Master thesis:



Comparison of Fish Communities at Two Danish Coastal Boulder reefs studied through Snorkel Visual Census, Stomach Analyse and Stable Isotopes



By Simone Lorange Jensen – 201206193 Department of Bioscience, Aarhus University

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## Preface

This study is a 60 ECTS master thesis at Aarhus university. This study was conducted from February to November 2017. The aim was to describe two fish communities and their feeding habits. Fieldwork was carried out from May to September at a newly established boulder reef at Aarhus Ø and an older boulder reef located at Aarhus east harbor. The methods used were snorkel visual census (SVC), stomach analysis and stable isotope analyses. Supervision and support during the thesis work was provided by Tomas Cedhagen and Peter Grønkjær, Department of Bioscience – Aquatic biology, Aarhus University.

Simone Lorange

Simone Lorange Jensen, Stud.nr. 201206193 Bioscience, Aarhus University, Denmark Submission date: 20.11.2017

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## Abstract

Boulder reefs are protected to preserve their rich diversity that gives them high ecological quality. In the 1900's a large part of the marine boulder reefs in Denmark were extracted because of the stone fishing industry. This caused the majority of the boulder reefs to disappear from the marine areas, especially in the coastal area.

The aim of this study was to compare the richness of two fish communities on two artificial boulder reefs in Aarhus Bay. Snorkel visual census (SVC) was used to study fish assemblage on the two reefs from May-September. The importance of boulder reefs for the feeding of different fish species was studied. It was analyzed by gut examinations and stable isotope analysis. The fish samples for these analyses were collected at the two boulder reefs from May-August.

The results of the SVC revealed no differences in assemblage of fish between the two stations. The reefs were dominated by the two-spotted goby (*Gobisculus flavescens*) and two labrids species, goldsinny wrasse (*Ctenolabrus rupestris*) and corkwinge wrasse (*Symphodus melops*). Fluctuations in temperatures or salinity did not affect the abundance of the three dominating species, nor of the Atlantic cod (*Gadus morhua*). Diurnal differences were only found for the Atlantie cod (*Gadus morhua*). Based on the stomach and stable isotope analysis of nitrogen and carbon, the majority of the food species were benthic. The major prey item appears to be crustaceans for both stations. Only one species, goldsinny wrasse (*Ctenolabrus rupestris*), ate more bivalves than crustaceans. The food species were often found on vegetation or hard bottom. A few species ((Broadnosed pipefish (*Syngnathus typhle*), Fifteenspine stickleback (*Spinachia spinachia*) and the two-spotted goby (*Gobiusculus flavescens*)) ate a pelagic diet. This study supports that fish species utilize the boulder reefs as a feeding ground but also showed that they are not limited to this habitat. This study therefore supports that boulder reefs are important to restore. Further investigations are needed to fully understand the ecology of fish species on boulder reefs.

## **Dansk Resumé**

Danske stenrev er kendt for deres høje diversitet af flora og fauna, som medfører at revene har høj økologisk kvalitet. Men tidligere blev danske stenrev genstand for omfattende stenfiskeri op gennem 1900-tallet. Dette betød at et stort antal stenrev er forsvundet fra de marine områder, især de kystnære områder blev berørt af dette.

Studiets formål var at sammenligne fiskesamfundene på to kunstige stenrev. De to udvalgte stenrev er henholdsvis et nyere anlagt stenrev ved Aarhus Ø fra 2012 og et ældre stenrev fra 1993, representeret af molerne ved Aarhus øst havn.

Sammenligningen af fiskesamfundene på de to rev er baseret på en undersøgelse af revenes artsrigdom. Observationer af artsrigdommen for de to fiskesamfund blev udført fra maj til september med snorkling (SVC – snorkeling visual census) som metode. Stenrevenes betydning som fødegrundlag for fiskearter blev analyseret med maveanalyser og stabile isotops analyser. Fra maj til August blev ruser, håndholdt net og harpun anvendt til fangstmetoder. De indsamlede fisk fik udtaget maver til bestemmelse af maveindhold samt muskelprøver til stabile isotops analyser af nitrogen og carbon.

Resultaterne fra SVC viste ingen signifikant forskel på de to fiskesamfundene på de to undersøgte stenrev. Begge stenrev var domineret af den toplettet kutling (*Gobisculus flavescens*) samt af labrid arterne, havkarusse (*Ctenolabrus rupestris*) og savgylt (*Symphodus melops*). Undersøgelsen viste ingen forskel i abundans af fisk for disse tre arter, eller for den atlantiske torsk (*Gadus morhua*) i forhold til udsving i temperatur og salinitet. Der blev kun fundet døgn forskelle for Atlantisk torsk (*Gadus morhua*), som var observeret signifikant mere hyppigt om natten. Maveanalysen og den stabile isotops analyse af nitrogen og carbon viste at størstedelen af byttet var bentisk. Alle fiskearter undersøgt havde et større indtag af muslinger end krebsdyr. I denne undersøgelse udgjorde bytte som lever på hård bund og makroalger den største del af fødeorganismerne. Den stabile isotops analyse viste at den trofiske struktur havde den atlantiske torsk (*Gadus morhua*) som toppredator, mens de to nålefisk arter (alm. tangnål (*S. Typhle*) og stor næbsnog (*N. Ophidion*)) og den toplettet kutling (*G. flavescens*) lå i bunden af fædekæden. Denne undersøgelse viste at de fleste fiskearter udnytter stenrevene til at finde føde, men deres fødesøgning er ikke begrænset til revene. Dette studie undersøtter derfor hvorfor stenrevene er vigtige at restaurerer og bevare.

## **1. Introduction**

The biodiversity in Danish waters has decreased over the last decades (Ejrnæs et al. 2011). The ocean is likely the ecosystem about we know the least and proper estimation is needed to assess the quality and condition of the biodiversity.

## 1.1. Ecological quality of boulder reefs

Boulder reefs are among the eight marine habitats that are protected by the habitats directive, under the water framework from 2000, and Denmark is obligated to preserve these reefs due to the scarcity of this kind of marine habitat in the Danish waters (Miljøstyrelsen 2001). Because in the early 1900's and peaking in the mid-1900's a large part of the marine boulder reefs in Denmark were extracted and the rocks were used for construction work. An estimated 34 km<sup>2</sup> of boulders were removed, leaving only about 1200 km<sup>2</sup> of boulder reefs left in the Danish waters (Dahl et al. 2003; Støttrup 2013). Extraction of boulders was banned by law in Denmark in 2010 (Ministry of Environment of Denmark 2009). Today boulder reefs are protected to preserve their rich diversity that gives them high ecological quality (Skov- og Naturstyrelsen 2000). The rich diversity was supported by a study in southern Kattegat which recorded 67 algae species and 19.000 animals representing 163 species on 4 m<sup>2</sup> boulder reef (Dahl et al. 2005).

Boulder reefs are found at different depths and consist of different kinds of rocks, gravel and sand. The fine sediment between the boulders has its own invertebrate- and fish faunas that can hide in the sand (Dahl et al. 2003). The cavities between the boulders create important hiding places and an environment for spawning and nursery.

Different types of macroalgae also create complex habitats and a lot of hiding places for fish (Støttrup 2013). Macroalgae constitute the majority of complex structures on a temperate boulder reef and density and species composition varies with depth and season. This is believed to be of great importance for the different fish species. This relation was supported by Levin and Hay (2002) who found that the fish densities varied with algae biomass and showed positive associations. For example, a Norwegian study showed the importance of kelp forests for the survival of fish. Removal of the kelp forest caused the juvenile cod (*Gadus morhua*) population to drop with more than 90% compared to an area where the kelp forest was intact (Lorentsen et al. 2010). This also supports the idea that this habitat has a nursery function and protects juveniles from predators. Another study on a temperate boulder reef in New Zealand (Perez-Matus and Shima 2010) showed that the occurrence and diversity of macroalgae contained the highest diversity of fish and the most abundant fish species

were wrasses. They found that juveniles preferred the vegetation of brown algae species while the adults mostly occurred outside the vegetation.

The complex habitats also have importance for juvenile cod (*Gadus morhua*). It is carnivorous and must actively search for prey. This forces them to choose between a safe shelter but nothing to eat - or to expose themselves to predators in search of prey (Støttrup 2013). Gotceitas et al. (1995) used large cod, which act as predators, studying the juveniles' behavior. The threat caused the juvenile cod to change behavior and seek for shelter in the vegetation cover of macroalgae when no predators were around, the juvenile cod preferred small rocks as hiding places whereas patches of kelp were the least preferred ones. The costs of hiding instead of seeking for food was a lower growth rate but a higher survival rate among the cod on boulder reefs (Garcia-Charton and Ruzafa 1998). The fish fauna on boulder reefs can, if they are forced to, choose between higher survival rate or a faster growth.

## **1.2. Biodiversity on Danish boulder reefs**

In shallow waters along the coast, the boulder reefs are exposed to plenty of light and to heavy waves. In such waters, boulder reefs are dominated by red, green and brown algae. If the exposures to waves are fierce only brown algae will grow on the reef. But if the wave activity is low, the boulder reef will have a rich diversity of algae species (Støttrup 2013). A shallow reef will besides sponges be colonized by algae, barnacles or mussels, sea anemones and polyps (Petraitis and Methratta 2006; Støttrup 2013). Garcia-Charton and Ruzafa (1998) found a significant positive relationship between the habitat complexity and the number of species and the mean of the total abundance of fish. This is supported by another study, which found that structural complexity and vertical relief were key factors for the functioning of an artificial as well as a natural reef and so, supports the diversity of fish (Granneman and Steele 2015). The knowledge of fish fauna on boulder reefs is often based on observations made by divers. There are no systematic studies of fish species on boulder reefs to be found. This might be because it's challenging to collect accurate data on the number of fish due to the many hiding places on a boulder reef. Additionally, some species shows different activity during the day.

A couple of projects in Denmark aim to restore lost habitats such as boulder reefs. The first Danish restoration project was the blue-reef project financed by EU in 2007. This project reestablished a boulder reef in shallow waters close to Læsø. The aim was to recreate a hollow forming reef and protect it from erosion. The stone fishing industry and trawl fishing has affected the waters around Læsø until the 1980's so that only a few rocks remained in the seabed. In 2012 the project found, as a result of the restoration, evident changes in the fish communities and that the reef functioned as a nursing area for cod and attracted fish from the neighboring areas. A fish community of 30 to 34 species was found. It was dominated by species from the wrasse family (labridae) such as goldsinny wrasse (*Ctenolabrus rupestris*). Wrasses increased most in numbers after the restoration. A new study has documented the effect of the restoration on the behavior of atlantic cod (*Gadus morhua*) also in the waters near Læsø. The atlantic cod (*Gadus morhua*) were tagged and released before and after the restoration. Ninetyfour percent of the tagged cod stayed in the area, compared to just 53% before the restoration. It was concluded that marine reefs function as a favorable cod habitat and should be used as a management tool to improve habitats for temperate fish species (Kristensen et al. 2017).

A study of the flora and fauna of two shallow rocky areas in Denmark were carried out at Samsø Bælt in 2005 by Dahl et al. (2005). They found a very high diversity of species as well as a high primary and secondary production. Another study in Denmark focused on four relatively deep reefs in Kattegat and a corresponding reef in Sweden. The vegetation cover decreased with depth and up to 39 faunal species were found on the boulder reefs (Lundsteen et al. 2008).

The significance of the size of an area on the fish community has only been studied in marine protected areas. Moland et al. (2013) showed an increase in the population density of the Atlantic cod population in a marine protected area of 1 km<sup>2</sup> compared with control areas.

Even though the restoration projects recreate lost habitats, there are still threats to consider for the biodiversity in our waters. These threats are believed to be eutrophication, unsustainable fishing, native species and climate change (Ejrnæs et al. 2011).

## **1.3. Importance for fish communities**

### **1.3.1.** Attraction or production

reefs are known to have high densities and consequently high catch rates of fish. This leads to the question of whether high densities of fish at an artificial reef are the result of habitat limitation or the result of behavioral preferences. This was investigated by Bohnsack (1989). He discussed the production hypothesis related to the underlying mechanisms of how the artificial reef provide a critical habitat which increases the abundance and biomass of fish. The reef also provides shelter from predation and provides a recruitment habitat for settling individuals. These reasons make the artificial reef a highly productive environment. The environments carrying capacity increases the production of fish, according to the production hypothesis (Fig. 1).



Figure 1. Predicted effects of the attraction and production hypotheses on catch. Increased catch is a function of the amount of deployed reef material (solid line). The attraction hypothesis (dotted line) predicts increase in catch followed by stock depletion and decline to levels below ambient catch without artificial reefs (dashed line). (Bohnsack 1989).

Another hypothesis is the attraction hypothesis where artificial reefs attract fish as the result of behavioral preferences – but do not significantly increase total fish biomass. The production hypothesis predicts a linear relationship between biomass production and catch (Fig. 1). On the contrary, the attraction hypothesis predicts that an initial increase in catch would be followed by a decline to a level without artificial reefs and will only increase again if migration is included (Fig. 1) (Bohnsack 1989). Bohnsack concluded that attraction and production works in unison, allowing boulder reef to increase fish productivity in two ways. This is by increasing the food resources or by creating hiding places for the juveniles and therefore decrease the mortality. Another way to look at production is to estimate the bottom-up energy on a boulder reef and identify how much of the production from the boulder reef that ends up as fish biomass.

## 1.3.2. Boulder reefs as a feeding ground

Flora and fauna on boulder reefs are important as a feeding ground for the fish community (Choat and Ayling 1987).

Macroalgae are also important for the occurrence and density of macro fauna. In a study from Norway they found up to 238 species of mobile benthos on one leaf of a *Laminaria*, such as snails and crustaceans, all of which are important prey for fish (Norderhaug et al. 2005; Christie et al. 2009). This indicates that boulder reefs are important as a feeding grounds. A study of an artificial reef conducted by Granneman and Steele (2015) supported this. They found that both the density and the biomass of fish were positively correlated with the density of invertebrates on the reefs.

Studies of feeding by fish species on Danish boulder reefs have been made in the blue-reef project at Læsø, where the stomach content of atæantic cod (*Gadus morhua*), seith (*Pollachius virens*) and goldsinny wrasse (*Ctenolabrus rupestris*) was analyzed (Stenberg et al. 2012). Crustaceans dominated in the cod stomachs and a variety of other fish species. The seith (*P. virens*) also ate a variety of fish species and goldsinny wrasse (*C. rupestris*) preyed on polychaetes. These results were from four years after the restoration.

A different and older study of feeding ecology in Danish waters was done by Blegvad (1916). He studied stomach content of fish from the inner Danish waters. He found that approximately half of the stomachs of cod (*Gadus morhua*) contained fish and benthos from boulder reefs. Large cod also took echinoderms (50%), dab (*Limanda limanda*) and whiting (*Merlangius merlangus*). The plaice (*Pleuronectes platessa*) was found in the fall and spring and its stomach contained juveniles of different mussels.

One study found that cod (*Gadus morhua*) temporarily focuses its feeding on the most abundant prey and cod is adapted to feed mainly near the bottom but can also catch prey epibenthic and endobenthic. It captures prey mainly by suction but is also adapted for seizing and biting (Mattson 1990).

A study at the Swedish west coast during the summer compared hard- and soft bottoms as feeding grounds (Stål et al. 2007). They tested if the content of a fish stomach reflected the availability of prey in different habitats such as soft and rocky bottoms. They also recorded the vegetation cover and benthos. On the soft bottoms the sea-grass (Zostera marina) was the dominant vegetation while on the rocky bottom kelp species such as Laminaria spp. dominated. Of the benthos snails (Gastropoda) and crustaceans (Amphipoda) dominated and a total of 17.800 benthos/m<sup>2</sup> were found on the hard-bottom in shallow waters. This decreased with depth but had the highest value compared to soft bottom. It indicated that concentrations of prey were much higher on hard than on soft bottoms. The dominant fish species was goldsinny wrasse (Ctenolabrus rupestris) (70% of all the fish sampled) on hard bottom in shallow waters. The biomass of prey was significantly higher on the hard than on the soft bottoms. They found the hard bottom to be an important hunting ground for fish such as goldsinny wrasse (Ctenolabrus rupestris), cod (Gadus morhua) and sea trout (Salmo trutta trutta) but also for plaice (Pleuronectes platessa) and Atlantic mackerel (Scomber scombrus). Stål et al. (2007) concluded that there was a larger fish biomass and a higher potential of food source on the hard bottom compared with the soft bottom in shallow waters (percent numbers from Støttrup et al. 2013).

Studies from around the world can provide a picture of what we can expect to find on the Danish boulder reefs. Some studies have analyzed the trophic levels of the fish communities on temperate reefs on the southerin hemisphere. A study from New Zealand found that on a temperate boulder reef the herbivorous fish represented 11% of the fish community, while 82% were carnivores and only 7% were omnivores (Jones 1988). This is believed to be similar to other temperate reef communities (Jones 1988). A study of the trophic structure on two shallow boulder reefs was made in the Azores in the Atlantic Ocean. The majority, 40% of the fish observed were carnivores. The second largest group was invertebrate feeders that accounted for 26% of the species, followed by omnivores with 21%. They also found that herbivores only accounted for 7% and planktivores for 5% (Bertoncini et al. 2010). We don't yet know if this is the case at boulder reefs in Denmark.

## 1.4. Study methods

## 1.4.1. Visual census method

The snorkel visual census (SVC) or Underwater visual census (UVC) has been widely used in the tropical and subtropical regions as a simple technique to measure environmental changes and estimate populations (Bertoncini et al. 2010; Dearden et al. 2010; Beck et al. 2014).

The shallow water fish communities are usually observed by amateur fisherman but conventional surveys, such as trawl and net fishing, are also used. More effective registration methods such as SVC is now recommended (Pedersen and Eskelund 2012; Kristensen et al. 2014).

In Denmark Pedersen and Eskelund (2012) have assessed snorkeling as a method to document fish species in coastal areas. They recommend SVC as a suitable method for biodiversity analyses.

Visual census techniques are not widespread in the northern temperate waters, but it has been applied in some recent studies in Sweden and in a few studies in Denmark (Pihl and Wennhage 2002; Pedersen and Eskelund 2012; Holm-Hansen 2015).

#### 1.4.2. Feeding analysis

In this study the following two methods will be used for the feeding analyses. The stomach content analysis gives a picture of the animals feeding during a short time span prior to capture (Hansen et al. 2012). Stable isotope analysis provides a long term (weeks to months) insight into the feeding ecology (DeNiro and Epstein 1978; DeNirot and Epstein 1981; Hansen et al. 2012). The disadvantage of isotope analysis is that it does not give precise data on the species ingested, hence a combination of stomach content and stable isotope analyses may aid to give a full picture of feeding ecology.

## 1.4.3. Gut examination

Study of diet based upon analysis of stomach content is an established practice in order to understand fish ecology. However, migration, seasonality and prey-availability may change over time. The method is simple and gives an insight into the current diet. Gut examination was used to study the food composition and consequently understand how the fish community utilizes its habitat. By using this method, it is possible to identify and record their types of food species, size, distribution and weight. A concern in stomach analysis is the different digestion rates among different prey taxa and the degree of digestion that can make it impossible to determine taxa. The digestion rate could be reduced by a time minimum of gillnet deployment (Stål et al. 2007). They recommended deployment of gillnet for five hours while others recommend deployment for only two hours (Stenberg et al. 2012). Digestion rate can be reduced by immediately placing fish on ice to prevent stomach content decomposition. Even with these precautions digestion can still influence the result because of the uncertainty as to when the fish actually has eaten its prey. Small food species are difficult to detect due to fast digestion. Crustaceans and insect larvae with chitinous exoskeleton remain in the stomach for a longer period of time (Wooton 1999). Even though all methods are used, there is no guarantee of a fully representative catch rate. Crow (1981) recommended 100 stomachs chosen randomly for a diet analysis while Gibson and Ezzi (1987) regarded 20-30 stomachs as sufficient.

#### 1.4.4. Stable isotopes

The stable isotopes of nitrogen and carbon provide a tool to evaluate the trophic structure and dynamics of ecological communities, but also provide information about resource and habitat use (Newsome et al. 2007; Fry 2009).

Isotopes are atoms with the same number of protons (positive charge) and electrons (negative charge), but they are different due to number of neutrons. The stable isotope <sup>13</sup>C consist of six protons and seven neutrons in the nucleus while the lighter stable isotope <sup>12</sup>C consist of six protons and six neutrons. Stable isotopes are energetically stable and persist in the same form and do not decay unlike radioactive isotopes.

The isotope analysis is a comparison between ratios of carbon and nitrogen, <sup>12</sup>C and <sup>14</sup>N and the heavier isotopes <sup>13</sup>C and <sup>15</sup>N. The content of an isotope in a sample is measured as parts per thousand (‰) and the notation for content of different isotopes is called delta ( $\delta$ ). The stable isotope  $\delta$  value is calculated using formula 1.1 (Peterson and Fry 1987).

$$\delta^{H}X(\%_{0}) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] * 1000 \qquad (1.1)$$

<sup>H</sup>X is either <sup>13</sup>C or <sup>15</sup>N and R is the ratio of <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N. R<sub>sample</sub> is the isotopic value of the ratio in the sample and R<sub>standard</sub> is the isotopic value of a known constant such as the <sup>15</sup>N/<sup>14</sup>N of atmospheric nitrogen or PDB for <sup>13</sup>C/<sup>12</sup>C.

The tissue of an organism will contain an isotopic composition of many materials and contain a "label" of the process that created it. It will be imprinted as distinct carbon and nitrogen signatures. Because of this imprinting of the signatures into the tissues, the isotopes can be used to quantify the consumed resources. The different turnover rates in the tissues must be taken into account. The turnover rate equals the time it takes to replace old tissue with new one. It is estimated by measuring the change in isotopic ratio from the ratio of the old diet to the ratio that reflects the new diet. It can be expressed as half time, the time it takes to reach half way to equilibrium with the new diet. A tissue with a fast turnover rate that can show short-term changes of diet is blood plasma (Fry 2009). The turnover rate of the tissue and the rate of the isotope is correlated with body mass. Large consumers such as long lived adult fish have muscle tissue turnover rates from months to years. As a result their isotopic signature is representative for their diet over long periods of time (Post 2002). Muscle tissue will therefore be the focus of this study.

Heavier isotopes of a given element increase in abundance compared with lighter isotopes through the process of isotope fractionation. Consumers do not integrate the heavier and light isotopes in the same amount. The heavier isotopes are accumulated because the lighter ones are more easily released. The isotopes <sup>13</sup>C and <sup>15</sup>N therefore increase throughout the trophic levels. The enrichment of isotopes between prey and predator is called trophic enrichment. The enrichment is 3-4 ‰ for  $\delta^{15}$ N (Post 2002; Sweeting et al. 2007). The heavier isotope of carbon changes only a little as it moves through the food web (+1‰) which is why it maybe can be used to evaluate the source of the consumer diet (DeNiro and Epstein 1978; Peterson and Fry 1987). Carbon can reveal if the food source is pelagic or benthic. Phytoplankton organisms are the primary pelagic carbon source and the base of the benthic food web is enriched by <sup>13</sup>C (less negative) compared to the base of the pelagic food web (Post 2002; Newsome et al. 2007).

Stable isotopic estimates of trophic position and food webs are believed to be a strong tool used to track energy and mass flow through ecological communities. However, it is recommended that stable isotopes are used with other methods such as direct diet analyses and gut examinations to give complete insight (Post 2002). Stable isotope analysis is also particular useful when limitations (e. g. empty stomachs) are present.

## 1.5. Aim

The aim of this study is to compare and describe the fish communities on two artificial boulder reefs, a newly established reef and an older reef. The new artificial reef is located at Aarhus Ø and the breakwater at the eastern part of Aarhus harbor represent the older boulder reef. This study will examine the two fish communities by comparing the richness of the species and their feeding habits at the two boulder reefs. This study will analyze how the reefs influence the feeding of different fish species by the use of gut examination and stable isotope analysis. These methods make it possible to distinguish between fish species utilization of the boulder reef and the surrounding area.

## 2. Method and Sampling

## 2.1. Locality Description

#### 2.1.1. Aarhus Bay

This project is located in Aarhus Bay. It is placed on the east coast of jutland, facing the Kattegat Sea. Brackish water flows from the Baltic sea and up through the inner Danish waters. Aarhus Bay is located at the outer part of this estuary and receives saltier water coming in from the North Sea. Aarhus Bay has a total area of 315 km<sup>2</sup> with depths around 12-15 meters in most parts of the bay. The bottom declines to 10 meters of depth 0.5-1 km from the shore. The deepest area is at Helgenæs with depths of 20-50 meters. The tidal range is limited to an interval of 20-50 cm every 12 hours (Nilsen et al. 1993; Christiansen et al. 1994). However, water level fluctuations are primarily determined by air pressure and wind direction.

## 2.1.2. Aarhus Ø

The artificial boulder reef is located at the north side of the newly constructed district called Aarhus Ø. The reef is a part of the harbor mooring and faces north. It was established in 2012 and expanded in 2013 by establishing islands made of 1100 kg rocks placed close to the shore. The top of the reef has a water depth of a meter, and expands ten meters out from the



Figure 2. Representative habitat, boulders covered with macroalgae at 1 m depth at Aarhus Ø in June 2017.

shoreline until a depth of 5-6 meters. The flora on the boulder reef consist of *Fucus* spp. surrounded by *Polysiphonia* spp. and *Ulva* (Tab. 1 + Fig. 2). In May the invasive brown alga *S. muticum* was small, but dominated from July to August, as it grew to 1-1.5 meters height (Tab. 1). Some *Laminaria* species were widely distributed and became common on the edge of the boulder reef.

The boulder reef restoration was conducted by the municipality of Aarhus and the Danish Society for the Conservation of Nature. The rocks are about half a cubic meter in size and were originally a part of some old boulder reefs in Aarhus bay but ended up being used for coastal protection and pier building.

## 2.1.3. Aarhus Harbor

The breakwater on the eastern part of the harbor of Aarhus represents an older boulder reef. The



Figure 3. Representative habitat, boulders covered with macroalgae at 1.5 m depth, in Aarhus Harbor in May 2017.

front of the breakwater faces southeast. The average depth in the area is about 10 meters, but the investigated area was just 2-4 meters deep. The marine flora was different from Aarhus Ø because here the rocks were covered with *Polysiphonia* spp., *Ceramium virgatum*, *Chondrus crispus*, spots with *Ulva*, *Cladophora* spp. A few *Laminaria* spp.

were spotted along with *Fucus* (Tab. 1 + Fig. 3). The cover stones of the breakwater are from a

quarry in Sweden and about a cubic meter in size. The first part of the breakwater is from 1993 and the rest from 1999. The reef is exposed to much wave action from ships.



Figure 4. Black arrows are marking the location of the two boulder reefs at Aarhus Ø and Aarhus east harbor.

Aarhus Harbor	Aarhus Ø
Rhodophyceae	Rhodophyceae
Ceramium virgatum	Ceramium virgatum
Chondrus crispus	Chondrus crispus
Furcellaria lumbricalis	Furcellaria lumbricalis
Phycodrys rubens	Phycodrys rubens
Polysiphonia elongata	Polysiphonia elongata
Polysiphonia fucoides	Polysiphonia fucoides
Rhodomela confervoides	Rhodomela confervoides
Dhaaanhaaaaa	DI I
Рпаеорпусеае	Pnaeopnyceae
Fucus serratus	Fucus serratus
Fucus serratus Laminaria sp.	Fucus serratus Laminaria sp.
Fucus serratus Laminaria sp. Sphacelaria cirrosa	Fucus serratus Laminaria sp. Fucus vesiculosus
Fucus serratus Laminaria sp. Sphacelaria cirrosa	Fucus serratus Laminaria sp. Fucus vesiculosus Sargassum muticum
Fucus serratus Laminaria sp. Sphacelaria cirrosa	Fucus serratus Laminaria sp. Fucus vesiculosus Sargassum muticum Sphacelaria cirrosa
Fucus serratus Laminaria sp. Sphacelaria cirrosa Chlorophyceae	Fucus serratus Laminaria sp. Fucus vesiculosus Sargassum muticum Sphacelaria cirrosa Chlorophyceae
Fucus serratus Laminaria sp. Sphacelaria cirrosa Chlorophyceae Cladophora sp.	Fucus serratus Laminaria sp. Fucus vesiculosus Sargassum muticum Sphacelaria cirrosa Chlorophyceae Cladophora sp.

 Table 1. Algae found in samples, observed during dives and recognized from pictures. For Auctor see Appendix 1.3.

## 2.2. Sampling

## 2.2.1. Snorkeling visual census

Between May and September 2017 a total of 29 snorkeling efforts were carried out, nineteen diurnals and ten nocturnal. All diurnal dives were carried out between 10-11.00 am and night dives were performed after sunset. At each location, a route of 200 meters along the coast was marked. An observer would swim along the route for approximately 30 minutes. Recorded species were counted and noted on a writing plate during the dive. Shoals of fish were estimated by visual subsampling.

Dive efforts were carried out unequally across the duration of the study. Two diurnal dives were carried out at each station in May, June and July. Only one diurnal was carried out in August and September, due to bad weather conditions and lack of time. During all months one nocturnal dive was carried out at each station. A 1500 lumen flashlight was used at the nocturnal dives. Objects along the shore were used for navigation while swimming. The observer swam close to shore in order to observe the fish that were living on the boulder reef for all dives. At Aarhus Ø the diver swam one meter above the reef, because the boulder reef consists of one big plateau. At Aarhus east harbor the depth varied and the diver would have to swim closer to the shore.

Before the field study a list of fish recorded near Aarhus harbor (from the Danish project Fiskeatlas) was used to determine which fish most likely would be seen at the reefs. By the use of drawings and images the diver learned distinctive features of these species. A total of 4 divers were involved in the fieldwork, but it was the same diver (1) who made the list of fish species.

Information regarding salinity, temperature and weather information such as wind speed and wind direction were noted at every dive to help determine any daily variation. The surface temperature and salinity was measured in the field and weather data was collected from www.yr.no.

## 2.2.2. Stomach analysis and stable isotope sampling

In this project stomachs were collected over a period of four months to give a temporal insight. The fish were caught in May-August. Every fourth week four to five days was spent in the field to collect data at both stations. Handheld net, harpoons and fykenets were used to collect fish for stomach analysis. In the fykenets, cod, catfish, salmon heads or flatfish was used as bait. The fykenets were placed in the water in the early hours of the morning and were emptied every two hours until late in the evening. The reason for this was to make sure, that the digestion rate wouldn't be too high for the stomach analysis. When taken out from the trap the stomach of each fish and a piece of muscle were immediately removed and the total length and standard length of the fish were measured. Large fish

were transported back to the laboratory on ice to prevent stomach decomposition and kept in a freezer until gut examinations. A piece of the muscle was removed from the fish to be used for stable isotopic analysis.

Table 2. Overview over methods used in the field

Visual census - snorkeling
Hand nets
Harpoon
Fishing rod

## 2.2.3. Gut examinations

Fish stomachs were kept in 4% formalin. Before examination of the stomach content it was washed in water and thereafter was placed in a petri dish with water in order to remove most of the formalin. The content was removed from the stomach and identified and afterwards kept in 80% alcohol. The content was described by number of the respective food species. A Leica MZ 6 stereomicroscope was used to determine the food species. When this was impossible the content was identified to nearest taxonomic level. In order to determine the types of mites, copepods and polychaetes a compound microscope was used. The literature used for identification of taxa is listed in Appendix 2.2.

A list of the respective species (fish species and food species) and their auctors be found in Appendix 1.1 + 1.2.

## 2.2.4. Stable isotope analysis

Samples of muscle tissue taken from the back of the fish and muscles from different prey species, such as crustaceans and bivalves, were dried at 50°C for three days. The muscle samples were ground into powder in a mortar. The muscle samples, 300-500 mg each, were packed into tin capsules and analyzed for  $\delta^{15}$ N and  $\delta^{13}$ C. Two replicates were prepared for each muscle tissue. Between preparation of each sample, contamination was prevented by burning the equipment and afterwards cleaning it with 99% ethanol between each sample. Standard gel A was also weighted and packed into the tin capsules. The muscles samples and gel A were sent to the Department of Biology at the University of Southern Denmark, where the samples were analyzed using a thermos scientific lRMS, Delta V advantage.

## 2.2.5. Data analysis and statistics

## Fish species abundance pattern

To compensate for the fluctuating visibility during the dives, the number of fish seen per dive was calculated to individuals/m<sup>2</sup> by using estimates of visibility to calculate the area scanned. This enabled comparisons between the dives. It has been taken into account that small fish, such as *G*. *flavescens*, couldn't be detected further away than 2 meters. When the visibility was 4 meters it would only be accounted for 2 meters in the calculations for small fish.

Correlations between observations and environmental parameters were investigated through Multiple linear regression (MLR) in R. A quadratic regression of temperature and salinity or a linear regression of temperature and salinity was used to analyze the abundance of the four most abundant species; *G. morhua, G. flavescens, S. melops and C. rupestris.* To diminish the effect of large variations in individual species abundance data were log transformed prior to analysis.

A CHI<sup>2</sup> test was used to test if the species richness differed between the two stations. Mann-Whitney U test was used to test if the abundance (individuals/m<sup>2</sup>) differed between the two stations.

Shannon index and Pielou's evenness were calculated for species richness for the two reefs. The Shannon index was calculated using formula 1.2.

$$H = \sum_{i=1}^{R} \frac{p_i}{\ln(p_i)} \quad (1.2)$$

p<sub>i</sub> is the proportion of total abundance represented by the i<sub>th</sub> species and R is number of species in the community. Pilou's evenness was calculated using formula 1.3 and 1.4.

$$J' = \frac{H}{H_{max}} \qquad (1.3)$$

$$H_{max} = -\sum_{i=1}^{S} \frac{1}{s} ln \frac{1}{s} = \ln(S) \quad (1.4)$$

S is the total number of species, H is the number derived from the Shannon index and  $H_{max}$  is the value of all species being equal.

A Whittaker plot was calculated. This illustrates the rank of species by their relative distribution.

## Gut analysis

The multivariate statistical program Primer (v. 5) was used to find Bray-Curtis similarity between all the species compared in the gut analysis and on size distributed *C. rupestris*. The data was presented as a Cluster analysis. Shannon diversity index and evenness were calculated for the stomach content (see formula 1.2-1.4). Parasites were identified to family, but not included in the analysis (see Appendix 2.3 for a list of parasites).

## Stable isotope analysis

The  $\delta^{15}N$  and  $\delta^{13}C$  values were normalized using Gel A with known isotope ratio values to correct for daily shifts ( $\delta^{15}N=5.4$  and  $\delta^{13}C=21.81$ ).

Comparison between groups were done partly by a visual comparison of an isotope biplot and partly through comparison of standard ellipses and standard ellipse area (Batschelet 1981). The SIBER packages used in R v. 3.4.2 was used to calculate standard ellipse and standard ellipse overlap between groups (Jackson et al. 2011).

Visualization of likely food composition was data calculated based on the thesis that the ration of carbon is enriched by 1‰  $\delta^{13}$ C (Caut et al. 2009) and that fish muscle tissue is enriched by 3.2‰  $\delta^{15}$ N (Sweeting et al. 2007). These are generalized enrichments.

## 3. Results

## 3.1. Fish species abundance analysis

Altogether fourteen species were observed during this survey. Thirteen species were observed at Aarhus  $\emptyset$  (AO) and ten at Aarhus east harbor (AH). Four species (*S. typhle*, *B. belone*, *P. flesus* and *P. gunnelus*) were observed only at AO and one species (*P. virens*) only at AH (Tab. 3). There was no difference in species richness ( $X^2 P=0.92$ ) or in the abundance (individuals/m<sup>2</sup>) of *G. morhua*, *G. flavescens*, *C. rupestris* and *S. melops* at the two stations (Mann-Whitney U test). But there was a significant difference in abundance of *G. morhua* between night and day (M.W. U test, critical value = 20, P<0.05). The highest rate of species was observed in August (seven species) and fewest in May (two species). The average richness was highest in June and July and lowest in May during the day. During the night the average richness was highest in September and lowest in June and August (Fig. 5). But this was not tested for significance.

Scientific name	Common name	Aarhus Harbor	Aarhus Ø	Total
Gadus morhua	Atlantic Cod	33	31	64
Syngnathus typhle	Broadnosed pipefish		1	1
Nerophis ophidion	Straightnosed pipefish	1	1	2
Spinachia spinachia	Fifteen-spined stickleback	2	9	11
Taurulus bubalis	Longspined bullhead	5	4	9
Gobiusculus flavescens	Two-spotted goby	4450	4426	8876
Ctenolabrus rupestris	Goldsinny wrasse	3754	2926	6680
Symphodus melops	Corkwinge wrasse	574	163	737
Pollachius virens	Saithe	1		1
Belone belone	Garfish		6	6
Chelon labrosus	Thicklip Grey Mullet	1	1	1
Hyperoplus lanceolatus	Greater sandeel	1	23	24
Pholis gunnellus	Gunnel		1	1
Platichthys flesus	Flounder		1	1
Total Abundance				16417
Species Richness				14

Table 3. Abundance of individual fish species at each station.



Figure 5. Monthly average diel species richness for both stations. The average species richness was highest in June-July and lowest in May. The nocturnal average species richness was highest in September and lowest in June and August.

Species richness varied between the diurnal and nocturnal efforts. Fewer species were observed at nocturnal dives than at diurnal ones. Four species were observed at night. One species, *P. flesus*, was only observed at night, while ten species were observed only by day (Fig. 6). Benthic species were only observed at night and demersal and pelagic species were more abundant by day (Fig. 7). The pelagic species represent 77% while demersal species represent 23% by day (Fig. 7).



■ Night ■ Day

Figure 6. Diurnal and nocturnal frequencies of species. Four species were observed at night and one of them was observed only at night. Ten species were only observed at day.



Figure 7. Diel variations in assemblage structure was not significant (X<sup>2</sup> P=0.07). Benthic fishes were only observed at night, while demersal fishes were most abundant at night and pelagic species most abundant during the day.

The distribution of the species at each station is illustrated in the Rank-Abundance diagram (Whittaker plot) (Fig. 8). The y-axis is logarithmic and the first species ranked on the x-axis was highly represented in the total abundance. This diagram shows that two species were highly abundant. The last six species were only observed one time at AO and two species only at AH, placing the species low in relation to abundance (Fig. 8). Evenness and dominance relationships were assessed from the slope of the curve. This curve is steep which indicates high dominance and low evenness. This is supported by results in Tab. 5.

The diversity index at both stations was calculated as a Shannon index (Tab. 4). It had a value of 0.719 at AO and 0.877 at AH. Both values indicate a low diversity of species by day. An evenness of 0.280 at AO indicates a low diversity because few species dominate (Tab. 4). It was the same pattern at AH, but evenness was a little higher at 0.399 (Tab. 4). At night the Shannon index is a little higher, 0.895 at AO and 1.016 at AH (Tab. 4). This indicates a relatively low diversity, but it is higher than during the day, however significance is not tested. The evenness indicates that there was less dominance of a few species (Tab. 4).



Figure 8. Rank-Abundance curve (Whittaker plot) for observed fish species at Aarhus Harbor and Aarhus Ø, illustrated as the relative distribution on a logarithmic y-axis.

	Aar	hus Ø	Aarhus	Harbor
	Day	Night	Day	Night
Richness	13	4	10	3
Shannon index	0.719	0.895	0.877	1.016
Evenness	0.280	0.646	0.399	0.925

Table 4. Diversity indices for fish. Richness, Shannon index and evenness for day and night at both stations.

The salinity and temperature increased over the first three months of this survey, but in September the salinity decreased slightly (Fig. 9).

There were no correlations between temperature, salinity and abundance of fish species (Tab. 5). Two models were tested for each species, one with linear regression and one with quadratic regression. Four species were tested since they were the most abundant species during the observations. Diurnal observations were done for *C. rupestris* and *S. melops*. For *G. flavescens* and *G. morhua* both nocturnal and diurnal observations were done.



Figure 9. The temperature (°C) and salinity (‰) for the timespan of the survey, from May to September. Light circles are salinity (‰) and dark circles are temperature (°C).

Table 5. Output from Multiple Linear Regression. P-values of quadratic and linear models. None of the models explained the abundance of the *Gadus morhua, Gobiusculus flavescens, Symphodus melops* and *Ctenolabrus rupestris.* SST = Sea Surface Temperature.

	SST + Salt			(SST+SST <sup>2</sup> ) + (Salt+Salt <sup>2</sup> )				
	F	DF	P-value	F	DF	P-value		
Gadus morhua	0.47	2,16	0.63	0.47	2,16	0.63		
Gobiusculus flavescens	2.28	2,21	0.12	2.4	2,21	0.11		
Symphodus melops	0.21	2,16	0.81	0.26	2,16	0.77		
Ctenolabrus rupestris	1.12	2,16	0.35	0.84	2,16	0.44		

## 3.2. Stomach content analysis

#### 3.2.1. Food composition

The contents of 252 stomachs from ten fish species were analyzed (Tabs. 6 + 7). Of the 252 examined stomachs 3.6% were empty, 30.6% contained a little content (few individuals of food species) and 65.9% were full. *Spinachia spinachia* and *P. virens* were only caught at AO, while the remaining eight species were caught at both stations. Seventyfour percent of the stomachs came from *C. rupestris* and the rest from nine other species (Tab. 7). The catch rate was higher at AO than at AH (Tab. 6).

Fifteen groups of 59 food species were identified from the guts. In total 59 food species

accounted for 17744 individuals (Appendix 2.3 – without parasites). Of the 59 taxa eight were macroalgae. Only ten taxa were identified to family. Of the polychaetes three families were identified from bristles, and of Amphipods only 7% were identified to family. During the examination, different types of eggs were found. They were grouped and consisted mainly of eggs from snail, fish and turbellarians.

<b>Month/location</b>	Aarhus Ø	Aarhus harbor	Total	
May	26	17	43	
June	51	20	71	
July	47	26	73	
August	47	18	65	
	171	81	252	

Table 7. Number of fish species caught for gut examination each month.

Fish species/Month	May	June	July	August	Total
Gadus morhua	3	18		8	29
Syngnathus typhle		2			2
Nerophis ophidion		1			1
Spinachia spinachia	1			4	5
Taurulus bubalis	1	6	3		10
Zoarces viviparus	1	1			2
Gobiusculus flavescens	2	1		6	9
Symphodus melops		3		4	7
Ctenolabrus rupestris	35	38	70	43	186
Pollachius virens		1			1
Total	43	71	73	65	252

For the eight fish species in Tab. 7 the relative distribution of the gut contents showed that their diet consisted of 14 different food groups. *Nerophis ophidion* and *P. virens* were excluded due to an empty stomach and a stomach with only parasites. All species consumed numerous crustaceans (Tab. 8). *Syngnathus typhle* at both stations consumed exclusively the copepod *D. nobilis* (Tab. 8 + Appendix 2.3). Almost the same, approximately 90%, was found in *G. flavescens* at both stations and in *S. spinachia* at AO (Tab. 8). The copepods *D. nobilis, T. longicornis* and the shrimp-stage of *C. maenas* were found inside the stomachs (Appendix 2.3).

At AH 80% of the diet of *G. morhua* consisted of crustaceans, but only 15.7% at AO where it mainly consumed gastropods and macroalgae (Tab. 8). The diet for *C. rupestris* at AH was the most diverse, and it had a diet that consisted of eleven different groups of food species compared with thirteen different food species groups at AO. The largest of the group was bivalves (Tab. 8). *Symphodus melops* contained ten different food species groups at AH but only five groups at AO - mostly crustaceans (Tab. 8). Half of *Z. viviparus*' diet consisted of crustaceans (Tab. 8).

Table 8. Relative distribution of number of food species in the gut contents for all fish species at both stations. AH = Aarhus Harbor, AO = Aarhus Ø, BP = Broadnosed pipefish (Syngnathus typhle), GW = Goldsinny wrasse (Ctenolabrus rupestris), CO = Cod (Gardus morhua), CW = Corkwinge wrasse (Symphodus melops), FS = Fifteen-spined stickleback (Spinachia spinachia), LB = Longspined bullhead (Taurulus bubalis), TG = Two spotted goby (Gobiusculus flavescens) and VE = Viviparous eelpout (Zoarces viviparus). The straightnosed pipefish and the seith are not included because of empty stomachs.

	C	20	В	Р	FS	L	В	V	Е	Т	G	С	W	0	GW
	AH	AO	AO	AH	AO	AH	AO	AH	AO	AH	AO	AH	AO	AH	AO
Halacarida	-	-	-	-	6.	-	-	3.6	1.9	-	-	3.	-	1.2	1.9
Ascidiacea	-	-	-	-	-	-	-	-	-	-	-	0.1	-	0.06	0.9
Bivalvia	-	3.92	-	-	1.5	-	-	35.7	20.8	-	-	15.6	-	42.5	53
Chaetognatha	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.01
Cladocera	-	-	-	-	-	-	-	-	-	7.4	-	-	-	-	0.01
Cnidaria	-	-	-	-	-	-	-	-	-	-	-	1.7	-	0.3	0.62
Crustacea	80	15.7	100	100	93.5	36.36	86.7	53.6	67.9	90.4	99.36	44.4	54.6	30.	23.14
Echinodermata	-	-	-	-	-	-	-	-	-	-	-	5.9	0.92	1.0	0.82
Egg	-	-	-	-	-	-	-	-	-	1.30	-	6.7	-	-	0.38
Gastropoda	-	29.4	-	-	-	-	-	3.57	3.8	0.3	0.68	20.6	27.3	16.2	11.81
Insecta	-	1.96	-	-	-	-	-	-	-	-	-	1.06	9.09	0.16	0.13
Macroalgae	5.7	31.4	-	-	-	45.5	-	3.57	3.8	-	-	4.9	9.09	6.18	5.31
Myriapoda	-	-	-	-	-	9.09	-	-	-	-	-	-	-	-	-
Polychaeta	5.7	5.9	-	-	-	-	13.33	-	1.9	-	-	-	-	0.16	0.14
Teleostei	8.6	5.9	-	-	-	9.09	-	-	-	-	-	-	-	-	-

The Shannon indexes for the gut contents of all species were generally high, except for *G*. *flavescens*. This indicates that the gut content for the most species were equally distributed between different groups of food species and there was no food species group dominating (Tab. 8). The *G*. *flavescens* ate only a few different taxa dominated by one food species group (crustaceans – copepods, Tab. 8) resulting in a low Shannon index and a low evenness (Tab. 9).

Table 9. Richness, Shannon index and evenness for all species' gut examination. AH = Aarhus Harbor, AO = Aarhus Ø, GW = Goldsinny wrasse (*Ctenolabrus rupestris*), <math>CO = Cod (*Gardus morhua*), CW = Corkwinge wrasse (*Symphodus melops*), <math>FS = Fifteen-spined stickleback (*Spinachia spinachia*), <math>LB = Longspined bullhead (*Taurulus bubalis*), <math>TG = Two-spotted Goby (*Gobiusculus flavescens*) and <math>VE = Viviparous eelpout (*Zoarces viviparus*).

	СО		L	В	FS	V	/E
	AH	AO	AO	AH	AO	AH	AO
Richness	4	8	4	5	3	5	7
Shannon index	0.716	1.7183	0.920	1.148	0.927	1.06	1.452
Evenness	0.516	0.826	0.663	0.713	0.833	0.658	0.746
	1	TG		CW		W	
	AH	AO	AH	AO	AH	AO	
Richness	6	3	13	5	15	17	
Shannon index	0.319	0.112	1.830	1.499	1.606	1.546	
Evenness	0.177	0.102	0.713	0.931	0.593	0.545	

#### Ctenolabrus rupestris

*Ctenolabrus rupestris* was the only fish species caught in high enough numbers to provide an insight of the diet between different size groups and a temporal insight. An overview with *C. rupestris* sorted into different size groups can be found in Appendix 2.1. The gut contents of *C. rupestris* sorted by size resulted in fourteen different food species groups (Tab. 10). The group Egg consisted of a variety of different types of eggs found in the stomachs, but the majority was believed to be snail eggs. For both stations the relative distribution of the gut contents in size groups showed that the small size groups (3-4 cm and 5-6 cm – at AH) and the largest ones (14-15 cm – at AO) contained few groups of food species. Few individuals of the smallest size groups and the largest size group was macroalgae, just followed by bivalves and gastropods (Tab. 10). At AH a higher proportion of gastropods than bivalves was eaten (Tab. 10).

The relative composition of the food for *C. rupestris* for May-August is shown in Tab. 11. At both stations the temporal variation in the diet of *C. rupestris* showed a shift from crustaceans to bivalves during the study. In May the intake of crustaceans was 95% at AH and 52% at AO but in August it fell to 17% at AH and 2% at AO.

	Aarhus Hai	rbor						
	3-4	5-6	7-8	8-9	9-10	10-11	11-12	
Halacarida	11.11	-	14.71	8.86	5.63	8	10.34	
Ascidiacea	-	-	-	-	1.41	4	3.45	
Bivalvia	-	-	14.71	17.72	18.31	4	10.34	
Campanulariidae	-	-	2.94	-	2.82	-	-	
Chaetognatha	11.11	-	-	-	-	-	-	
Macroalgae	-	-	20.59	15.19	16.90	28	20.69	
Cladocera	-	-	-	-	-	-	-	
Insecta	22.22	-	2.94	5.06	1.41	-	-	
Crustacea	55.56	100	32.35	32.91	30.99	24	20.69	
Egg	-	-	-	-	-	-	-	
Gastropoda	-	-	11.76	18.99	16.90	16	24.14	
Echinodermata	-	-	-	-	5.63	8	6.90	
Polychaeta	-	-	-	1.27	-	4	3.45	
Cnidaria	-	-	-	-	-	4	-	
	Aarl	nus Ø						
	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15
Halacarida	12.50	10.59	6.39	5.47	3.66	-	-	-
Ascidiacea	-	-	1.83	1.56	3.66	11.36	3.45	-
Bivalvia	9.38	17.65	15.07	18.75	10.98	13.64	17.24	20
Campanulariidae	-	4.71	0.46	2.34	4.88	-	-	-
Chaetognatha	-	-	0.41	-	-	-	-	-
Macroalgae	15.63	11.76	22.83	18.75	15.85	18.18	31.03	60
Cladocera	-	-	-	0.78	-	-	-	-
Insecta	3.13	8.24	5.02	5.47	3.66	4.55	3.45	-
Crustacea	46.88	36.47	27.85	18.75	20.73	22.73	17.24	-
Eggs	-	-	-	-	2.44	2.27	-	-
Gastropoda	9.38	8.24	18.72	24.22	28.05	13.64	20.69	-
Echinodermata	-	-	0.46	1.56	2.44	9.09	0	-
Polychaeta	3.13	2.35	0.91	2.34	3.66	4.55	6.90	-
Cnidaria	-	-	-	-	-	-	-	-

Table 10. Relative distribution of the gut contents of 186 *Ctenolabrus rupestris* by size groups (cm) for Aarhus Harbour and Aarhus Ø.

	Aarhus Harbor				Aarhus Ø			
	May	June	July	August	May	June	July	August
Halacarida	0.94	1.37	0.83	4.06	2.43	6.3	0.39	1.63
Ascidiacea	-	0.68	0.06	-	14.16	0.09	0.64	-
Bivalvia	-	24.65	53.43	45.62	3.98	28.66	62.59	61.69
Chaetognatha	0.13	-	-	-	0.22	-	-	-
Macroalgae	0.94	23.3	6.76	6.33	5.53	7.48	3.33	7.53
Cladocera	-	-	-	-	-	-	0.02	-
Insecta	0.8	0.68	-	0.16	1.32	2.66	0.46	0.233
Crustacea	95.16	26.03	19.11	17.21	52.88	46.78	23.85	2.26
Eggs	-	-	-	-	1.99	1.62	-	-
Gastropoda	-	21.91	18.43	25.16	10.39	5.71	7.34	24.72
Nemertea	-	-	-	-	0.44	0.05	-	-
Echinodermata	-	-	1.24	1.46	-	-	1.14	0.93
Polychaeta	0.53	-	0.12	-	1.10	0.29	0.09	-
Cnidaria	1.48	1.37	0.03	-	5.53	0.35	0.14	0.93

Table 11. The relative distribution of the gut contents of *Ctenolabrus rupestris* for each station in May, June, July and August.

## 3.2.2. Prey type

The majority of all food species were benthic (79.7%). Of the benthic species 59% were sessile associated with hard substrates (Tab. 13).

At both stations G. morhua ate mainly mobile prey taken at the bottom (Figs. 10 + 12 +Tab. 12). Ninety percent of the stomach content from AH were generalist food species and over 50% from AO were food species associated with soft bottom (Fig. 11). At AH the prey organisms were mainly the benthic generalist C. maenas and benthic gastropods at AO. The gastropods found in the gut contents were both associated with vegetation and soft bottom. One endobenthic species, A. virens found on the soft bottom together with a few pelagic fish species, was also found in the stomachs of G. morhua (Tab. 12 + Appendix 2.3). Syngnathus typhle only ate pelagic species (Fig. 10) such as the copepod D. nobilis (Tab. 12 + Appendix 2.3). Spinachia spinachia ate mostly mobile pelagic prey (92%) (Figs. 10 + 12). They were mostly (97%) generalist mobile food species such as C. maenas (shrimp stage) (Tab. 12 + Fig. 11 + Appendix 2.3). Taurulus bubalis only ate benthic food species (100%) at both stations (Fig 10). The majority of them were mobile food species (Fig. 12). For both stations most of the food species (80%) were associated with vegetation (Fig. 11). For both stations Z. viviparus ate only benthic prey (100%) (Fig 10). For both stations the stomach content were mainly mobile prey (Fig. 12). The benthic prey was mostly associated with vegetation (60%) at AH (Fig. 11). At AH the mobile benthic prey I. granulosa (27%) was found in the stomachs while more epibenthic amphipods (28.3%) (e.g. D. nobilis) were found in the stomachs from AO (Tab. 12 + appendix 2.3). Gobiusculus flavescens from both stations ate mainly pelagic food species (62% at AH and 86% at AO) (Fig. 10). Gobiusculus flavescens also ate benthic food species (37% at AH and 13% at AO) (Fig. 10). Symphodus melops mostly ate mobile benthic food species (96%) at AH but only mobile benthic food species (100%) at AO (Figs. 10 + 12). At AH the majority of the food species were generalists (59%) (Fig. 11) such as, e.g., T. basteri and C. maenas (Shrimp stage) (Tab. 12 + Appendix 2.3). While S. melops at AO the majority of the food species were associated with vegetation (40%) (Fig. 11), mainly the epibenthic amphipod A. jurinei (Tab. 12 + Appendix 2.3). Ctnelobarus rupestris mainly ate benthic food species at both stations (74% at AH and 81% at AO) (Fig. 10). The majority were small individuals of *M. edulis*, a species associated with hard bottom (Fig. 10 + Tab. 12 + Appendix 2.3). This can explain the high intake of sessile food organisms at both stations (Fig. 12).

The food species were sorted into groups according to literature (Jensen 1978; Bondesen 1984; Green and Macquitty 1987; Køie et al. 1991; Bondesen 1994; Hayward and Ryland 1995).

Table 12. Species found in gut contents sorted as mobile and sessile fauna for both habitats. Benthic species are divided into four groups: generalist, vegetation, hard bottom and soft bottom dwellers. Generalist = a species that does not prefer vegetation, hard or soft bottom, but can be found in all three habitats. The species were grouped according to literature (Jensen 1978; Bondesen 1984; Green and Macquitty 1987; Køie et al. 1991; Bondesen 1994; Hayward and Ryland 1995) and personal communication with Boy Overgaard Nielsen. Insects, parasites, eggs, fish scales and a fish spine have been omitted.

Benthic			
Generalist	Vegetation	Hard bottom	Soft bottom
Mobile fauna			
Thalassarachna basteri	Apherusa jurinei	Psammechinus miliaris	Cerastoderma edule
Carcinus maenas	Caprella linearis	Clunio marinus	Crangon crangon
Pagurus bernhardus	Corophium bonnelli	Glyptotendipes barbipes	Bittium reticulatum
Polynoidae	Dexamine spinosa		Strigamia maritima
Nemertea	Microdeutopus gryllotalpa		Ophiura albida
	Hyale pontica		Alitta virens
	Leptocheirus pilosus		Nereididae
	Melita pellucida		
	Idotea balthica		
	Idotea granulosa		
	Lacuna parva		
	Rissoa parva		
	Spirobis spirorbis		
	Spirorbis		
Sessile fauna			
Laomedea flexuosa	Musculus subpictus	Musculus discors	Mya arenaria
		Mytilus edulis	
		Dendrodoa	
		grossuaria Styela coriacea	
		Semibalanus	
		balanoides	
Pelagic			
Temora longicornis			
Diarthrodes nobilis			
Evadne nordmanni			
Podon leuckarti			
Ctenolabrus rupestris			
Sagitta sp.			



Table 13. Relative distribution of food species type for all individuals found in gut contents in fish species.



■ Benthic ■ Pelagic

Figure 10. The relative distribution of benthic and pelagic food species in the gut content of all fish species.



Figure 11. Relative distribution of the gut content for all fish species that ate benthic food species. Food species are sorted into four groups; generalist, vegetation, hard bottom and soft bottom dwellers.



Figure 12. The relative distribution of food species from gut content sorted into mobile and sessile prey type.

## 3.2.3. Trophic analysis

The analysis of food contents was carried out using Bray-Curtis similarity which resulted in two groups. *G. flavescens* (TG) and *S. typhle* (BP) were clustered together, with a Bray-Curtis similarity at 40. At the two stations *S. typhle* had a similarity of 100 (Fig. 13). This indicates that *G. flavescens* and *S. typhle* belonged to the same trophic category. Both species had a high content of crustaceans in their gut (Tab. 8). The rest of the species clustered together which indicates that they had the same diet with only minor differences (Fig. 13).



Figure 13. Bray-Curtis similarity of gut content shown as cluster analysis of all species. AH = Aarhus Harbor, AO = Aarhus Ø, BP = Broadnosed pipefish (Syngnathus typhle), GW = Goldsinny wrasse (Ctenolabrus rupestris), CO = Cod (Gadus morhua), CW = Corkwinge wrasse (Symphodus melops), FS = Fifteen-spined stickleback (Spinachia spinachia), LB = Longspined bullhead (Taurulus bubalis), TG = Two-spotted goby (Gobiusculus flavescens) and VE = Viviparous Eelpout (Zoarces viviparus).

There were no major differences between size groups or stations in the Bray-Curtis similarity for *C. rupestris* (Fig. 14). Two groups that stand out were the small sized group, from 3-4 cm and 5-6 cm, which were only caught at AH and the largest size group, from 14-15 cm, which was only caught at AO. For the most of the size groups of *C. rupestris* the Bray-Curtis similarity was higher than 73.



Figure 14. Bray-Curtis similarity on gut analysis showed as cluster analysis for selected samples of *Ctenolabris rupestris* by size for each station. GW = goldsinny wrasse, AH = Aarhus Harbor, AO = Aarhus Ø, 1213 = from 12 cm to 13 cm.

## 3.3. Stable isotope analysis

## 3.3.1. Stable isotope composition of species

Stable isotopes of nitrogen and carbon were analyzed from muscle tissue from fish species caught at AO and AH from June to August. Fish caught in May could not be used due to contamination by formalin by mistake.

Table 14. Overview of number of samples for fish species and samples of food species analyzed for stable isotopes of nitrogen and carbon.

Fish species	AO	AH	Food species	AO	AH
G. morhua	21	5	M. edulis	3	3
N. ophidion	1		S. balanoides	2	
S. typhle	1	1	P. elegans	1	
S. spinachia	4		C. maenas	1	
T. bubalis	5	1	Alitta virens	1	
Z. viviparus	1	1			
G. flavescens	4	3			
S. melops	1	5			
C. rupestris	48	30			
P. virens	1				
The stable isotope analysis showed a difference between the two stations (Fig. 15). This could be a reflection of the two stations were located at different locations. The fish species S. typhle, N. ophidion, G. flavescens and S. spinachia had similar  $\delta^{15}$ N values ranging from 12.1-12.5‰ and was consequently placed at the bottom of the food chain with regard to the fish species. But S. typhle and N. ophidion differed in  $\delta^{13}$ C. Gobiusculus flavescens and S. typhle are grouped together showing values at -22.1 to -22.9‰. Spinachia spinachia and N. ophidion are grouped together with values at -19.5 to -19.8‰. This was supported by the standard ellipses which showed a high overlap between the two pipefish (S. typhle and N. ophidion) species and G. flavecsens (Tab. 15). The overlap of the ellipses indicates a similar use of resources (Tab. 15). Symphodus melops from AO showed a very different value of both  $\delta^{15}$ N and  $\delta^{13}$ C than S. melops AH. Symphodus melops at AO was lower in the food chain with  $\delta^{15}$ N values around 12‰ and  $\delta^{13}$ C values -23‰. While S. melops at AH was higher in the food chain with  $\delta^{15}$ N values around 14‰ and  $\delta^{13}$ C values -21‰. The small overlap between S. melops, the two pipefish species (S. typhle and N. ophidion) and G. flavecsens and the high overlap between S. melops, C. rupestris and T. bubalis (Tab. 15) indicates they use similar ressources. Symphodus melops at AH grouped together with C. rupestris AH and P. virens AO with a value of  $\delta^{15}$ N at 13.7‰. Ctenolabrus rupestris AO and Z. viviparus grouped together with a value of  $\delta^{15}$ N around 14‰ and lower  $\delta^{13}$ C value around -21.5‰. The two wrasse species had a high overlap which indicates a use of the similar resources (Tab. 15). The same is found for the pipefish species and G. *flavescens* (Tab. 15). In the top of the food chain were G. morhua AO + AH with similar  $\delta^{13}$ C values at 19.3‰ but different  $\delta^{15}$ N at 15.33‰ and 14.88‰. The food species *M. edulis* from AO and *M*. *edulis* from AH showed a difference in  $\delta^{15}$ N and  $\delta^{13}$ C, which could indicate that the two stations had different conditions. The A. virens had the highest level of  $\delta^{13}$ C (less negative). Carcinus maenas and *P. elegans* had higher values of  $\delta^{15}N$ , at 20% and 19%, than the other food species.



Figure 15. Average stable isotope biplots of nitrogen stable isotope ( $\delta^{15}$ N) versus carbon stable isotopes ( $\delta^{13}$ C) of the fish species *G. morhua*, *N. ophidion*, *S. typhle*, *S. Spinachia*, *T. bubalis*, *Z. viviparus*, *G. flavescens*, *S. melops*, *C. rupestris and P. virens* at each station (AO and AH). And for the food species *M. edulis*, balanoidae, *P. elegans*, *C. maenas* and Polychaeta. Error bars shows standard deviation.

	GW	CW	LB	BP+SP	СО	FS	TG
GW							
CW	0.672						
LB	0.75	0.47					
BP+SP	-	0.25	-				
СО	-	-	0.086	-			
FS	-	-	-	-	-		
TG	-	0.23	-	0.78	-	-	

Table 15. Calculated overlap fo the standard ellipses by SIBER analysis of the stable isotopes for *G. morhua*, *N. ophidion*, *S. typhle*, *S. spinachia*, *T. bubalis*, *Z. viviparus*, *G. flavescens*, *S. melops*, *C. rupestris and P. virens* for both station. See Appendix 3.1 for the figure of the ellipses.

# 3.3.2. Visualization of likely food composition

Fig. 16 illustrates the likely food composition, computed from data represented in Fig. 15. The new data calculated was based on the thesis that the ration of carbon is enriched by 1‰  $\delta^{13}$ C (Caut et al. 2009) and that fish muscle tissue is enriched by 3.2‰  $\delta^{15}$ N (Sweeting et al. 2007). These are generalized enrichments. *Ctenolabrus rupestris, G. flavescens* (AH), *P. virens* (AO) and *S. melops* (AH) moved to the position of *M. edulis*. This indicated a diet including *M. edulis*. *Gadus morhua* moved closer to *C. maenas* and *P. elegangs* indicating a diet including crustaceans. *Syngnathus typhle, S. melops* (AO), *G. flavescens* (AO), *S. spinachia* and *N. ophidion* moved under *M. edulis* indicating a diet at lower trophic levels.



Figure 16. Average stable isotope biplots of nitrogen stable isotope ( $\delta^{15}$ N) versus carbon stable isotopes ( $\delta^{13}$ C) but enrichment was subtracted 1.0‰ of  $\delta^{13}$ C and the enrichment of 3.2‰ of  $\delta^{15}$ N for the fish species *G. morhua*, *N. ophidion*, *S. typhle*, *S. spinachia*, *T. bubalis*, *Z. viviparus*, *G. flavescens*, *S. melops*, *C. rupestris and P. virens* at each station (AO and AH). And the food species *M. edulis*, S. balanoides, *P. elegans*, *C. maenas* and *A. virens*. Error bars shows standard deviation.

# 3.3.3. Ctenolabrus rupestris

*Ctenolabrus rupestris* varied in  $\delta^{13}$ C between the stations. *Ctenolabrus rupestris* from AO had a higher  $\delta^{15}$ N values around 14‰ and higher  $\delta^{13}$ C values around -20.7 to -20.3‰ than those caught at AH. The fish caught in June, July and August from the two stations had values of  $\delta^{15}$ N that varied slightly. There was a high overlap between months except for August AH, July AO and August AO (Tab. 16). The overlap indicates a similar exploration of resources.



Figure 17. Average stable isotope biplots of nitrogen stable isotope ( $\delta^{15}N$ ) versus carbon stable isotopes ( $\delta^{13}C$ ) for *C. rupestris* for June, July and August for AH and AO. Error bars show standard deviation.

Table 16. Overlap of standard ellipses calculated by SIBER analysis for the stable isotope analysis for June, July and August for both stations for *C. rupestris*. See Appendix 3.1 for the figure of the ellipses.

	June AH	July AH	Aug. AH	June AO	July AO	Aug. AO
June AH						
July AH	1.04					
Aug AH	0.41	0.33				
June AO	0.76	0.49	0.029			
July AO	0.41	0.22	-	0.43		
Aug. AO	0.33	0.43	-	0.32	0.19	

The size groups of *C. rupestris* showed the same pattern as the analyses of stomach content sorted by months (Fig. 18). AH had low values of  $\delta^{15}$ N and differed in  $\delta^{13}$ C. The size groups from AO had a higher  $\delta^{15}$ N and  $\delta^{13}$ C values compared to the size group from AH which had lower values. The larger size group from AH (11-12cm and 12-13cm) was close to the size groups of AO and had a higher  $\delta^{13}$ C values than those of the smaller size groups from AH. The small sized groups had the

lowest value of  $\delta^{15}$ N, while the two largest size groups (13-14cm and 14-15cm) had the highest value of  $\delta^{15}$ N and lowest of  $\delta^{13}$ C for AO (Fig. 18). For both stations, a small overlap was formed between small size groups and larger size groups (Tab. 17).



Figure 18. Average stable isotope biplots of nitrogen stable isotope ( $\delta^{15}N$ ) versus carbon stable isotopes ( $\delta^{13}C$ ) of *C. rupestris* for size groups of cm. Error bars show standard deviation.

Table 17.	Calculated	overlap of the s	standard ellip	ses by SIBEF	R analysis for	the stable	isotpoe ar	nalysis all si	ze groups a	it each
station fo	r C. rupestris	s. See Appendix	3.1 for the fig	ure of the elli	ipses.					

				AH						AC	)		
		7-8	8-9	9-10	10-11	11- 12	7-8	8-9	9-10	10-11	11-12	12-13	13-15
	7-8												
	8-9	0.11											
AH	9- 10	-	0.034										
	10- 11	0.054	0.36	0.074									
	11-12	0.19	0.55	0.074	1.11								
	7-8	0.09	0.09	-	0.03	0.82							
10	8-9	-	0.101	0.02	0.39	0.63	0.085						
AU	9-10	0.03	0.07	-	0.12	0.48	0.15	0.33					
	10-11	-	-	-	0.11	0.36	-	0.19	0.15				
	11-12	-	-	0.01	0.28	0.59	-	0.27	0.14	0.31			
	12-13	-	-	-	0.08	0.15	-	0.017	-	0.12	6.90E-02		
	13-15	-	-	-	-	2.00E-03	-	-	-	-	-	-	

# 4. Discussion

## 4.1. Fish species analysis

#### 4.1.1. Fish Species abundance patterns

The fish communities at both stations in Aarhus Bay were dominated by two wrasse species (*C. rupestris* and *S. melops*) (Tab. 3). No significant difference between abundance of them was detected at the two stations. Like in this study, other studies have also found *C. rupestris* and *S. melops* at hard bottom habitats (Gjøsæter 2002; Dahl et al. 2005; Lundsteen et al. 2008; Andersson et al. 2009; Stenberg et al. 2012; Skiftesvik et al. 2015). However, they have also been observed in lower numbers on soft bottoms in other studies (Andersson et al. 2009).

*Gobiusculus flavescens* was the most abundant species at both stations and increased highly in numbers from August, probably due to reproduction. Similarly, other studies have documented *G*. *flavescens* to be abundant at shallow rocky habitats and they observed an increased in numbers due to reproduction in August (Magill and Sayer 2002; Wilhelmsson et al. 2006; Andersson et al. 2009).

Another species observed in this study was *G. morhua*. Sixtyfour individuals were observed through 19 dives, with no significant difference between the two stations. A study by Kristensen et al. (2017) from the waters near Læsø in Kattegat found an increase in the Atlantic cod which remained in the area of a restored reef, suggesting marine boulder reefs is a favorable cod habitat.

A species not observed at the dives but found in stomach analyses was *P. virens*. Juveniles of *G. morhua* and *P. virens* have been observed on vegetated rocky substratum (Pihl and Wennhage 2002). During night dives in the present study only juvenile cod were observed.

In this study *S. spinachia* was observed in May and again in August. In May a big individual (11.4 cm) was caught at AO and smaller individuals were first observed again in August, hiding in the top of the alga *S. muticum. Spinachia spinachia* breeds in spring or early summer and the females die afterwards (Kaiser and Croyt 1991).

At the bottom of the reef between algae, *T. bubalis* was observed nine times from May to September. The Danish project Fiskeatlas has also regularly observed this species at AO (personal communication, Henrik Carl).

Several species in this study were only observed once or twice. This could be due to food availability, migration and reproduction. In May and July five *B. belone* were observed at AO. In the early spring it migrates from the eastern Atlantic Ocean to the North Sea and again to Kattegat in order to spawn in shallow waters in the seaweed belt during May and June (Ojaveer 2017). *Chalon* 

*labrosus* was only spotted once in July. It is regarded as a regular summer guest along the North Sea coasts and the Danish belt Sea (Muus and Nielsen 1999).

Two species of pipefish were observed in this survey, *S. typhle* and *N. ophidion*. Two *S. typhle* and one *N. ophidion* were observed in June swimming above the vegetation. Pipefish are common in seagrass habitats and were probably observed due to the vegetation on the boulder reef. In a Swedish study on the reproductive ecology of pipefish the species were breeding in May and June. They also found that *S. typhle* and *N. ophidion* swam above the vegetation to search for a mate (Vincent et al. 1995).

In this study the two stations were dominated by few species at diurnal dives. There were no significant differences in richness or abundance of species between the two stations in Aarhus Bay. This result was expected because the two stations are placed closely to each other. Only *S. typhle, P. flesus* and *P. gunnellus* were observed only once at AO. Average depth was higher at AH than at AO. This could be the reason why benthic species such as *P. flesus* and *P. gunnellus* were only observed at AO. The observed species in this survey were all recorded in the Danish project Fiskeatlas database for Aarhus harbor (personal communication, Henrik Carl).

#### 4.1.2. Diel variations

The circadian rhythms of fish species is coupled to predation risk, food availability and intraspecific competition (Reebs 2002). At both stations only a few species were observed to be nocturnal. Taurulus bubalis, G. morhua, G. flavescens and P. flesus were observed at night on the boulder reef (Fig. 5). Gadus morhua showed a significant nocturnal activity, but was also observed at diurnal dives. In this study juvenile cod were observed at nocturnal dives, while larger cod were observed at diurnal dives. At AO, there was a lot of activity during the day and especially in the afternoon because of swimmers and other activities. These disturbances could be the reason why this study observed significantly more cod at night than by day. It was my observation that cod were only observed during the day after a period of no disturbance at AO. The diel pattern of G. morhua is well studied and others have found juvenile cod to migrate into shallow waters at night or to have had a crepuscular activity in search for food (Pihl 1982; Paulsen 2001). The shallow areas can act as a nursing ground (Pihl 1982) because food is abundant at the reefs and the reefs lowers visibility which consequently reduces predation risk (Gotceitas et al. 1995; Reubens et al. 2014). A Norwegian study of the behavior of cod showed that larger cod searched for food during the day (Løkkeborg 1998) and another study found that juvenile cod (age group 0 and 1) had a diurnal feeding (Keats and Steele 1992).

In this study *T. bubalis* was observed at both diurnal and nocturnal dives. Other species of Cottidae have been reported to show primarily diurnal activity (Nickell and Sayer 1998) while others report a catch rate highest at night (Pihl and Wennhage 2002).

In my study, the majority of *G. flavesecens* were observed at diurnal dives, but a few individuals were observed at nocturnal dives. This was also observed by Thetmeyer (1997) but also found that the semi pelagic *G. flavescens* had a diurnal behavior coupled to predator avoidance and increased food availability.

The labrid species observed in this study all had a diurnal rhythm and crepuscular activity. In one study *Ctenolabrus rupestris* showed crepuscular activity (Nickell and Sayer 1998) but diurnal activity is well known in labrid species (Videler 1988; Gerkema et al. 2000). On the Swedish west coast *S. melops* was also found to have a diurnal rhythm, believed to be feeding activity (Pihl and Wennhage 2002).

Overall the pelagic species were abundant at diurnal dives while the demersal species were abundant at night (Fig. 7). Only one benthic species, *P. flesus*, was observed in this study. This could be due to low visibility at the bottom of the boulder reef where *P. flesus* is usually found (Mendonca et al. 2009). In this study the sand bottom in front of the boulder reef was not observed during the dives. This study found that species richness at diurnal dives was higher than at nocturnal dives. Other studies have found significant more species at night (Pedersen and Eskelund 2012; Holm-Hansen 2015).

### 4.1.3. Physiochemical factors

Changes in environmental temperature is known to affect the physiology and metabolism and therefore the distribution of fish species (Tirsgaard et al. 2015). Both the quadratic and linear regressions showed no correlation with the four most abundant species, *G. morhua, C. rupestris, S. melops* and *G. flavescens*, with temperature and salinity (Tab. 5). The cod observed in this study were observed between the temperatures of 11°C to 19°C. The labrids *C. rupestris* and *S. melops* did not show any change in abundance according to ambient temperature and salinity (Tab. 5). *Ctenolabrus rupestris* and *S. melops* were observed at temperatures ranging from 9 to 19°C and at every diurnal dive during the five months study period, indicating that these species did not leave the boulder reef. The measured fluctuations in temperature and salinity were probably just not high enough to affect them. The composition of fish assemblage in shallow areas have been found to a great extent to be dependent on water temperature and salinity (Hoff and Ibara 1977; Thorman 1986). High temperatures have been found favorable for smaller juvenile cod while for the larger juveniles favorable temperature were 10°C (Tirsgaard et al. 2015). A study found that a cold-water

wrasse species, *Coris julis*, from the Mediterranean nearshore areas showed inactivity and increased resting at temperatures over 23°C, but remained in its preferred habitat, if no competition were present (Milazzo et al. 2013). Fish species are to some extent able to cope with the stress imposed by abiotic factors. These factors beyond their limit in an intertidal zone caused a tradeoff between energetic costs and gain (Somero 2002).

The overall pattern for species richness showed that the maximum of species observed in a single dive effort was in August (Fig. 5). August was the month with the highest SST (Fig. 7). In another study the average number of fish species on the Swedish west coast were found to be positively correlated with temperatures exceeding 15°C (Thorman 1986).

The salinity of oceanic water is around 35‰, but in coastal areas the salinity can vary due to fresh water run-off. This is seen in large scale from the Baltic Sea to the North Sea, which Aarhus Bay is placed in the middle of. The salinity in the study area ranges from 16 to 30 ‰ (Fig. 9) and is in the range of brackish water. In this study five species (*S. typhle, N. ophidion, Z. vivparus, S. spinachia and P. gunnellus*) are defined as truly estuarine resident while nine species (*C. rupestris, S. melops, G. morhua, G. flavescens, P. virens, B. belone, C. labrosus and H. lanceolatus, T. bubalis*) are defined as marine species (Elliott 1995). Of the fourteen species observed during this study almost half were estuarine species and the other half marine species. *Spinachia spinachia,* observed two out of the five months of the survey, is known to prefer brackish waters (Elliott 1995; Mendonca et al. 2009). *Gadus morhua* was categorized in a study as a migrant species, which use estuaries primarily as habitats for juveniles and then spend most of their adult life at sea (Elliott 1995).

Dissolved oxygen concentration is believed to be the most important predictor of fish abundance (Maes et al. 2004). I did not include oxygen concentration as a factor in this study but assumed the effect of oxygen levels were of minor concern because of the location of the study was rocks and not the deeper sea bottom. The shallow coastal area would probably be very well mixed. In the latest years Aarhus Bay has experienced hypoxia in the late summer and fall. This year, from 24 August to 20 September 2017, the bay experienced a moderate oxygen depletion (2-4 mg/l) in the area around Aarhus Harbor, with only one site of severe hypoxia in Knebel Vig (Hansen et al. 2017b; a).

#### 4.1.4. Method efficiency

The species recorded in this study are resident in coastal areas and not targeted by conventional fishing methods. It is difficult to conduct surveys at boulder reefs because traditional methods such

as trawl can not be used. This study was conducted at relative shallow depths, because it had to be covered by SVC. Sargassum muticum was a dominant alga at AO. From July and onwards did this alga grew rapidly and became very abundant with an estimated height of more than one meter (Fig. 19). This could have affected the visibility and thereby the task of making a list of fish species. However, I observed S. spinachia more easily because they swam at the top of S. muticum. Edgar et al. (2004) found the factor of reduced visibility in vegetated habitats to be negligible. Pelagic species in the surface can be hard to observe while simultaneously observing benthic species (Pedersen and Figure 19. Representative habitat, boulders Eskelund 2012).



covered with S. muticum at 1 m depth at Aarhus Ø in August 2017.

I found that the behavior of fish seemed less affected when I swam very slowly and avoided fast movements. I observed that the fish, particularly wrasses, were very curious and came very close to me. Divers and snorkelers are known to affect the behavior of fish while during surveys, but no significant and consistent changes occur (Dearden et al. 2010).

Some species were observed regularly by the Danish project Fiskeatlas at Aarhus Harbor, Salmo trutta trutta, Pomatoschistus minutus, Gobius niger, Myoxoceohalus scorpius and Syngnathus rostellatus (one species they have registered this year) (personal communication, Henrik Carl) but were never observed by me. This could be due to the large numbers of G. flavescens or, maybe, limited experience from my side. Taurulus bubalis were observed but could have been mistaken for Myoxocephalus scorpius. I am convinced that in this case it was T. bubalis due to its smaller size compared with the bigger M. scorpius. All cottids caught for the gut analysis were T. bubalis, which supported the observations. An observation done by SVC cannot be checked by a more experienced diver. A study addressed this problem and found that trained non-experts can provide reliable data, and that the difference between expert and non-expert only differed by 10% (Hassell et al. 2013).

One method that I decided not to use was underwater video. Other studies have used cameras to quantify small scale distribution of fish and it affects the behavior of fish less than snorkelers (Harvey and Shortis 1998; Dearden et al. 2010; Hansen 2012). But by recommendation this was excluded due to the problems with filming the whole area at once and that cameras do not allow depth of field. The chance of a shoal of gobies lingering in front of the camera, and covering for the sight is also high. It is also only possible to film during daylight.

# 4.1.5. Conclusion

My results of the SVC showed no differences in fish assemblage between the two stations. For all five months of this study both boulder reefs were dominated by *G. flavecsens* and two labrids species, *C. rupestris* and *S. melops*. Neither temperature nor salinity had any impact on abundance of fish species. Only one species, *G. morhua*, was observed significantly more at night than by day. Several species were observed once or just a few times. This could be caused by migration due to food availability or reproduction.

# 4.2. Stomach content analysis

#### 4.2.1. Diet analysis

Only one species, *C. rupestris*, was caught in the numbers recommended to give a sufficient picture of food utilization (Crow 1981; Gibson and Ezzi 1987). For *C. rupestris* 186 stomachs were collected, but for the other species occurring in this study only a few individuals were caught and this prevented any analysis of temporal changes in diet over the sampling period. I decided that even with the inconsistent samplings of stomachs for some species the collected stomachs could still provide a picture of what to expect for their diet. The fish species included in this analysis were all observed during my dives. Some food categories are quickly digested and consequently, difficult to detect. Other food species such as crustaceans have chitinous exoskeletons, which remain in the stomachs for longer (Wooton 1999).

## Gadus morhua

*Gadus morhua* had different food composition at the two stations, but most food species were benthic and mobile (Figs. 10 + 12). At AH 80% of the diet consisted of mobile crustaceans. At AO the diet was more diverse with much macroalgae and gastropods. *Gadus morhua* from AO, 28-31 cm long, took the largest number of gastropods. These *G. morhua* also contained macroalgae. The species of gastropods were *B. reticulatum, L. parva* and *R. parva. Bittium reticulatum* is associated with soft substratum while *L. parva* and *R. parva* are associated with vegetation on hard substrate (Hayward and Ryland 1995). *Lacuna parva* is often found on *F. vesiculosus* (Hayward and Ryland 1995), an alga living at both stations. *Rissoa parva* is usually found on finely branched algae, e.g., *Polysiphonia* species (Hayward and Ryland 1995) and they were dominant at both stations. Crustaceans found in the stomachs were mainly *C. maenas* but also *C. crangon. Carcinus maenas* is

a benthic mobile species, very common in most areas along the coast and is known to be night active (Køie et al. 1991; Hayward and Ryland 1995). *Crangon crangon* is an epibenthic species usually found on sand bottom and is usually hiding in the sand during the day (Køie et al. 1991; Hayward and Ryland 1995). The prey species indicate that *G. morhua* is opportunistic and searches for prey both at the boulder reefs as well as sand bottom near the reef. I found whole fish and fish spines in the stomachs (Appendix 2.3). In this study I conclude that *G. morhua* was opportunistic and generalist which consumed primarily benthic mobile prey. Other studies have also found that crustaceans are the main diet for *G. morhua* (Blegvad 1916; Pihl 1982; Keats and Steele 1992; Stenberg et al. 2012) while a study in southern Norway found that juvenile *G. morhua* offen took prey associated with seaweed e.g. gastropods (Fjøsne and Gjøsæter 1996). Feeding cycles of *G. morhua* is also piscivorous (Blegvad 1916; Stenberg et al. 2012). One study showed that cod temporarily specializes its feeding on the most abundant prey. *Gadus morhua* is adapted to feed mainly near the bottom where it can catch prey epibenthic or endobenthic (Mattson 1990).

The amount of prey could be biased because most cod contained only one big *C. maenas* while others contained several gastropods, which will then account for a higher content when data were presented as frequencies. Numbers of prey can cause overestimation of the small prey organisms ingested in large amounts (Hyslop 1980).

#### Syngnathus typhle and Nerophis ophidion

The two pipefish analyzed for stomach contents were *S. typhle* and *N. ophidion*. Only three individual samples were examined and the one stomach of *N. ophidion* was empty. But the two stomachs of *S. typhle* contained 100% crustaceans (Tab. 8), the harpacticoid copepod *D. nobilis* which is associated with algae (Hicks and Grahame 1979). This indicates that *S. typhle* is a zooplanktivore. A study in Denmark showed that young individuals of *S. typhle* mainly ate copepods, but adults would also eat small fish and juveniles of Gobiidae (Blegvad 1916).

#### Spinachia spinachia

*Spinachia spinachia* was only observed at AO. The diet mainly consisted of pelagic species (92%) but also few benthic species (7.5%) (Fig. 10). The majority was crustaceans (93.5%) (Tab. 8), mainly the shrimp stage of *C. maenas*, just followed by *D. nobilis*, associated with algae (Appendix 2.3). So, the diet of *S. spinachia* consist of both pelagic and benthic prey. Mendonca et al. (2009) found that the diet of *S. spinachia* consisted of 90% harpacticoids in a shallow lagoon in the UK.

# Taurulus bubalis

The epibenthic species *T. bubalis* diet consisted only of mobile benthic species (Figs. 10 + 12). At AO the majority of the diet was crustaceans and at AH it were crustaceans and macroalgae. The majority of the benthic species found in the gut contents were associated with vegetation (80%) (Fig. 11). The diet analysis of *T. bubalis* showed that this species is a benthic generalist. In this study *T. bubalis* had eaten species from the entire area of the reef and its surroundings. Barret et al. (2016) found that *T. bubalis* in the UK ate large amounts of crustaceans and gastropods but was primarily piscivorous.

## Zoarces viviparus

The benthic species Z. viviparus contained benthic food species (Fig. 10) of which the majority was associated with vegetation (Fig. 11). It took crustaceans (53% at AH and 67.9% at AO) and bivalves (35% at AH and 20% at AO) (Tab. 8). Numerous M. edulis (Bivalvia) and I. granulosa (Crustacea) were found together with a few individuals of T. basteri (Halacarida) and L. parva (Gastropda), all species associated with hard bottom and algae. Species associated with soft bottom were also found, such as A. virens and B. reticulatum. Zoarces viviparus searches for its prey among algae and ate numerous crustaceans such as Gammaridae and Idothea. Blegvad (1916) found M. edulis and other gastropods in individuals caught at Skagen, Denmark. The diet of Z. viviparus indicates that it is a generalist hunting mostly for benthic species, both active and sessile fauna among vegetation. This is also supported by small amounts of algae found in the stomachs. The algae were probably ingested while hunting for food species in the vegetation.

#### Gobiusculus flavescens

The diet of *G. flavescens* was mostly pelagic (Fig. 10). The stomach content found at AO mainly contained copepods (approx. 90%) (Tab. 8) and small amounts of gastropods but at AH Cladocera were also found (7.4%) (Tab. 8). The majority of the copepods were *T. longicornis* but *D. nobilis* was also found (Appendix 2.3). Both are pelagic species (Nielsen and Hansen 1999) but *D. nobilis* is known to be associated with algae (Hicks and Grahame 1979). Other pelagic species were *E. nordmanni* (Cladocera) and the shrimp-stage of *C. maenas* (Crustacea) of which the majority was found in stomachs found at AH. This indicates that *G. flavescens* is primarily a zooplanktivore, which is supported by Fosså (1991). However, at both stations a few individuals of gastropods, *B. reticulatum* and *L. parva*, were found. These benthic species are and found on sand bottom and algae

(Hayward and Ryland 1995). The shoals of *G. flavescens* were observed among the algae where *G. flavescens* could have spotted *L. parva. Bittium reticulatum* could have been spotted in sandy patches between the boulders.

# Symphodus melops

The diet of *S. melops* was very diverse, but almost all food species were benthic (Tab. 9 + Fig. 10). The diet consisted mainly of crustaceans, gastropods and at AH also bivalves. The diet was more diverse at AH (Tab. 9). This could be caused a larger number of individuals caught at AH. Of the crustaceans, the majority were gammarids (Amphipoda). Overall *S. melops* ate a high proportion of mobile benthic prey which is usually associated with vegetation. Prey were slow moving such as gastropods or sessile such as bivalves (Figs. 10 + 11 + 12 + Appendix 2.3). A few species associated with soft substrate indicated that *S. melops* uses the boulder reef as feeding grounds though it might also find food in the sandy patches between the boulders and on the surrounding sand bottom. I found *S. melops* to be a generalist eating a large variety of food species. An old study from Nyborg fjord also found that *S. melops* primarily ate crustaceans, gastropods and bivalves. They also found gammarids, *Idothea*, copepods, insect larvae (Chironomidae) and *M. edulis* and a small amount of juvenile Gobiidae in the stomachs (Blegvad 1916).

# Ctenolabrus rupestris

The diet of *C. rupestris* consisted of benthic sessile food species (Fig. 10 + Fig. 12). It contained many crustaceans but the majority of the stomach content consisted of small bivalves (Tab. 8). The stomach content had a high diversity (Tab. 9) and *C. rupestris* was the species with the highest intake of different taxa (Tab. 9). For both stations, numerous food species from hard bottom were found (60%) (Fig. 11). *Ctenolabrus rupestris* mostly ate benthic sedentary prey, primarily small *M. edulis* (0.5-1.5 mm), but benthic mobile prey associated with algae was also found in the stomach content. *Ctenolabris rupestris* locates prey epibenthic or hyperbenthic and on hard bottom and vegetation in the littoral zone. During the dives *C. rupestris* was often observed with algae in the mouth. This could probably be because of food species located on the algae. *C. rupestris* uses the boulders and the algae as a feeding ground, but the results also showed that it is capable of finding prey elsewhere such as on a sand bottom or in the pelagial. I found *C. rupestris* to be a feeding generalist.

The diet of C. rupestris resembles the results from other studies. They found bivalves,

gastropods, crustaceans, amphipods, decapods and fish (Gobiidae) in its diet - species that are slowmoving, sedentary or active and mostly taken on or close to the bottom (Blegvad 1916; Sayer et al. 1995; Fjøsne and Gjøsæter 1996; Stål et al. 2007).

The size groups did not show any significant differences in their diet (Fig. 11). The small sized *C. rupestris* (3-4cm and 5-6cm) were only caught at AH. Their diet consisted of few taxa, mainly crustaceans (Amphipoda and Copepoda). The size groups from 7-8 to 13-14cm contained a variety of different taxa. At AH the majority (approx. 30%) of this diet was crustaceans while only approximately 20% at AO. At AO and AH, the intake of algae was almost as high as crustaceans or higher. This indicates, as earlier mentioned, that *C. rupestris* eats prey associated with the vegetation. The largest size class (14-15cm) was only caught at AO and it only ate bivalves and algae. A study from Scotland found that the food of juvenile *C. rupestris* was dominated of harpacticoid copepods and suggested that they forage on epibenthos (Sayer et al. 1995). This corresponds to my finding of *D. nobilis* in the guts of small sized *C. rupestris* in my study.

The diet of *C. rupestris* at both stations showed a shift from crustaceans to bivalves over the four months during my study. In May, the intake of crustaceans was 95% at AH and 52% at AO but declined to 17% at AH and 2% at AO in August. This large amount of crustacenas in the late spring was probably caused by the spring bloom of phytoplankton in March-April (Nielsen and Hansen 1999). Copepods feed on phytoplankton but could decline in numbers because of increased predation. Sayer et al. (1995) also found that small newly settled mussels (2-4 mm) dominated in the stomachs of *C. rupetris* in June and July. The diet was more diverse at AO than at AH, this could be a bias caused by more individuals caught at AO.

The high consumption of benthic food species indicates that most fish species in this survey use the boulder reefs as a feeding ground. Both stations studied had a dense vegetation cover that create a mosaic of different microhabitats which might support numerous of food species available. Also, the small amount of empty stomachs (3.6%) indicates the rich availability of food. A study from the Swedish west coast found a larger number of species and abundant prey on rocky bottoms compared to soft bottoms. This was due to a dense vegetation cover at the rocky habitat (Stål et al. 2007). Another study also supports my observations that fish found on boulder reef live of benthos (Stenberg et al. 2012). The food availability in a specific habitat can indicate the quality of habitat which reflects the habitat requirements of the fish (Wennhage et al. 2007).

#### 4.2.2. Trophic categories

Bray-Curtis similarity was calculated to determine the trophic categories for the fish species. *Gobiusculus flavescens* and *S. typhle*, according to Bray-curtis similarity are in their own trophic category while the rest of the species belong to another. This is supported by my results that showed *G. flavescens* and *S. typhle* to be zooplanctivores while the other species had a more diverse, generalist diet.

#### 4.2.3. Food species patterns

The copepods *T. longicornis* and *D. nobilis* were found at both stations. *Diarthrodes nobilis* dominated in May and June while *T. longicornis* dominated in July and August. *Diarthrodes nobilis* had their population peak after the spring bloom and decreased in numbers in July and August probably due to predation. *Tamora longicornis* dominates later in the season. This peak could be due to the second plankton bloom in the late summer. This bloom occurs because of wind and degradation of the thermocline which brings new nutrients to the surface (Nielsen and Hansen 1999).

The dominant amphipod at both stations was *C. bonnelli*. Other amphipods frequently recorded were *M. grylloptalpa* and *M. pellucida*. The content of amphipods found in the stomachs declined over the timespan at AO but at AH the number of amphipods was more stable. This could be because of predation or differences in number of gut contents examined for each station.

*Carcinus maenas* was found in its zoëa and shrimp stages from May. Very small adults were found in the stomachs from August. The zoëa are pelagic before becoming to the shrimp and adult stage (Nielsen and Hansen 1999).

# 4.2.4. Method efficiency

The fykenets used in this study were selective. Smaller fish such as Gobiidae were too small to be caught due to mesh size. Six species were caught in the fykenets, while smaller fish were caught by a handhold net. Only a few small individuals of *C. rupestris* were caught because the holes in the fykenets were big enough to allow them to escape. The small number of individuals caught would probably also have escaped, if the fykenets were not frequently emptied repeatedly.

The gut examination might have underestimated small food species due to quick digestion. This was largely avoided by short interval of emptying of the fykenets and quick transfer of the stomachs to formalin.

#### 4.2.5. Conclusion

Two species, *S. typhle* and *G. flavescents*, had a pelagic diet. The last six species, *S. spinachia*, *G. morhua*, *T. bubalis*, *Z. viviparus*, *S. melops* and *C. rupestris*, all ate a benthic diet. Two trophic categories were found among all fish species. *Gobisuculus flavescens* and *S. typhle* were zooplanktivorous while the rest were considered generalists. All species consumed much crustaceans. Only one species, *C. rupestris*, ate more bivalves than crustaceans. The majority of the food species were benthic. I found that boulder reefs can provide fish species with food. Several of them seemed to utilize both the boulder reef and the surrounding area.

#### 4.3. Stable isotope analyses

# 4.3.1. Difference between stations

In my study, I found that four species (*S. melops, T. bubalis, G. flavescens and G. morhua*) caught at AH had a higher value of  $\delta^{15}$ N than the species caught at AO (Fig. 15). This could indicate that the fish species from AH is part of a different food chain, then those from AO. The two different food chains probably reflect that samples were collected in two different areas. *Mytilus edulis* is, based on carbon isotopes, planktivory consuming in marine coastal environment and is considered to have lower  $\delta^{13}$  C relative to benthic consumers such as gastropods. therefore *M. edilus* serve as a baseline organism for pelagic derived production. The differences in *M. edulis* values could reflect different conditions at each station. Next to AO is the outrun from Aarhus river, bringing freshwater from inland areas. *Alitta virens* can be used as a baseline for benthic derived prey in fish species.

#### 4.3.2. Stable isotope composition of fish species

Gadus morhua had the highest trophic position and was the top predator in this ecosystem (Fig. 15). It would be expected to have a higher position if its main prey was only consisting of fish. The position of *G. morhua* however, had lower  $\delta^{13}$ C values could indicate a more benthic diet, supported by stomach content. The higher  $\delta^{15}$ N values in *G. morhua* at AH could be caused by ingestion of larger food species such as fish, polychaetes and decapods. The ingestion of crustaceans is shown in Fig. 16. The lower  $\delta^{15}$ N values at AO could be caused by the ingestion of small individuals of *C. maenas*, gastropods, bivalves and algae.

*Taurulus bubalis* (AH and AO), *C. rupestris* (AO) and *Z. viviparus* were next in the trophic positions under *G. morhua. Taurulus bubalis* (AO) and *C. rupestris* (AO) had a wide range of  $\delta^{13}$ C values, which could implie a generalistic feeding mode. The high overlap between these two species indicates a similar use of resources (Tab. 15). The generalistic feeding corresponds with other studies (Fig. 15) (Fjøsne and Gjøsæter 1996; Norderhaug et al. 2005; Hielscher et al. 2015). *Taurulus* 

*bubalis* and *Z. viviparus* would be expected to have a higher position if their prey included fish. The position of *Taurulus bubalis* and *Z. viviparus* however, was lower indicating a more benthic diet. Similar findings were discovered by Hielscher et al. (2015). The high trophic position of *T. bubalis* (AH and AO), *C. rupestris* (AO) and *Z. viviparus* and the low trophic position for *G. flavescens* could be explained by other studies that a strong predation of juvenile Gobiidae by these species (Blegvad 1916; Norderhaug et al. 2005).

Symphodus melops (AH), P. virens (AO) and C. rupestris (AH) are grouped together in the next trophic positions (Fig. 15). Ctenolabrus rupestris (AH and AO) have a wide range of  $\delta^{13}$ C values, which could indicate a generalistic feeding mode or be because of sample size. Symphodus melops (AH) was different from S. melops (AO). Symphodus melops from AH and AO were different. At AH they has a more benthic diet and was almost one trophic position higher (3.2‰) than S. melops from AO. Pollachius virens had a more pelagic diet than G. morhua which was expected because P. virens is a more pelagic species than G. morhua. Symphodus melops (AH) and C. rupestris are closer to M. edulis in Fig. 16, supporting earlier findings of bivalves in large proportion in the gut contents (Appendix 2.3).

In the bottom of the trophic positions S. typhle (AO and AH), N. ophidion and G. flavescens, S. spinachia and surprisingly S. melops (AO) were found. Syngnathus typhle and, G. flavescense had a clearly pelagic diet, which corresponds with the gut content. Gobiusculus flavescens had a slightly more pelagic diet at AO than at AH. The stomach had large amounts of pelagic copepods. Symphodus melops also had a pelagic diet according to the stable isotope analysis, but this was only partly corresponded to the gut contents because pelagic species such as C. maenas (shrimp stage) in combination with larger amounts of gastropods were found in the gut content. Gut examination can underestimate small pelagic species such as copepods due to their faster digestion rate. This can explain the differences in the stable isotope results and gut content for S. melops. Stable isotope represents the diet over time and S. melops may have eaten a more pelagic diet before this study. Only one individual of S. melops at AO was analyzed, so the result cannot be assumed to be representative of the whole population. Nerophis ophidion and S. spinachia have similar  $\delta^{15}$ N values but very different  $\delta^{13}$ C value. The higher  $\delta^{13}$ C value reflects a considerably more benthic diet. This does not corresponds to the gut contents for S. spinachia where almost only pelagic species were found (Fig. 10). Nerophis ophidion had an empty stomach and its content could have been digested. According to the stable isotope analysis N. ophidion ate benthic prey. Other studies have found N. ophidion to eat small Mytilus, Gammaridae and Idothea (Blegvad 1916) which could explain the values that reflect a benthic diet.

#### 4.3.3. Ctenolabrus rupestris

According to the stable isotope analysis of *C. rupestris* there was a difference between the two stations. It ate a more pelagic diet at AH than at AO (Fig. 17). For all samples, the arguments for enrichment (+1‰ and +3.2‰) subtracted will move *C. rupestris* down to *M. edulis* (Fig. 16). *Mytilus edulis*, as earlier mentioned, has a pelagic diet and all samples of *C. rupetris* ate a higher enrichment in  $\delta^{13}$ C pointing towards a more benthic diet. At AH *C. rupestris* contained more benthic prey in June and July such as polychaetes, echinoderms, and gastropods (Tab. 12). For the individuals caught in August there were no indications of a more pelagic diet. Pelagic food could have been digested by the time the stomach examination took place. *Ctenolabrus rupestris* from August at AO had a high proportion of gastropods which could explain the highest enrichment in  $\delta^{13}$ C. In June was a large amount of amphipods and copepods which could explain the position (Fig. 17 + Tab. 11). The greater depths at AH could explain the higher exploitation of the water column compared with AH. At AO the reef is very shallow with a large amount of larger algae such as *Fucus* and *Laminaria* which create microhabitats for benthic and algae associated prey. This might explain the benthic diet values.

The same pattern was found for *C. rupestris* divided into size groups, which showed larger enrichment of  $\delta^{13}$ C for the samples from AO. The size groups were distributed into a pattern of different trophic positions (Fig. 18). The smallest size groups (7-9cm) had the lowest enrichment of  $\delta^{15}$ N and consequently the lowest trophic position. This indicates that small individuals of *C. rupestris* ate from the lower part of the food chain such as amphipods and copepods, which were both found in the stomachs (appendix 2.3). The larger size groups (13-15cm) had the highest enrichment of  $\delta^{15}$ N and consequently, the highest trophic position (Fig. 18) because they ate from a higher position of the food chain than the smaller sized *C. rupestris*. Larger sized *C. rupestris* are big enough to prey on juvenile Gobiidae. Juveniles of Gobiidae were frequently observed in large shoals at each station. This could cause a higher enrichment of  $\delta^{15}$ N. A study argued that the  $\delta^{15}$ N of fishes typically increases with body size due to size based changes in diet, since larger fish usually feed on larger prey (Sweeting et al. 2007). This seems to be the case also in my study. It was a small enrichment and *C. rupestris* was located between the benthic and the pelagic feeding fish species, indicating that *C. rupestris* is a generalist. This conclusion supported my results from the gut examinations and by other studies (Sayer et al. 1995; Fjøsne and Gjøsæter 1996; Stål et al. 2007).

## 4.3.4. Conclusion

The stable isotope results enabled insight into the intertidal food web. The trophic structure resulted in *G. morhua* as the top predator and the pipe fish species and *G. flavecsens* in the bottom. The

species with a pelagic diet were the zooplanktivorous *S. typhle* and *G. flavescens. Gadus morhua* had an entirely benthic diet. The rest of the species (*C. rupestris, T. bubalis and Z. vvivparus, S. melops, N. ophidion, S. spinachia* and *P. virens*) had a diet varying between benthic and pelagic prey. *C. rupestris* at AO had a more benthic diet than at AH. Smaller individuals of *C. rupestris* ate a more pelagic diet than the larger ones.

# 4.4. Attraction or production

Bohnsack discussed if artificial reefs attract fish populations due to behavioral preferences and increased production of reef fish (Bohnsack 1989). This study showed that the fish species caught do indeed utilize the boulder reef as a food resource. This supports the production hypothesis. The few fish species eating pelagic prey were also attracted by the reef. This was due to both food choices and behavior. The pipefish ate a pelagic copepod associated with algae and pipefish are known to be found among vegetation. Gobius flavecsens also ate food associated with the reef, but also reproduction was observed at the reefs during the summer months. The choice to reproduce at the reef could support the production hypothesis that the reef provide shelter from predation. But the fish species could also reproduce other areas and the juveniles migrate to the boulder reef for shelter. Behavioral preferences are hard to exclude. Fishes may use reef structures for orientation and to avoid unproductive foraging areas (Bohnsack, 1989). For migrating species this could be the case. The labrid species use the reef to find food, but a small amount of food species was found at the soft bottom. When the labrids showed they also were able to find food in other habitats that could indicate a behavioral preference to live at the reef. But as Bohnsack (1989) argue, attraction and production does not exclude each other due to differences between species. Both hypothesis can probably affect fish species.

# 5. Conclusion

The fish communities at the two boulder reefs in Aarhus Bay were dominated by G. flavecsens and two species of labrids, C. rupestris and S. melops. These three species together with G. morhua showed no changes in abundance due to changes in temperatures or salinity. Diurnal differences were only found for G. morhua. Based on the stomach and stable isotope analysis of nitrogen and carbon, the majority of the food species were benthic. The major prey item appears to be crustaceans for both stations. Only one species, C. rupestris, ate more bivalves than crustaceans. The food species were often found on vegetation or on hard bottom. A few species (S. typhle, S. spinachia and G. flavescens) ate a pelagic diet. The trophic structure resulted in G. morhua as the top predator and the pipe fish species and G. flavecsens in the bottom. This study supports that fish species utilize the boulder reefs as a feeding ground but also showed that they are not limited to this habitat. This study therefore supports why the boulder reefs are important to restore. This study also found no significance evidence of differencies of fish fauna or utilization of the new and older boulder reefs. These findings indicate that, only five years after reconstruction of the boulder reef at Aarhus Ø, this reef can support the fish fauna. Further investigations are needed to fully understand the ecology of fish species on boulder reefs. Seasonal patterns of the fish assemblage could provide further information about the exploration of the reefs.

# 6. Literature

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**Appendix 1. Species and Auctor** A 1.1. Fish species and auctor. All fish species mentioned in the report.

Species	Auctor	Species	Auctor		
Belone belone	Linnaeus, 1760	Platichthys flesus	Linnaeus, 1758		
Chelon labrosus	Rissoa, 1827	Pleuronectes platessa	Linnaeus, 1758		
Ctenolabrus rupestris	Linnaeus, 1758	Pollachius virens	Linnaeus, 1758		
Gadus morhua	Linnaeus, 1758	Salmo trutta trutta	Linnaeus, 1758		
Gobiusculus flavescens	Fabricius, 1779	Scomber scombrus	Linnaeus, 1758		
Hyperoplus lanceolatus	Le Sauvage, 1824	Spinachia spinachia	Linnaeus, 1758		
Limanda limanda	Linnaeus, 1758	Symphodus melops	Linnaeus, 1758		
Merlangius merlangus	Linnaeus, 1758	Syngnathus rostellatus	Nilsson, 1855		
Myoxocephalus scorpius	Linnaeus, 1758	Syngnathus typhle	Linnaeus, 1758		
Nerophis ophidion	Linnaeus, 1758	Taurulus bubalis	Linnaeus, 1758		
Pholis gunnellus	Linnaeus, 1758	Zoarces vivparus	Linnaeus, 1758		

A. 1	1.2.	Food	species	from	gut	content	and	auctor.
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Species	Auctor	Species	Auctor
Allita virens	Sars, 1835	Laomedea flexuosa	Alder, 1857
Allomelita pellucida	Sars, 1882	Leptocheirus pilosus	Zaddach, 1844
Apherusa jurenei	H. Milne Edwards, 1830	Microdeutopus gryllotalpa	Costa, 1853
Bittium reticulatum	da Costa, 1778	Musculus discors	Linneaeus, 1767
Caprella linearis	Linneaeus, 1767	Musculus subpictus	Cantraine, 1835
Carcinus maenas	Linneaeus, 1758	Mya arenaria	Linneaeus, 1758
Cerastoderma edule	Linneaeus, 1758	Mytilus edulis	Linneaeus, 1758
Clunio marinus	Haliday, 1855	Ophiura albida	Forbes, 1839
Corophium bonnelli	H. Milne Edwards, 1830	Pagurus bernhardus	Linneaeus, 1767
Crangon crangon	Linneaeus, 1758	Podon leuckarti	Sars, 1862
Dendrodoa grossularia	van Beneden, 1846	Psammechinus milaris	Muller, 1771
Dexamine spinosa	Montagu, 1813	Rissoa parva	da Costa, 1778
Diarthrodes nobilis	Baird, 1845	Semibalanus balanoides	Linneaeus, 1767
Evadne nordmanni	Lovén, 1836	Spirorbis spirorbis	Linneaeus, 1758
Glyptotendipes barbipes	Stæger, 1839	Strigamia maritima	Leach, 1817
Hyale pontica	Rathke, 1847	Styela coricea	Alder & Hancock, 1848
Idotea balthica	Pallas, 1772	Temora longicornis	Muller O.F., 1785
Idotea granulosa	Rathke, 1843	Thalassarachna basteri	Johnston, 1836
Lacuna parva	da Costa, 1778		

# A 1.3. Algae species and auctor.

Species	Auctor	Species	Auctor
Ceramium virgatum	Roth, 1797	Polysiphonia fucoides	(Hudson) Greville, 1824
chondrus crispus	Stackhouse, 1797	Rhodomela confervoides	(Hudson) P. C. Silva, 1952
Fucus serratus	Linnaeus, 1753	Sargassum muticum	(Yendo) Fensholt, 1955
Fucus vesiculosus	Linnaeus, 1753	Sphacelaria cirrosa	(Roth) C. Agardh, 1824
Furcellaria lumbricalis	J. V. Lamouroux, 1813	Ulva lactuca	Linnaeus, 1753
Phycodrys rubens	(Linnaeus) Batters, 1902	Zostera marina	Linnaeus, 1753
Polysiphonia elongata	(Hudson) Sprengel, 1827		

# Appendix 2. Gut examination

Cm/Month	Μ	lay	Ju	ne	Ju	ly	Aug	gust
	Ø	Н	Ø	Н	Ø	Н	Ø	Н
3-4		1						
4-5								
5-6		1						
6-7								
7-8	5	3	1			2	2	2
8-9	3	5	5	4	4	7	8	4
9-10	11	3	10		13	8	11	2
10-11	1	1	6	3	14	2	5	2
11-12			4		7	3	3	1
12-13	2		2		3			
13-14			1		2		1	
14-15					1			

A 2.1. Number of caught goldsinny wrasse (*Ctenolabrus rupestris*) sorted into size (cm) for each station each month by total length.  $\emptyset$  = Aarhus  $\emptyset$ . H = Aarhus harbor.

A 2.2. Literature used to determine food species from gut content.

Species		Used for determination
Halacaridae	1.	Green, J. and Miranda Macquitty (1987). Halacarid Mites – keys and
		notes for the identification of the species. The Linnean Society of
		London and the Estuarine and Brackish-Water sciences Association.
		The Bath Press.
Amphipoda	1.	Jensen, Kurt (1978). Tanglopper, Illustreret nøgle til danske
		tanglopper(Amphipoda). BIOKON aps.
	2.	Oldevig, Hugo (1933). Sveriges Amphipoder. Elanders Boktruckeri
		Aktiebolag
	3.	Sars, G.O. (1895). An account of the Crustacea of Norway, Vol.1.
		Amphipoda(plates). ALB Cammermeyers forlag.
Ascidiacea	1.	Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
		Planter. 2 udgave. Gyldendal A/S.
	2.	Lutzen, Jørgen G. (1967). Danmarks Fauna Bd. 75 – Sækdyr. Gads Forlag.
Bivalvia	1.	Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
		Planter. 2 udgave. Gyldendal A/S.
	2.	Bondesen, Paul (1984). Danske Havmuslinger. Naturhistorisk Museum,
		Århus. Rosendahl Bogtrykkeri.
Campanulariidae	1.	Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
		Planter. 2 udgave. Gyldendal A/S.
Chironomidae + Insecta	1.	Boy Overgaard Nielsen
Macroalgae	1.	Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
		Planter. 2 udgave. Gyldendal A/S.

	2. Birgit Olesen
Cladocera + Copepoda	1. Nielsen, Torkel Gissel og Per Juel Hansen. (1999). Dyreplankton i
	danske farvande. TEMA-rapport fra DMU, 28/1999. Scanprint as.
Decapoda	1. Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
	Planter. 2 udgave. Gyldendal A/S.
	2. Stephensen, K. (1910). Danmarks Fauna. Storkrabs, Skjoldkrebs. Gads
	Forlag.
Gastropoda	1. Bondesen, P. (1994). Danske havsnegle. Naturhistorisk Museum, Århus.
	Rosendahl Bogtrykkeri.
Myriapoda	1. Hayward, P. J. And J. S. Ryland. (1995). Handbook of the Marine Fauna
	of North-West Europe. Oxford University press.
Echinodermata	1. Køie, M., Å. Kristiansen and S. Weitmeyer. 1991, 2014. Havets Dyr og
	Planter. 2 udgave. Gyldendal A/S.
	2. Mortensen, T. H. (1924). Danmarks Fauna – Pighuder(Echinodermer).
	Gads forlag.
Polychaeta	1. Dahl, Friderich, Maria Dahl, Fritz Peus. (?). Die Tierwelt Deutschlands –
	Annelida, Borstenwurmer, Polychaeta. Gustav Fischer Verlag Jena. P 19.
	2. Hayward, P. J. And J. S. Ryland. (1995). Handbook of the Marine Fauna of
	North-West Europe. Oxford University press.

A 2.3. Overview over species in gut contents for all species. BP = Broadnosed pipefish (*S. typhle*), CO = Cod (*G. morhua*), CW = Corkwinge wrasse (*S. melops*), FS = Fifteen-spined stickleback (*S. spinachia*), GW = Goldsinny wrasse (*C. rupestris*), SE = Seith (*P. virens*), LB = Longspined bullhead (*T. bubalis*), TG = Two-spotted Goby (*G. flavescens*), VE = Viviparous eelpout (*Z. viviparus*). For *C. maenas* is both zöea and shrimp stage and adults included.

	В	Р	CO		С	W	FS	0	σw	SE	L	B	TG	VI	Ξ	Total
	AH	AO	AH	AO	AH	AO	AO	AH	AO	AO	AH	AO	AH AO	AH	AO	
Algae																
Chlorophyceae				2				9	46		1			1		59
Chlorophyceae sp.				1				9	41		1			1		53
Cladophora sp.									5							5
Ulva lactuca				1												1
Rhodophyceae			2	11	37	1		297	509		4				1	882
Ceramium virgatum					26			212	161							399
Delesseria sanguinea				4	3			14	61							82
Rhodomela confervoides				3	4			62	193							262
Chondrus crispus			2	4	4	1		10	113		4				1	139
Phaeophyceae				3				6	30							19
Sargassum muticum				3				5	11							19
Ascidiacea																
Dendrodoa grossularia					1			2	101							104
Styela coriacea								1								1
Bivalvia																
Cerastoderma edule					4											4
Musculus discors					3				1							4
Musculus subpictus					7			2	18							27
Mya arenaria				1	3				6							10
Mytilus edulis				1	101		1	2124	5829					10	1	8067
Chaetognatha																
Sagitta sp.								1	1							2
Chironomidae																
Clunio marinus					8	1		6	84							99
Glyptotendipes barbipes									2							2
Cladocera																
Evadne nordmanni													40			40
Podon leuckarti									1							1
Cnidaria																
Laomedea flexuosa					13			9	68							90
Crustacea																
Decapoda																
Carcinus maenas			27	5	73	3	38	29	259				24 2			460
Crangon crangon			1	3					3							7
Semibalanus balanoides								1				1				2
Pagurus bernhardus															5	5
Isopoda																
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Idotea balthica				3		1	1							5		
Idotea granulosa				69		14	17		3 3	3		15		121		
Amphipoda																
Apherusa Jurinei				26										26		
Caprella linearis				7		10	4							21		
Corophium bonnelli				17		176	219		1					413		
Dexamine spinosa				1		5	2						8	16		
Microdeutopus gryllotalpa				64		65	64							193		
Gammaridae sp.						9	45						8	62		
Hyale pontica				7				1						8		
Leptocheirus pilosus				9										9		
Melita pellucida				42		21	39		8					110		
Copepoda																
Copepodit						544								544		
Temora longicornis						583	1161			415	93			2252		
Diarthrodes nobilis	10	1		20	,	24 39	739			48	51			932		
Echinodermata																
Psammechinus miliaris				7		51	90							148		
Ophiura albida			3											3		
Egg																
Egg				51			35			7				93		
Halacarida																
Thalassarachna basteri				23	4	62	209					1		299		
Insecta																
Ichneumonidae sp.						2	4							6		
Coleoptera sp.							2							2		
Formicidae sp.			1											1		
Gastropoda																
Bittium reticulatum			9	20	2	20	215			1			1	268		
Lacuna parva			1	39	1	180	868			1	1	1		1092		
Rissoa parva			5	97		611	221							934		
Myriapoda																
Strigamia maritima									1					1		
Nemertea																
Nemertea sp.							3							3		
Parasites																
Nematoda																
Nematoda sp.		7	4	12	1	27	56	7	3				1	118		
Platyhelminthes																
Rhabdocoela sp.							1							1		
Bothriocephalus sp.									2 4	1				6		

Trematoda sp.				2						44							46
Polychaeta																	
Neanthes virens			2					4	3			1				1	11
Nereididae sp.				2				1	6								9
Polynoidae sp.				1					2			1					4
Spirorbidae sp.								3									3
Spirorbis spirorbis									5								5
Teleost																	
Ctenolabrus rupestris			2														2
Teleost sp. (fish scales)				1	7			111	123		1		3				246
Teleost sp. (spine)			1	2							1						4
Hovedtotal	10	1	42	57	771	8	68	5026	11092	44	21	22	539	147	28	25	17915

	Aarhus Harbo	or				Aarhus Ø				Total
		May	June	July	August	May	June	July	August	
Algae										
Chlorophyceae										
Chlorophyceae sp.			11				9	31	2	53
Cladophora							5			5
Ulva lactuca									1	1
Phaeophyceae										
Sargassum muticum									14	14
Rhodophyceae										
Ceramium virgatum			30	178	30		19	68	74	399
Chondrus crispus	5		13		1	12	77	10	1	119
Delesseria sanguinea				4	13	5	12	21	27	82
Rhodomela confervoides			13	47	6	1	26	51	118	262
Ascidiacea										
Dendrodoa grossularia				2	1	64	2	35		104
Styela coriacea			1							1
Bivalvia										
Cerastoderma edule					4					4
Musculus discors			3				1			4
Musculus subpictus				2	7	9	2	7		27
Mya arenaria					3	1		5	1	10
Mytilus edulis			100	1807	328	9	579	3390	1854	8067
Chaetognatha										
Sagitta sp.		1				1				2
Chironomidae										
Clunio marinus		4	7		3	4	54	19	8	92
Glyptotendipes barbipes						2				2
Cladocera										
Evadne nordmanni			40							40
Podon leuckarti								1		1
Cnidaria										
Laomedea flexuosa	11		8	1	7	25	7	8	28	95
Crustacea										
Decapoda										
Carcinus maenas		2	3	1	146	2	3	223	79	459
Crangon crangon					1	1	3	2		7
Semibalanus balanoides				1				1		2
Pagurus bernhardus						5				5
Isopoda										
Idotea balthica		1	3					1		5

A 2.4. Overview over species in gut contents for all species sorted in to months for each station. For *C. maenas* is both zöea and shrimp stage and adults included.

Idotea granulosa			80	8		13			6	1		13	121
Amphipoda													
Apherusa jurinei					28						3		31
Caprella linearis	8	5		1	3			4					21
Dexamine spinosa					6			9	1				16
Gammaridae sp.	7	2						24	29				62
Hyale pontica		8											8
Leptocheirus pilosus		1			8								9
Melita pellucida	2	48		9	4			2	20	25			110
Corophium bonnelli	64	14		47	68			125	63	16	16		413
Microdeutopus gryllotalpa	50	38			41			32	10	20	2		193
Copepoda													
Copepodit	544												544
Temora longicornis		50		583	365			68	151	1008	26		2251
Diarthrodes nobilis	32	84			1			104	682		29		932
Echnodermata													
Ophiuroidea													
Ophiura albida												3	3
Parechinidae													
Psammechinus miliaris				42		16				62		28	148
Egg													
Egg			58					9	33				100
Gastropoda													
Bittium reticulatum			29			12		2	77	89		59	268
Lacuna parva			16	115		90		0	29	281		561	1073
Rissoa parva			3	509		196		46	10	29		141	917
Halacarida													
Thalassarachna basteri	7	13		28	38		11		128	21	53		299
Insecta													
Coleoptera sp.										2			2
Formicidae sp.								1					1
Ichneumonidae sp.		2								4			6
Myriapoda													
Strigamia maritima			1										1
Nemertea													
Nemertea sp.								2	1				3
Parasites													
Nematoda													
Nematoda sp.	8	20		14	11		21		10	17	17		118
Platyhelminthes													-
Turbellaria sp.								1					1
Bothriocephalus sp.			2						2	2			6
Trematoda sp.									46				46

Polychaeta									
Neanthes virens	6				5				9
Nereididae sp.			1		1	3	2	2	9
Polynoidae sp.						1	3		4
Spirorbidae sp.			3						3
Spirorbis spirorbis					1	3	1		5
Teleost									
Ctenolabrus rupestris				2					2
Teleost sp.		5	95	23	1	55	52	18	249
Hovedtotal	754	709	3498	1475	610	2159	5508	3178	17915

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## Appendix 3. Stable isotope analysis.

3.1. Output from SIBER analysis



Ellipses formed by SIBER analysis for G. morhua, N. ophidion, S. typhle, S. Spinachia, T. bubalis, Z. viviparus, G. flavescens, S. melops, C. rupestris and P. virens for both station and for the food species M. edulis and crustaceans.



Ellipses formed by SIBER analysis for *C. rupestris* for June, July and August for both stations.



Ellipses formed by SIBER analysis for all size groups at each station for C. rupestris.