeata*	S/F
		Ophiuridae	*Ophiura albida*	S/F
	Echinoidea	Echinocyamidae	*Echinocyamus pusillus*	S
		Spatangoida*		S
		Strongylocentrotidae	*Strongylocentrotus droebachiensis*	S/F
	Holothuroidea	Cucumariidae	*Cucumaria frondosa*	S/F
		unknown	*Holothuroidea_unknown1*	S/F
		Psolidae	*Psolus phantapus*	S
Chordata	Actinopterygii	Clupeidae*		S
		Anarhichadidae	*Anarhichas sp.*	F
		Gadidae	*Gadus morhua***	S/F
		Gadidae	*Trisopterus esmarkii***	F
		Gadidae	*Pollachius virens*	S
		Holidae	*Pholis gunnellus*	S
		Stichaeidae	*Lumpenus lampretaeformis***	F
		Stichaeidae	Unidentified	S
		Ammodytidae	*Ammodytes marinus***	S/F

Phylum	**Class**	**Family**	**Species**	**Location**
Chordata	Actinopterygii	Cottidae	*Triglops murrayi***	S
		Lotidae	*Ciliata mustela***	S
		Pleuronectidae	*Hippoglossoides platessoides***	F
		Pleuronectidae	*Hippoglossus hippoglossus*	F
		Unidentified		S/F
	Ascidiacea*			S/F
Mollusca	Bivalvia	Anomiidae*		S
		Cardiidae	*Acanthocardia echinata*	F
		Hiatellidae*		S
		Myidae	*Mya sp.*	S/F
		Mytilidae	*Modiolula phaseolina*	S
			Mytilus edulis	S/F
			Unidentified	S
		Nuculanidae*		S/F
		Pectinidae	*Palliolum tigerinum*	F
		Unidentified		S/F
	Gastropoda	Aporrhaidae	*Aporrhais pespelecani*	F
		Buccinidae	*Buccinum undatum*	S/F
			Neptunea antiqua	S/F
		Littorinidae	*Lacuna vincta*	S
			Littorina sp.	S
		Margaritidae	*Margarites helicinus*	F
		Velutinidae*		S
	Polyplacophora	Mopaliidae	*Tonicella rubra*	S/F
Nemertea*				S
Annelida	Polychaeta	Ampharetidae	*Melinna sp.*	S
		Aphroditidae	*Aphrodita aculeata*	F
		Arenicolidae	*Arenicola marina*	S
		Eunicidae	*Eunice pennata*	S
			Unidentified	F
		Flabelligeridae*		S/F
		Glyceridae	*Glycera sp.*	S/F
			Unidentified	S/F
		Nereididae	*Unidentified*	S
		Onuphidae	*Nothria conchylega*	F
		Opheliidae	*Ophelia sp.*	S
		Phyllodocidae	*Phyllodoce groenlandica*	S
			Unidentified	S
		Polynoidae	*Eunoe nodosa*	S
			Eunoe sp	S
			Unidentified	S/F
		Serpulidae	*Hydroides sp.*	S
			Spirobranchus triqueter	S/F
			Spirorbis sp.	S
		Terebellidae*		S/F
		Unidentified		S/F
Arthropoda	Maxillopoda	Balanomorpha*		S

Phylum	Class	Family	Species	Location
Arthropoda	Malacostraca	Euphausiacea	*Euphausiacea**	S/F
			Meganyctiphanes norvegica	S/F
			Thysanoessa inermis	S
		Oregoniidae	*Hyas araneus*	S
			Hyas cf. coarctatus	S/F
			Hyas sp.	S
		Cancridae	*Cancer pagurus*	S
		Portunidae	*Carcinus maenas*	S/F
		Lithodidae	*Lithodes maja*	S/F
		Paguridae	*Pagurus pubescens*	S/F
		Munididae	*Munida rugosa*	S
		Galatheidae	*Galathea sp.*	S
		Crangonidae	*Sclerocrangon boreas*	S
		Hippolytidae	*Eualus cf. pusiolus*	S/F
			Eualus gaimardii	S
			Hippolyte cf. Varians	S
			Hippolyte sp.	S
			Spirontocaris liljeborgiii	F
			Unidentified	S/F
		Pandalidae	*Pandalus cf. montagui*	S/F
			Pandalus sp.	S/F
		Pasiphaeidae	*Pasiphaea multidentata*	S/F
		Mysidae	*Heteromysis cf. formosa*	F
			Praunus cf. inermis	F
			Schistomysis sp.	S
		Idoteidae	*Idotea cf. neglecta*	S
			Idotea sp.	S
		Janiridae	*Janira maculosa*	S
		Amphipoda*		S
		Caprellidae	*Caprella sp.*	S
		Hyperiidea*		S/F
		Acidostomatidae	*Acidostoma sp.*	S
		Ampithoidae*		S
		Calliopiidae*		S
		Epimeriidae*		S
		Gammarellidae	*Gammarellus sp.*	S
		Ischyroceridae	*Parajassa pelagica*	S
		Liljeborgiidae	*Liljeborgia pallida*	S
		Melitidae	*Melita dentata*	S
			Melita palmata	S
			Unidentified	S/F
		Stenothoidae*		S

3.2 Spatial difference in diet

The diet of cod in both study sites was clearly dominated by fish with a relative weight (A_W) of 59% and 48% in Saltstraumen and Skjerstadfjorden, respectively, and relative importance index (RI) of 24-25% (Table 3.2). Other prey taxa with high RI in Saltstraumen include Ophiuroidea (16%), Brachyura (11%) and Polychaeta (7%), whereas Echinoidea (9%), Polychaeta (8%), Bivalvia (7%) and Caridea (6%) showed high RI values in Skjerstadfjorden. All other taxa contributed less than 6% (RI) in both study sites. Euphausiacea (A_N= 23%) and Mysidae (17%) were the most abundant prey taxon in Skjerstadfjorden but only occurred in 2% and 9% of stomachs (O_{ef}), respectively, and had low RI values.

In Saltstraumen *Pollachius virens* (A_W= 23%) and Clupeidae (9%) were the main fish species in the diet of cod, whereas *Gadus morhua* (16%) and *Trisopterus esmarkii* (8%) dominated in the diet Skjerstadfjorden. Notably, unidentified fish in the diet accounted for 24 and 18% (A_W) in Saltstraumen and Skjerstadfjorden, respectively. Other important prey taxa in Saltstraumen include *Hyas coarctatus* and *Carcinus maenas* (Brachyura), *Ophiopholis aculeata* (Ophiuroidea), and Polynoidae and Nereididae (Polychaeta). The prey taxa *Strongylocentrotus droebachiensis* (Echinoidea), *Lithodes maja* (Lithodidae), *Mytilus edulis* (Bivalvia), *Pandalus sp*. (Caridea), and Polynoidae, Glyceridae and Onuphidae (Polychaeta) were important in Skjerstadfjorden.

Across all size classes, the multivariate cluster analysis revealed no clear separation between the cod diets in the two study sites (Appendix 5). Nevertheless, a significant difference was found between the diet composition of cod in Saltstraumen and Skjerstadfjorden (ANOSIM based on square rooted prey abundance data; $p = 0.0005$, species level; $p = 0.0004$, grouped taxa). The taxa contributing the most to these differences were Actinopterygii (unidentified), *O. aculeata*, Unknown, *S. droebachiensis* and *H. coarctatus* (in descending order; SIMPER, species level;). In Saltstraumen, Actinopterygii (unidentified), *O. aculeata* and *H. coarctatus* occurred more often, whereas Unknown and *S. droebachiensis* occurred more often in Skjerstadfjorden.

Table 3.2 Relative frequency of occurrence (O_{ef}), relative abundance (A_n), relative weight (A_w), Relative importance index (RI), and results of SIMPER (contribution to dissimilarities of taxa, %) for taxa in the diet of *Gadus morhua* in Saltstraumen and Skjerstadfjorden, sorted by RI of Saltstraumen. Number of stomachs analysed in parentheses. (*= unidentified)

Taxa	Saltstraumen (150)				Skerstadfjorden (47)				SIMPER
	O	A_N	A_W	RI	O	A_N	A_W	RI	Contrib. (%)
Actinopterygii	65.3	13.1	58.7	25.0	60.5	5.4	48.0	24.0	1.7
Ophiuroidea	38.7	41.9	7.5	16.1	16.3	2.8	0.2	4.1	1.9
Brachyura	36.0	8.0	15.7	10.9	7.0	0.5	5.8	2.8	8.0
Polychaeta	32.0	5.0	0.6	6.9	30.2	7.6	1.4	8.3	8.6
Holothuroidea	17.3	2.1	9.4	5.2	7.0	0.5	1.3	1.9	3.7
Caridea	24.0	4.2	0.4	5.2	25.6	3.7	0.7	6.3	7.4
Bivalvia	22.0	2.2	1.8	4.8	18.6	13.1	4.6	7.6	6.8
Unknown	21.3	1.5	3.0	4.7	23.3	2.2	3.4	6.1	6.2
Amphipoda	13.3	3.7	0.0	3.1	2.3	0.1	0.0	0.5	2.9
Caprella sp.	8.7	8.3	0.0	3.1	-	-	-	-	4.4
Gastropoda	13.3	1.3	0.4	2.7	16.3	1.0	1.8	4.0	3.2
Echinoidea	9.3	3.0	1.1	2.4	14.0	15.9	12.4	8.9	6.4
Galatheoidea	9.3	0.9	0.2	1.9	-	-	-	-	-
Paguroidea	8.0	0.6	0.2	1.6	9.3	1.0	0.2	2.2	2.2
Decapoda	7.3	0.5	0.1	1.4	7.0	0.4	0.0	1.6	-
Isopoda	4.0	1.2	0.0	0.9	-	-	-	-	-
Balanomorpha	4.0	0.5	0.2	0.9	-	-	-	-	-
Euphausiacea	2.7	0.8	0.0	0.6	2.3	22.8	0.2	5.3	-
Crustacea	2.7	0.2	0.0	0.5	4.7	0.3	0.0	1.0	-
Porifera	2.0	0.1	0.0	0.4	2.3	0.1	1.2	0.8	-
Hyperiidea	2.0	0.1	0.0	0.4	2.3	0.6	0.0	0.6	-
Nemertea	1.3	0.1	0.0	0.3	-	-	-	-	-
Mysida	0.7	0.3	0.0	0.2	9.3	17.3	0.3	5.7	-
Ascidiacea	0.7	0.1	0.1	0.2	9.3	3.1	2.4	3.1	2.7
Actiniaria	0.7	0.1	0.2	0.2	-	-	-	-	-
Lithodidae	0.7	0.0	0.0	0.1	4.7	1.4	16.2	4.7	2.0
Asterias rubens	0.7	0.0	0.0	0.1	-	-	-	-	-
Chitonida	0.7	0.0	0.0	0.1	2.3	0.1	0.0	0.5	-

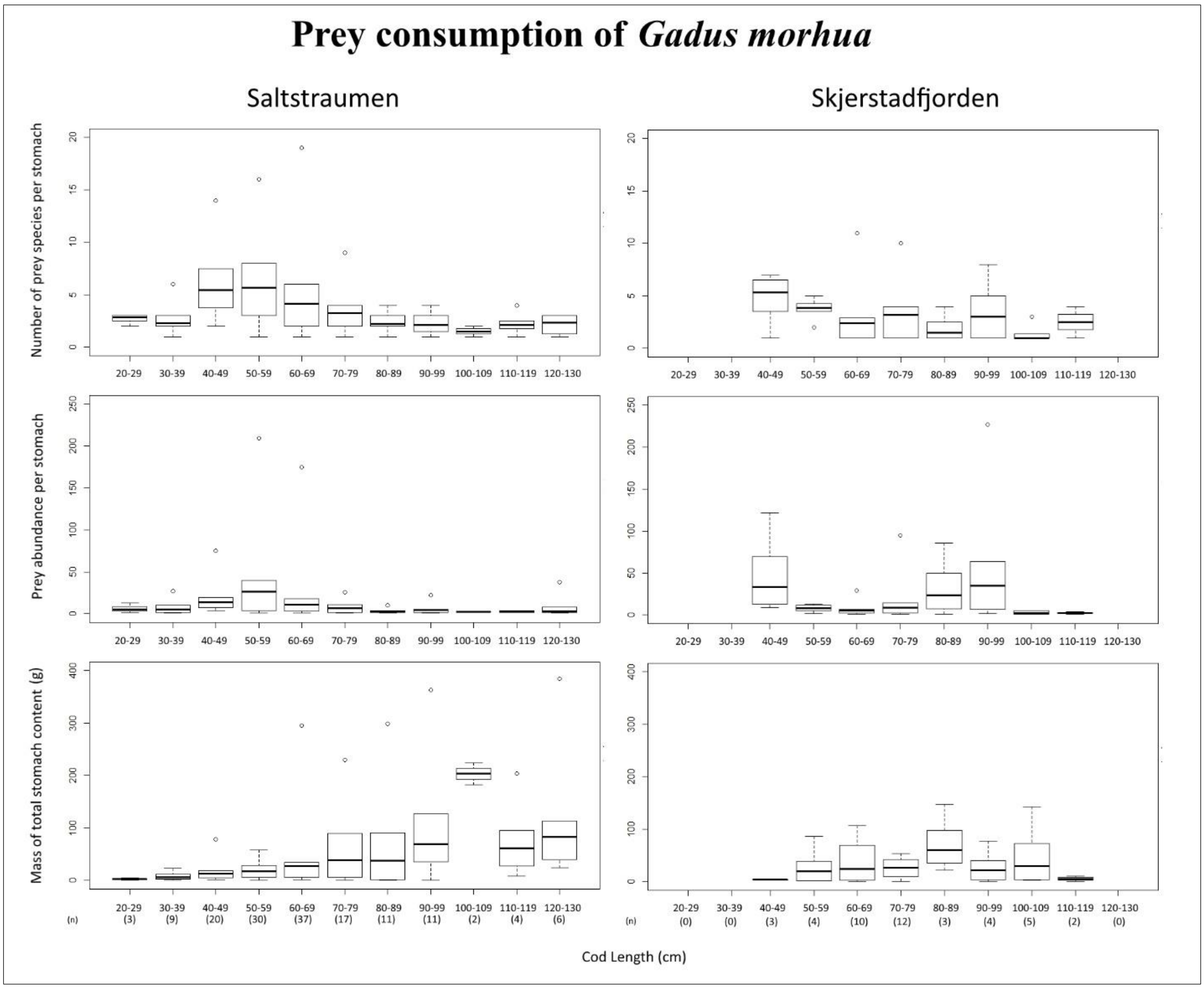

Figure 3-1 Number of prey species, prey abundance and mass of total stomach content in the different size classes of *Gadus morhua* in Saltstraumen and Skjerstadfjorden. Number of stomachs analysed in parentheses.

3.3 Spatial difference in diet between size classes

The number of prey spices, prey abundance and mass of total stomach content per stomach varied with cod length (Fig. 3.1). In Saltstraumen, number of prey species and prey abundance was highest in the cod size class 50-59 cm and decreased again with increasing cod length. In fact, cod length was significantly negatively correlated with number of prey species (kendal, tau = -0.23, $p < 0.001$) and prey abundance (tau = -2.02, $p < 0.001$). Not surprisingly, mass of total stomach content significantly increased with cod length (tau = 0.29, $p < 0.001$).

In contrast, no significant correlation between cod length and number of prey species, prey abundance or mass of total stomach content was found in Skjerstadfjorden, probably because of too low sample sizes. Nevertheless, number of prey species was highest in the 40-59 cm size class and decreased slightly with cod length. The highest prey weight was found in size class 80-89 cm and there was only a slight increase with cod length. Prey abundance, however, was highest in 40-49 and 80-99 cm cod and showed no obvious trends. In the further analysis, cod was grouped into the following size classes; 20-39 cm, 40-69 cm, 70-99 cm and 100-130 cm, to increase the sample sizes for diet comparison. There were, however, no samples from Skjerstadfjorden in the 20-39 cm size class.

Size class 20-39 cm

The diet in 20-39 cm cod in Saltstraumen was clearly dominated by fish with a relative weight (A_W) of 59% and a relative importance index (RI) of 53%, followed by Ophiuroidea (RI= 17%), Caridea (10%) and Brachyura (8%) (Table 3.3). All other taxa contributed less than 8% (RI). Polychaeta (O_{ef}= 25%) were often preyed upon, despite low RI. The main fish species in the diet was Pollachius virens (A_W= 16%), although unidentified fish accounted for 36% (A_W). Other important prey taxa include *Ophiopholis aculeata* (Ophiuroidea), *Hyas coarctatus* (Brachyura), *Eualus pusiolus* (Caridea), and Nereididae, Polynoidae and Serpulidae (Polychaeta).

Size class 40-69

The diet of 40-69 cm cod in Skjerstadfjorden was also clearly dominated by fish with a relative weight (A_W) of 58% and a relative important index (RI) of 27%, followed by Mysidae (RI= 13%), Caridea (10%) and Polychaeta (10%; Table 3.3). In contrast, the diet of 40-69 cm cod in Saltstraumen was dominated by Ophiuroidea with a relative importance index (RI) of 20% and a relative abundance (A_N) of 47%, followed by fish (RI= 17%). Fish, however, had the highest relative weight (A_W) of 38%, although this was lower than for fish in Skjerstadfjorden. Other important taxa were Brachyura (RI= 12%) and Polychaeta (8%). All other taxa contributed less than 6% (RI) in both locations. Despite low RI values, Lithodidae (16%) and Echinoidea (11%) had the highest relative weight (A_W). Other frequently consumed taxa were include Gastropoda (O_{ef}= 24%) and Paguroidea (24%) in Skjerstadfjorden, whereas in Saltstraumen Caridea (O_{ef}= 30%) and Bivalvia (26%) were often preyed upon. Notably, Mysidae had the highest relative abundance (A_N= 51%) in Skjerstadfjorden, which also was 14x higher than in all other size classes in both locations.

The dominant prey fish species in 40-69 cm cod in Skjerstadfjorden was *Pollachius virens* (A_W= 15%), whereas *Trisopterus esmarkii* (22%) and *Gadus morhua* (14%) dominated in Saltstraumen. Unidentified fish, however, contributed to 21% and 12% (A_W) in Skjerstadfjorden and Saltstraumen, respectively. Other important prey taxa in Skjerstadfjorden include *Heteromysis cf. formosa* (Mysidae), *Strongylocentrotus droebachiensis* (Echinoidea), *Pandalus sp*. (Caridea), *Pagurus pubescens* (Paguroidea), *Lithodes maja* (Lithodidae), and Glyceridae and Polynoidae (Polychaeta). The prey taxa *Ophiopholis aculeata* (Ophiuroidea), *Hyas coarctatus* (Brachyura), *Eualus pusiolus* (Caridea), Nereididae and Polynoidae (Polychaeta), and Mytilidae (Bivalvia) were important in Saltstraumen.

A significant difference was found between the diet composition of the 40-69 cm cod size class between Saltstraumen and Skjerstadfjorden (ANOSIM based on square rooted prey abundance data; $p = 0.001$, species level; $p = 0.017$, grouped taxa). The taxa contributing the most to these differences were *Ophiopholis aculeata*, Actinopterygii (unidentified), *Heteromysis cf. formosa*, *Hyas coarctatus* and Unknown (in descending order; SIMPER, species level). In Saltstraumen, *Ophiopholis aculeata, Hyas coarctatus* and Unknown occurred more often, whereas Actinopterygii (unidentified) and *Heteromysis cf. formosa* occurred more often in Skjerstadfjorden.

Size class 70-99 cm

Fish was clearly the most important prey in the diet of 70-99 cm cod in both locations, but of higher importance in Saltstraumen than in Skjerstadfjorden. In Saltstraumen, fish had a relative importance index (RI) of 33% and a relative weight (A_W) of 57%, whilst in Skjerstadfjorden, it was 20% and 44%, respectively (Table 3.3). Other prey taxa with high RI in Saltstraumen include Holothuroidea (12%) and Brachyura (11%), whereas in Skjerstadfjorden Echinoidea (13%) and Bivalvia (12%) showed high RI values. All other taxa contributed less than 9% (RI) in both locations. Despite low RI, Euphausiacea had the highest relative number (A_N) of 34% in Skjerstadfjorden, which was also 13x higher than in all other size classes in both locations. In contrast, Ophiuroidea (RI= 7%) was of less importance as prey for size class 70-99 cm in Saltstraumen than for smaller cod. Other taxa often preyed upon in Saltstraumen were Bivalvia

(O_{ef}= 18%) and Caridea (15%), while Polychaeta (26%) and Caridea (16%) were common prey in Skjerstadfjorden.

In 70-99 cm cod in Saltstraumen, *Pollachius virens* (A_W= 33%) and Clupeidae (13%) were the dominating prey fish species, whereas *Gadus morhua* (23%) dominated in Skjerstadfjorden. Unidentified fish accounted for 10% (A_W) at both locations. Other important prey species in Saltstraumen include *Ophiopholis aculeata* (Ophiuroidea), *Hyas coarctatus* and *Carcinus maenas* (Brachyura), *Eualus pusiolus* (Caridea), *Cucumaria frondosa* (Holothuroidea) and Mytilidae (Bivalvia). The prey taxa *Strongylocentrotus droebachiensis* (Echinoidea), *Mytilus edulis* (Bivalvia), and Polynoidae and Onuphidae (Polychaeta) were important in Skjerstadfjorden.

A significant difference was found between the diet composition of the 70-99 cm cod size class between Saltstraumen and Skjerstadfjorden (ANOSIM based on square rooted prey abundance data; $p = 0.019$, species level; $p = 0.017$, grouped taxa). The taxa contributing the most to these differences were Actinopterygii (unidentified), S. droebachiensis, *Mytilus edulis*, Unknown and *Ophiopholis aculeata*, (in descending order; SIMPER, species level). In Saltstraumen, Actinopterygii (unidentified) and *Ophiopholis aculeata* occurred more often, whereas S. droebachiensis, *Mytilus edulis* and Unknown occurred more often in Skjerstadfjorden.

<u>Size class 100-130 cm</u>

The diet of 100-130 cm cod in Saltstraumen was solely dominated by fish with a relative weight (A_W) of 95% and a relative importance index (RI) of 77% (Table 3.3). In contrast, Lithodidae dominated by weight (A_W= 58%) in Skjerstadfjorden, followed by fish (40%). Despite this, fish had the highest relative important index (RI) of 38%, followed by Lithodidae (29%). All other taxa contributed less than 8% (RI and A_W) in both locations. Notably, Lithodidae only occurred in one of the seven stomachs in Skjerstadfjorden. The most important fish species in Saltstraumen was *Pollachius virens* (A_W= 16%) and Clupeidae (11%), with unidentified fish accounted for 68% and 36% (A_W) in Saltstraumen and Skjerstadfjorden, respectively.

A significant difference was found between the diet composition of the 100-130 cm cod size class between Saltstraumen and Skjerstadfjorden (ANOSIM based on square rooted prey abundance data; $p = 0.064$, species level; $p = 0.054$, grouped taxa). The taxa contributing the most to these differences were Actinopterygii (unidentified), *Pollachius virens*, *Lithodes maja* and Clupeidae (in descending order; SIMPER, species level). In Saltstraumen, Actinopterygii (unidentified), *Pollachius virens* and Clupeidae occurred more often, whereas *Lithodes maja* occurred more often in Skjerstadfjorden.

Table 3.3 Relative frequency of occurrence (O_{ef}), relative abundance by numbers (A_N), relative abundance by weight (A_W), Relative importance index (RI) and results of SIMPER (contribution to dissimilarities of taxa, %), for taxa in diet of Gadus morhua size classes in Saltstraumen, sorted by average RI. Number of of stomachs analysed in parentheses. (*= unidentified)

	Saltstraumen (12)				Saltstraumen (87)				Skerstadfjorden (17)				SIMPER	Saltstraumen (39)				Skerstadfjorden (19)				SIMPER	Saltstraumen (12)				Skerstadfjorden (7)				SIMPER
Taxa	O_{ef}	A_N	A_W	RI	O_{ef}	A_N	A_W	RI	O_{ef}	A_N	A_W	RI	Contrib (%)	O_{ef}	A_N	A_W	RI	O_{ef}	A_N	A_W	RI	Contrib (%)	O_{ef}	A_N	A_W	RI	O_{ef}	A_N	A_W	RI	Contrib (%)
Actinopterygii	58.3	36.0	58.5	34.6	59.8	8.6	38.4	16.9	70.6	9.8	57.8	27.0	12.4	69.2	23.1	56.6	32.8	52.6	2.3	43.8	20.2	15.1	100.0	82.1	94.7	77.2	57.1	31.6	39.8	37.5	39.8
Ophiuroidea	25.0	28.1	21.4	16.9	54.0	46.8	19.6	19.1	17.6	2.4	0.1	3.9	16.0	20.5	17.6	0.9	8.6	15.8	2.7	0.2	3.8	7.3	-	-	-	-	14.3	10.5	0.2	7.3	6.8
Lithodidae	-	-	-	-	1.1	0.0	0.1	0.2	5.9	2.4	16.3	4.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.3	26.3	58.4	28.9	12.4
Brachyura	16.7	6.7	10.9	7.8	49.4	7.7	19.6	12.1	11.8	0.8	0.2	2.5	9.2	20.5	12.6	18.8	11.4	5.3	0.4	12.1	3.6	6.5	8.3	1.5	4.1	3.9	-	-	-	-	-
Caridea	33.3	9.0	0.5	9.7	29.9	4.1	0.8	5.5	41.2	7.3	0.5	9.6	9.2	15.4	4.2	0.4	4.4	15.8	1.7	1.1	3.8	3.8	-	-	-	-	14.3	10.5	0.1	7.3	5.6
Polychaeta	25.0	7.9	0.6	7.6	44.8	5.3	1.7	8.2	41.2	7.3	2.1	9.9	9.1	12.8	2.9	0.1	3.5	26.3	7.9	1.4	7.3	7.0	8.3	1.5	0.0	2.8	14.3	5.3	0.0	5.7	9.7
Echinoidea	-	-	-	-	13.8	2.3	1.5	2.8	5.9	7.3	10.5	4.6	3.5	5.1	10.5	1.4	3.7	26.3	20.6	18.6	13.4	11.9	-	-	-	-	-	-	-	-	-
Bivalvia	16.7	2.2	3.3	5.0	26.4	1.9	2.7	4.9	11.8	0.8	1.2	2.7	3.7	17.9	3.4	2.1	5.2	31.6	19.4	8.8	12.2	11.6	8.3	9.0	0.0	4.8	-	-	-	-	3.4
Mysida	-	-	-	-	1.1	0.3	0.0	0.2	11.8	51.2	0.8	12.5	6.8	-	-	-	-	10.5	1.9	0.1	2.6	3.6	-	-	-	-	-	-	-	-	-
Unknown	16.7	2.2	0.5	4.4	26.4	1.3	6.5	5.4	17.6	3.7	4.6	5.1	6.0	12.8	2.5	1.1	3.6	31.6	1.3	3.6	7.5	6.8	16.7	3.0	1.0	5.8	14.3	5.3	0.5	5.8	8.6
Holothuroidea	16.7	2.2	0.4	4.4	16.1	1.2	6.7	3.8	5.9	0.4	0.2	1.3	2.9	25.6	10.1	16.9	11.6	10.5	0.6	2.7	2.8	5.5	-	-	-	-	-	-	-	-	-
Caprella sp.	16.7	2.2	0.0	4.3	12.6	9.8	0.1	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Euphausiacea	-	-	-	-	2.3	0.6	0.0	0.5	-	-	-	-	-	5.1	2.9	0.0	1.8	5.3	34.4	0.5	8.2	4.2	-	-	-	-	-	-	-	-	-
Gastropoda	8.3	2.2	3.9	3.3	16.1	1.1	0.3	2.8	23.5	1.6	5.0	5.9	3.5	10.3	2.5	0.6	3.0	15.8	0.8	0.1	3.4	2.9	8.3	1.5	0.1	2.8	-	-	-	-	
Paguroidea	-	-	-	-	9.2	0.4	0.5	1.6	23.5	3.3	0.5	5.3	3.5	10.3	2.1	0.2	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascidiacea	-	-	-	-	1.1	0.1	0.4	0.3	5.9	0.4	0.3	1.3		-	-	-	-	10.5	4.0	4.4	3.9	3.4	-	-	-	-	14.3	10.5	1.0	7.5	5.6
Amphipoda	8.3	1.1	0.0	2.1	18.4	4.2	0.1	3.6	5.9	0.4	0.0	1.2	3.9	7.7	1.3	-	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galatheoidea	-	-	-	-	14.9	1.0	0.5	2.6	-	-	-	-	2.0	2.6	0.4	0.0	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decapoda	-	-	-	-	10.3	0.5	0.3	1.8	5.9	0.4	0.0	1.2	-	5.1	0.8	-	1.3	10.5	0.4	0.1	2.2	2.8	-	-	-	-	-	-	-	-	-
Isopoda	-	-	-	-	6.9	1.4	0.0	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyperiidea	-	-	-	-	-	-	-	-	-	-	-	-	-	7.7	1.3	0.0	2.0	5.3	1.0	0.0	1.3	-	-	-	-	-	-	-	-	-	-
Crustacea*	-	-	-	-	3.4	0.2	0.0	0.6	5.9	0.4	0.0	1.2	-	-	-	-	-	5.3	0.2	0.0	1.1	-	8.3	1.5	0.0	2.7	-	-	-	-	-
Porifera	-	-	-	-	3.4	0.1	0.0	0.6	-	-	-	-	-	-	-	-	-	5.3	0.2	2.5	1.6	-	-	-	-	-	-	-	-	-	-
Balanomorpha	-	-	-	-	5.7	0.5	0.1	1.0	-	-	-	-	-	2.6	0.8	0.4	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Actiniaria	-	-	-	-	-	-	-	-	-	-	-	-	-	2.6	0.8	0.4	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chitonida	-	-	-	-	1.1	0.0	0.0	0.2	-	-	-	-	-	-	-	-	-	5.3	0.2	0.0	1.1	-	-	-	-	-	-	-	-	-	-
Nemertea	-	-	-	-	2.3	0.1	0.1	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asterias rubens	-	-	-	-	1.1	0.0	0.0	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4 Discussion

The marine protected area of Saltstraumen consists of a strong tidal current with an ecosystem of high diversity and abundance, which is remarkably different from adjacent regions. Information on the fauna is, however, solely based on observations by divers and underwater photos, not from systematic monitoring or research projects (Reiss, unpubl. data). In this area, Atlantic cod (*Gadus morhua*) is an abundant species and their diet usually reflect the prey organisms available in the ecosystem (Svåsand et al. 2000). Therefore, the diet of Atlantic cod was investigated by using a citizen science approach (i) to assess the diet composition in Saltstraumen MPA in relation to the different life stages and (ii) to investigate the small-scale differences in diet composition between Saltstraumen and Skjerstadfjorden outside Fauske.

The results showed that fish and crustaceans dominated the diet in both location in terms of weight (A_W). There was, however, a significant difference in diet composition between the two locations, across all size classes, and between the 40-69 cm and 70-99 cm size classes at the two locations. In Saltstraumen, fish was of higher importance in 20-39 cm cod than in 40-69 cm cod, and then increased in importance with cod length for >70 cm cod. Crustaceans, on the other hand were more important in 40-69 cm cod than in 20-39 cm cod, but quantities decreased with cod length in >70 cm cod. In contrast to Saltstraumen, importance of fish decreased with increasing cod size in Skjerstadfjorden, whereas importance of crustaceans increased from 40-99 cm cod to >99cm cod. Fish was also more important for 40-69 cm cod in Skjerstadfjorden than in Saltstraumen whilst the opposite was the case for >70 cm cod. The results also showed that cod length was significantly negatively correlated with number of prey species and prey abundance, and significantly positively correlated with mass of total stomach content in Saltstraumen.

4.1 General diet composition and spatial difference in diet between size classes

Across all size classes the diet of cod was found to be dominated by fish and crustaceans in Saltstraumen and Skjerstadfjorden (Table 3.2). This is consistent with findings of many studies of cod diet composition in the North-East Atlantic (e.g. Klemetsen 1982, Du Buit 1995, Jaworski et al. 2006, Magnussen 2011, Pachur et al. 2013). Crustaceans and fish taxa, however, differed between Saltstraumen and Skjerstadfjorden. Differences on large spatioal scales in the diet were found between locations in the North-east Atlantic (e.g. Klemetsen 1982, Du Buit 1995, Jaworski et al. 2006, Magnussen 2011, Pachur et al. 2013), and was also observed along the Norwegian coast (Svåsand et al. 2000).

Gobies and labrids were of high importance as prey in southern and western parts of Norway, whilst herring and capelin were important prey fish north of Vestfjorden (Svåsand et al. 2000). Furthermore, examples of small-scale spatial difference in cod diet has been found within the Ullsfjord-Sørfjord fjord system (Kanapathippillai et al. 1994) and the fjord Balsfjord (Klemetsen 1982), both in Northern Norway. The diet of <30 cm cod in Ullsfjord was dominated by fish (A_W= 60-90%), whereas polychaetes, crustaceans, fish and echinoderms (mostly brittle stars) were the dominant prey taxa in 30-60 cm cod in Sørfjord (Kanapathippillai et al. 1994). In Basfjord, however, *P. borealis* was the dominant prey at the Tennes station, capelin at Svartnes and small euphausids at Ramfjornes (Klemetsen 1982).

Clupeidae (A_W= 9%; O_{ef}= 11%) was also found in the diet of cod in Saltstraumen, which is consistent with the diet found in cod in Northern Norway. However, the most important fish species in Saltstraumen was *Pollachius virens* (23%; 12%), which is highly abundant in this area. Nevertheless, large numbers of small Clupeidae (species unidentified) migrated into Saltstraumen during the last days of sampling (own observations), and Clupeidae could therefore have a larger importance in the diet than this study indicated. In fact, a dramatic shift in cod diet towards Atlantic herring and Atlantic mackerel, as well as increased feeding rates, was observed in Cape Cod, whenever migrating schools of these species were observed in the area (Smith et al. 2007).

In Skjerstadfjorden, on the other hand, *Gadus morhua* (A_W= 16%) and *Trisopterus esmarkii* (8%) were the most important fish species, but were only found in 5% and 2% (O_{ef}) of stomachs, respectively. In contrast to Saltstraumen, no Clupeidae were found in the cod stomachs from Skjerstadfjorden, maybe because of late arrival of clupeids in the inner-fjord (private communication with local anglers). Nevertheless, cannibalism by cod is common in many locations, and frequencies increase with increasing cod length (Bogstad et al. 1994). In the

Barents Sea, Icelandic waters and in Newfoundland waters cannibalism occurred in 0-2% (O_{ef}) of stomachs, accounting for 0-9% (A_W) of prey weight in 1975-92 (Bogstad et al. 1994), while it was 80% (A_W) of prey weight in >60 cm cod in Sørfjord, Northern Norway (Kanapathippillai et al. 1994). The frequency of cannibalism, however, was found to increase with increasing abundance of juvenile cod (Bogstad et al. 1994), and there are several spawning sites of cod in Skjerstadfjorden (Dahle et al. 2014). However, the results of the fish species might be biased by the poor identification success of highly and partly digested fish (in both locations), because a high number remained unidentified due to problems with DNA extraction. Unidentified fish was found in 47% (O_{ef}) of stomachs in both locations, and accounted for 24% and 18% (A_W) of prey weight in Saltstraumen and Skjerstadfjorden, respectively.

The diet of cod shifts from small invertebrates to fish with increasing size of cod, and fish dominates the diet of older individuals (Hop et al. 1992, Kanapathippillai et al. 1994, Link et al. 2002, Smith et al. 2007). This was not entirely the case in this study. In Saltstraumen, 20-39 cm cod preyed on higher quantities (A_W) of fish than in 40-69 cm cod, fish then increased in importance with cod length >70 cm cod as expected. Crustaceans, on the other hand were more important in 40-69 than in 20-39 cm cod, but quantities decreased with cod length in >70 cm cod. A similar diet pattern was found in Sørfjord, where fish was more important in 20-30 cm cod terms of weight (A_W) than 30-60 cm cod, and crustaceans were more important in 30-50 cm cod than in 20-30 cm cod (Kanapathippillai et al. 1994). In contrast to Saltstraumen (and Skjerstadfjorden), polychaetes accounted for 40% (A_W) in 20-30 cm cod and ~20% in 30-60 cm cod in Sørdfjord.

The 25 km long Sørfjord, is separated from Ullsfjord with a 300 m wide and 8 m deep sill. The area close to the sill is characterized by strong tidal currents, resembling Saltstraumen, whilst the inner part of Sørfjord has a reservoir about 130 m deep, similar to Skjerstadfjorden. Differences in cod diet was found between the inner part and the region close to the sill (Kanapathippillai et al. 1994). Close to the sill, cod diet mainly consisted of benthic invertebrates, such as anthozoans, brittle stars and bivalves. In Saltstraumen, however, there are high densities of sessile filter feeders, but only Holothuroidea and Porifera were found in the cod diet, in 17% and 2% (O_{ef}) of stomachs, respectively. Instead, the associated mobile fauna, such as crabs (Brachyura) and particularly brittle stars (Ophiuroidea), seems to supplement fish in the diet of cod, and pelagic or bentho-pelagic invertebrates seems to be of little importance in the overall diet.

In contrast, cod diet in the inner part of Sørfjord, mainly consisted of bentho-pelagic and pelagic prey such as shrimps, herring and euphausids (Kanapathippillai et al. 1994). In comparison, mysids and euphausids were abundant in the diet of cod across all size classes in Skjerstadfjorden, but only accounted for 0.2-0.3% of prey weight (A_W). This is, however, likely to be biased compared to less easily digestible prey. Nevertheless, benthic organisms such as Brachyura, bivalves, Anomura (e.g. *Lithodes maja*) and sea urchins seem to be of higher importance than pelagic or bentho-pelagic invertebrates in Skjerstadfjorden.

In contrast to Saltstraumen, the relative weight (A_W) of fish decreased with increasing cod size in Skjerstadfjorden, whereas relative weight of crustaceans increased from 18% for 40-99 cm cod to 59% for >99cm cod. Fish was also more important for 40-69 cm cod in Skjerstadfjorden than in Saltstraumen whilst the opposite was the case for >70 cm cod. A possible reason for this is that cod show a clear preference for decapods whenever they are numerous, which can contribute to up to 80-90% (A_W) of total prey weight (Zamarro 1985, Du Buit 1995). In Skjerstadfjorden, *Lithodes maja* accounted for 58% (A_W) of total prey weight. It is occasionally chough in traps in Skjerstadfjorden (private communication with local anglers), however the abundance and distribution of *L. maja* is not known. Nevertheless, there were only seven stomachs in the 100-130 cm size class in Skjerstadfjorden, and *Lithodes maja* was only found in one of them, however in large quantities (weight and number). Another possible reason is low abundances of available prey fish, compared to Saltstraumen where *Pollachius virens* is highly abundant.

The total number of taxa found in the stomachs was highest in 40-99 cm cod at both locations. These size classes also had the highest number of stomachs analysed, and the number of taxa increased with sample size (data not shown). Moreover, sample size was more than 3 times as high in Saltstraumen as in Skjerstadfjorden, which resulted in a bias in total number of taxa, both in small and large cod, and between locations. Mean number of taxa per stomach was 4.2 in Saltstraumen and 3.0 in Skjerstadfjorden, which is high compared to Sørfjord, where mean was 2.5 taxa per stomach (Kanapathippillai et al. 1994).

4.2 Citizen science approach

From the 372 samples collected in this study, 104 (46 and 57 from Skjerstadfjorden and Saltstraumen, respectively) were sampled and handed in by the public. The remaining 256 (13 from Skjerstadfjorden and 256 from Saltstraumen) were collected by directly approaching anglers and sample their fish. The direct approach recruited more participants to the study than posters and flyers, as samples handed in by the public were primarily from people previously approached. In general, posters and collecting stations were not noticed by anglers until informed, even though the stations in Saltstraumen were marked with 5 m tall beach flags. The large number of recreational anglers made the direct approach an efficient way to collect samples in Saltstraumen.

Compared to Saltstraumen, Skjerstadfjorden had low numbers of recreational anglers, and these were mainly locals. This made the approaches selected for this study less effective. A better approach at this location might have been to recruit a given number of anglers and provide them with specific training. In this way, they would get all information needed and it would be easier to communicate the importance of the study. Low catch rates was another reason for the low sample numbers from Skjerstadfjorden (private communication with anglers).

The use of citizen science to collect samples in this study, have likely led to higher variability of the meta data, since samples were collected by many different anglers. Length measurements, catch time, position on the map, fish species and fishing gear used may therefore be inaccurate or missing. Despite these limitations, the citizen science approach enabled the study of cod diet composition in an efficient and sustainable way. At the same time the awareness among the general public for the respective research and the fjord ecosystem in general was raised.

4.3 Conclusion

The present study showed small-scale spatial difference in cod diet between the Marine Protected Area in Saltstraumen and Skjerstadfjorden, both in general and between size classes. Across all size classes the diet in Saltstraumen was dominated by fish, crabs, sea cucumbers and brittle stars, whereas fish, crabs, bivalves, *Lithodes maja* and sea urchins, dominated the diet in

Skjerstadfjorden. Mysids and Euphausids were also important in Skjerstadfjorden, whereas polychaetes were important in both locations. Cannibalism was found in both locations, but was twice as frequent in Skjerstadfjorden.

The high densities of sessile filter feeders observed in Saltstraumen, only partly contributed to the diet of cod, with only Holothuroidea and Porifera found in 17% and 2% (Oef) of the stomachs, respectively. Instead, the associated mobile fauna, such as crabs (Brachyura) and brittle stars, seems to supplement fish in the diet of cod, and pelagic or bentho-pelagic invertebrates seem to be of little importance in the diet. Especially the intermediate size class (40-69 cm) of cod in Saltstraumen fed on brittle stars, which were less important prey in Skjerstadfjorden. It remains unclear to what extent the high diversity of benthic invertebrates in Saltraumen is also reflected in the diet of cod, since the low numbers of stomach samples from Skjerstadfjorden hampered a thorough comparison of the sites. Nevertheless, my results indicate that differences in the benthos and fish fauna between Saltstraumen and Skjerstadfjorden also lead to differences in the diet of cod on relatively small spatial scales. The implications of these findings for e.g. the condition of local populations, the fitness of cod individuals, and potential effects of ecosystem changes on the food web (e.g. increase of aquaculture) should be in the focus of future investigations.

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6 Appendices

Appendix 1

Poster

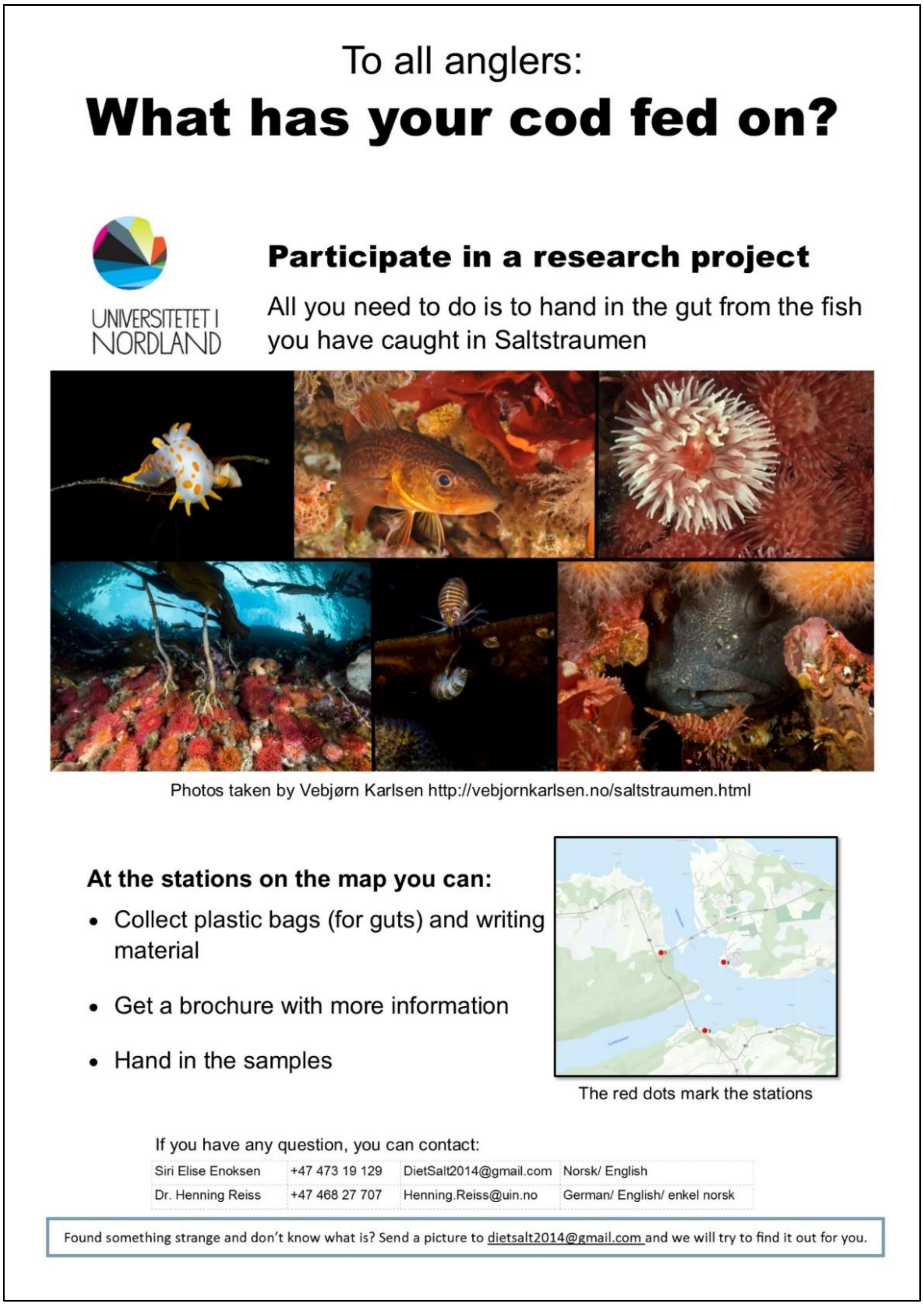


Figure 6-1 Poster used to inform the public about the citizen science project

Appendix 2

Leaflet handed out to anglers

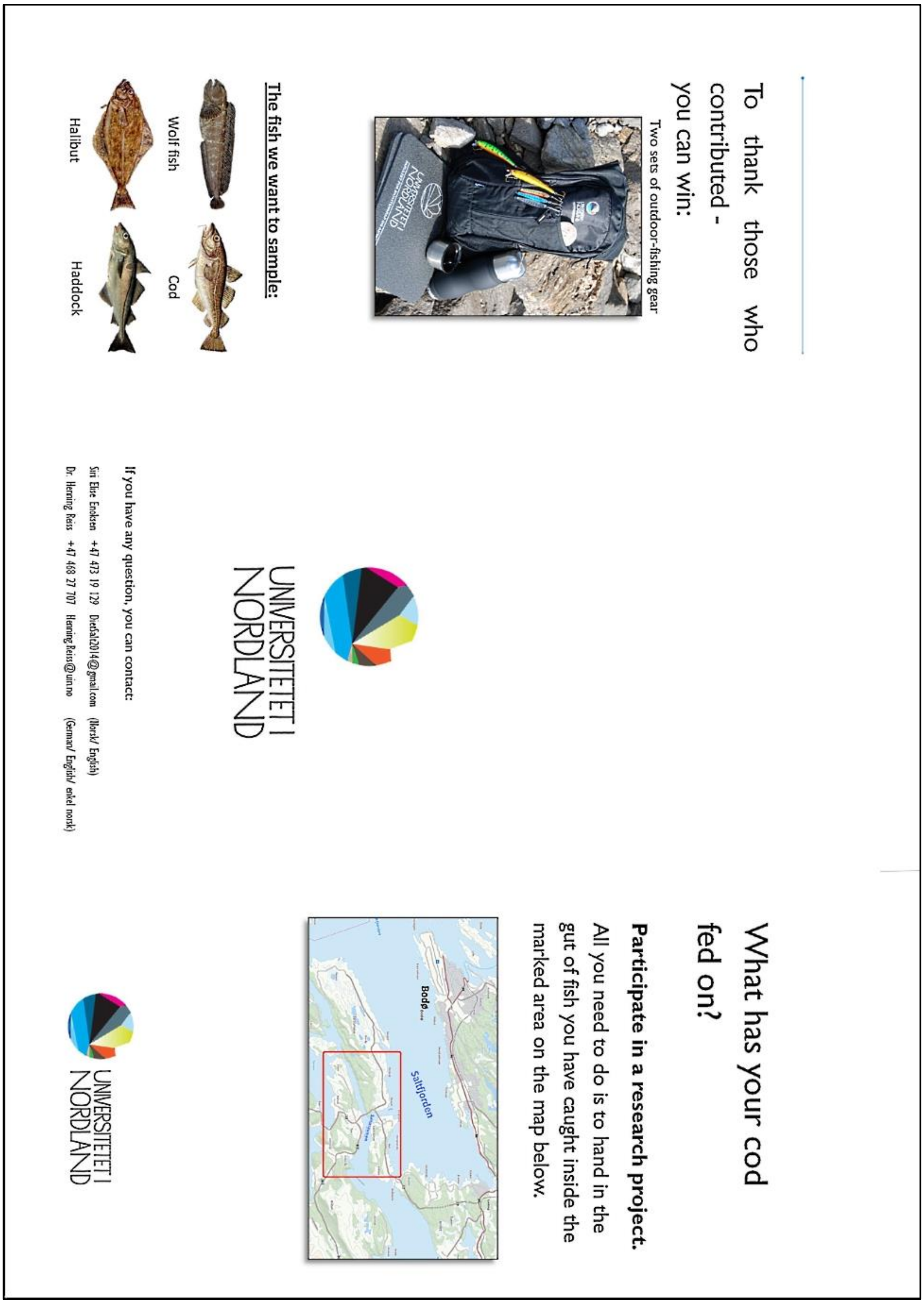


Figure 6-2 Leaflet handed out to anglers to inform the public about the citizen science project, and give instructions on how to participate, page one.

What has your cod fed on?

UNIVERSITETET I NORDLAND

All you need to do is:

- Put the guts of the fish in the provided zip-lock bags
- Fill out on the attached paper: date, time, fish species, fish length and what fishing gear you have used
- Mark on the map where the fish was caught
- Deliver the sample(s) where you got the sample bags or at one of the collecting stations on the map to the right

Zip-lock bags, measuring tape and writing material can be obtained at the collecting stations.

It is important that the samples are handed in as quickly as possible after your fishing trip. We will then freeze the samples, because digestion continues.

About the project:

This research project aims to collect guts from cod, haddock, wolf fish and halibut in Saltstraumen and in Skjerstadfjord outside Fauske.

This is done to study what these fish species feed on and is the first step in assessing the marine food web of the region and will contribute to the protection of these ecosystems.

The collection of guts will take place in July 2014.

Sustainable and efficient:

By collecting the guts of fish already caught and make use of the parts of the fish which otherwise are thrown away, we can carry out research in the most sustainable and efficient way. This requires that you are willing to help us.

Map of Saltstraumen showing the locations of the three collecting stations

We hope that as many as possible wish to participate in this project - thank you!

We will raffle off two sets of outdoor-fishing equipment among the participants as a small thank you. Just provide your name and e-mail address on the sample bags.

Found something strange and don't know what is? Send a picture to dietsalt2014@gmail.com and we will try to find it out for you.

Figure 6-3 Leaflet handed out to anglers to inform the public about the citizen science project, and give instructions on how to participate, page two.

Appendix 3

Form on sample bags

Dato / *date*:_____/_____-14 Kl / *time*:___________

Art (type fisk) / *species (type of fish)*:_______________________

Lengde (cm) / *length (cm)*:________________

Fisket med / *equiptment used*:

□ Garn / *net*

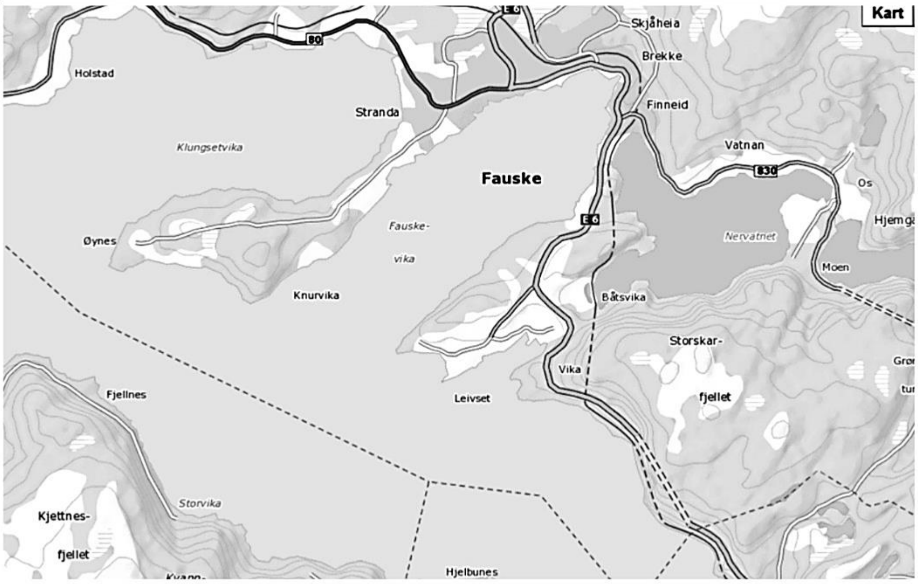
□ Jukse el. Håndsnøre / *hand line*

□ Annet (spesifiser) / *other specify)*:___________________

□ Stang / *fishing rod (*

Hvor ble fisken fanget? (Merk av på kartet under) / *where was the fish caught? (Mark with a cross on the map below)*:

NB! Innvoller fra en fisk pr pose / *gut from one fish pr bag*



If you wish to participate in the lottery please provide:

Name: E-Mail:

(all personal data will be kept confidential and won't be stored or transferred)

Figure 6-4 Form on sample bags, used for gathering meta data in the citizen science project.

Appendix 4

Results from DNA barcoding

Table 6.1 List of species from DNA barcoding after BLAST search in the databases of National Center for Biotechnology Information (NCBI) and The Barcode of Life (BOLD). (*= no hit)

		NCBI				BOLD
No.	**Species**	**Total score**	**Query cover**	**Identity**	**Accession code**	**POP (%)**
1	Ciliata mustela	1197	0.99	0.99	KJ204803	99.8
2	Pollachius virens	1201	1.00	0.99	FR751399	100
3	Gadus morhua	1173	1.00	0.99	HG514359	100
4	Trisopterus esmarkii	1205	1.00	1.00	KJ205233	100
5	Trisopterus esmarkii	1205	1.00	1.00	KJ205233	100
6	Gadus morhua	1066	1.00	1.00	KJ204880	100
7	Hippoglossoides platessoides	1197	0.99	0.99	JN312184	100
8	Pollachius virens	1203	0.99	1.00	FR751399	100
9	Pholis gunnellus	728	1.00	0.99	KJ205118	99.5
10	Pholis gunnellus	1194	0.99	0.99	KJ205110	99.8
11	Chirolophis wui	1027	1.00	0.95	KC748089	*
12	Pollachius virens	1205	1.00	1.00	FR751399	100
13	Gadus morhua	1205	1.00	1.00	HG514359	100
14	Pholis gunnellus	1184	1.00	0.99	KJ205115	99.5
15	Pholis gunnellus	1199	1.00	0.99	KJ205115	99.8
16	Ammodytes marinus	1199	1.00	0.99	KJ204678	99.8
17	Pollachius virens	1205	1.00	1.00	FR751399	100
18	Gadus morhua	1199	1.00	0.99	HG514359	99.8
19	Triglops murrayi	1197	1.00	0.99	KC015975	100[a]
20	Pollachius virens	1205	1.00	1.00	KC015814	100
21	Chirolophis wui	1022	1.00	0.95	KC748089	*
22	Lumpenus lampretaeformis	1199	1.00	0.99	KJ204997	99.8
23	Pollachius virens	1205	1.00	1.00	FR751399	100
24	Ammodytes marinus	1201	1.00	0.99	KJ204680	100
25	Ammodytes marinus	1205	1.00	1.00	KJ204680	100[a]
26	Gadus morhua	1205	1.00	1.00	HG514359	100[a]
27	Pholis gunnellus	1182	1.00	0.99	KJ205115	99.4
28	Pollachius virens	1205	1.00	1.00	FR751399	100
29	Pholis gunnellus	1205	1.00	1.00	KJ205110	100
30	Pollachius virens	1205	1.00	1.00	FR751399	100
31	Pollachius virens	1205	1.00	1.00	FR751399	100
32	Pollachius virens	1205	1.00	1.00	FR751399	100
33	Chirolophis wui	1022	1.00	0.95	KC748089	*
34	Pollachius virens	1199	1.00	0.99	KC015814	100
35	Pollachius virens	1205	1.00	1.00	FR751399	100
36	Pollachius virens	1201	1.00	0.99	FR751399	100
37	Pollachius virens	1205	1.00	1.00	KC015814	100
38	Pollachius virens	1205	1.00	1.00	FR751399	100
39	Pollachius virens	1205	1.00	1.00	FR751399	100
40	Pollachius virens	1205	1.00	1.00	FR751399	100
41	Pollachius virens	1205	1.00	1.00	FR751399	100
42	Pholis gunnellus	1199	1.00	0.99	KJ205110	100
43	Pollachius virens	1205	1.00	1.00	FR751399	100
44	Pollachius virens	1205	1.00	1.00	FR751399	100
45	Pollachius virens	1201	1.00	0.99	FR751399	100
46	Pholis gunnellus	1197	1.00	0.99	KJ205110	100
47	Pholis gunnellus	1201	1.00	0.99	KJ205110	100
48	Pollachius virens	1184	1.00	0.99	FR751399	99.8

Appendix 5

Result from cluster analysis

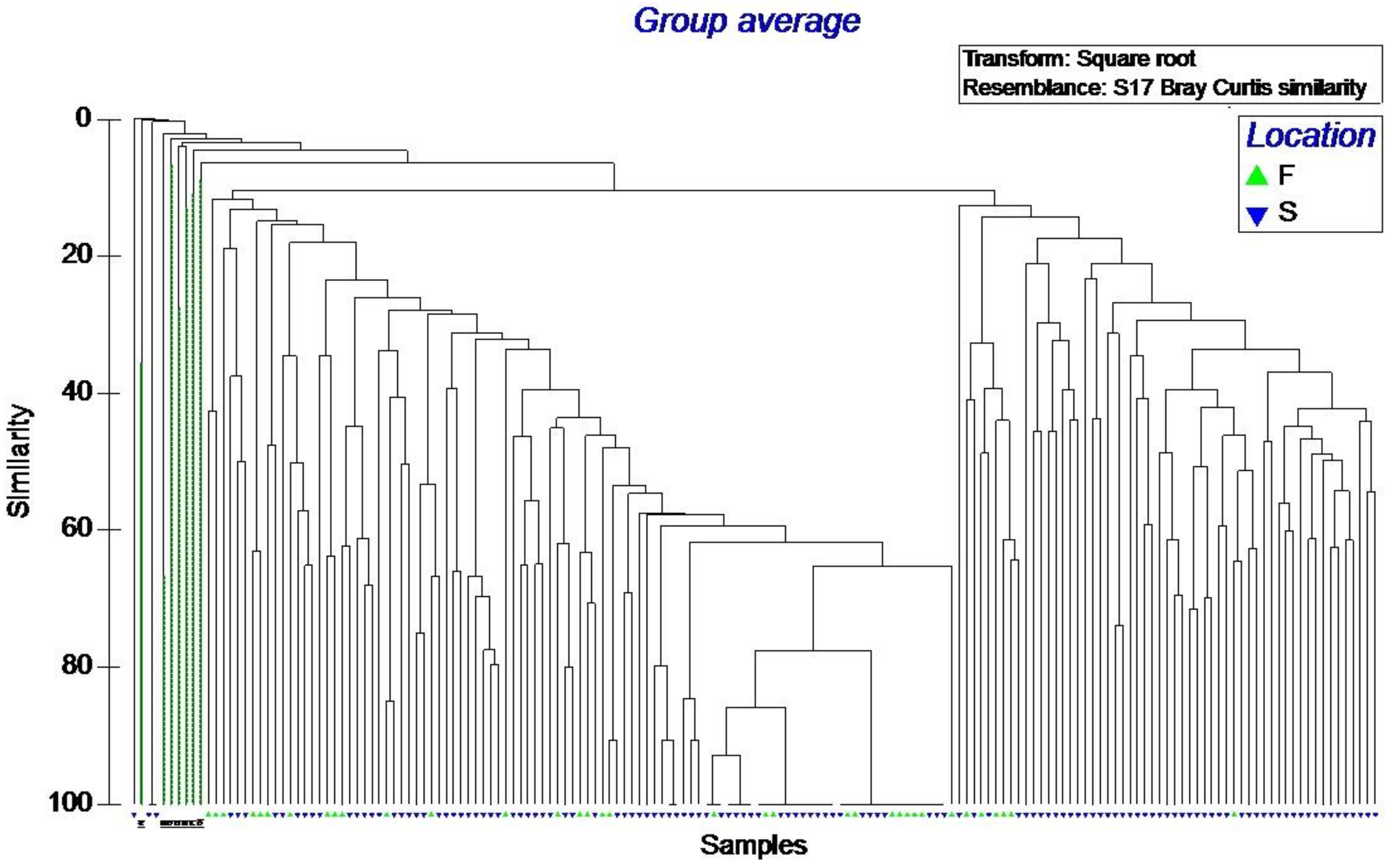


Figure 6-5 Cluster diagram of square root transformed data on species level. (F= Skjerstadfjorden, S= Saltstraumen)