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Invasive round goby, *Neogobius melanostomus* - suitable prey for native cod, *Gadus morhua*?

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Illustration: Karl Jilg/ArtDatabanken, SLU

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Invasive round goby, *Neogobius meanostomus* – suitable prey for native cod, *Gadus morhua*?

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Abstract

Invasive species is a worldwide issue that is considered to be the one of the main reasons to losses of biodiversity. The round goby, *Neogobius melanostomus*, is an invasive species in the Baltic Sea that originates from the Black- and Caspian Sea. It was probably introduced via ballast water and since the discovery of the first specimens in 1990 round goby has spread and is now established along a majority of the Baltic coasts.

To investigate the success of an invasion it is important to understand the competitive effects from the intruder but also the predator-prey interaction between the introduced species and native predators. Round goby competes with native fish species for resources but it has also been shown to be an important prey to native piscivorous predators such as cod, *Gadus morhua*, perch, *Perca fluviatilis* and great cormorant, *Phalacrocorax carbo*.

The present study was carried out to investigate cod predation on round goby in the Karlskrona archipelago over seasons. The aim of the study was to see if round goby is a common prey for cod in this area and if round goby make up a part of the diet all year round or just certain parts of the year. Cod condition over the seasons were also investigated to get an idea of the quality of round goby as prey.

Round goby looks very similar to the native black goby, *Gobius niger*, and to be able to separate the two species in stomach content an anatomical study was carried out. Stomach content from 116 cod caught in the Karlskrona archipelago was then analysed.

Round goby was more common during the spring periods compared to the winter season. Cod condition was also higher during the spring periods compared to the winter season. Differences in round goby presence might be the result of a seasonal shift in cod diet or lack of spatial overlap between the two species. For cod condition the differences might be due to round goby consumption but further analyses have to be carried out to exclude other explanations for seasonal change in cod condition.

Keywords: Round goby, invasive species, cod, predator-prey interaction.

Sammanfattning

Invasiva arter är ett världsomfattande problem och ses som en av huvudorsakerna till minskad biodiversitet. Svartmunnad smörbult, *Neogobius melanostomus*, är en invasiv art i Östersjön som härstammar från Svarta- och Kaspiska havet. Den kom troligen via ballastvatten och sedan de första individerna upptäcktes 1990 har arten spridit sig och etablerade populationer finns nu längs de flesta kusterna i Östersjön.

För att kunna undersöka framgången vid en invasion är det viktigt att förstå, dels konkurrensen från den nya arten gentemot inhemska arter, dels interaktionen mellan den introducerade arten och inhemska predatorer. Svartmunnad smörbult konkurrerar om resurser med inhemska arter men har också visat sig vara ett viktigt byte för inhemska fiskätande predatorer som torsk, *Gadus morhua*, abborre, *Perca fluviatilis* och storskarv, *Phalacrocorax carbo*.

I denna studie undersöktes torskpredation på svartmunnad smörbult i Karlskronas skärgård mellan säsonger. Syftet var att se om svartmunnad smörbult är ett vanligt byte och huruvida den utgör en del av dieten över hela året eller bara under enstaka perioder. Torskkondition över säsongerna undersöktes också, för att få en uppfattning om svartmunnad smörbults kvalitet som byte.

Svartmunnad smörbult är mycket lik den inhemska svarta smörbulten, *Gobius niger*, och för att kunna separera dem i maginnehåll utfördes en anatomistudie. Totalt analyserades maginnehåll från 116 torskar fångade i Karlskrona skärgård.

Förekomsten av svartmunnad smörbult i maginnehållet var högre under vårperioderna jämfört med vintern. Konditionen för torskarna var också högre under vårperiodena jämfört med vintern. Variationen av förekomsten av svartmunnad smörbult mellan säsonger tros bero på en förändrad diet hos torsken eller brist på rumsligt överlapp mellan de två arterna. Variationen i torskkondition kan bero på konsumtion av svartmunnad smörbult, men för att utesluta andra orsaker behöver ytterligare studier utföras

Nyckelord: svartmunnad smörbult, invasiva arter, torsk, byte-predator interaktion

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1 Introduction

Throughout the majority of the Earth's history, mountains and oceans have created significant natural barriers, thereby preventing the spread of species to new environments and enabling ecosystems to evolve under relatively isolated conditions (Lowe et al., 2000). Intensified trade and travel has decreased the significance of these barriers and created a worldwide phenomenon of human-mediated dispersal of non-native species (Almqvist et al., 2010). Intentional or unintentional transport of species across the globe may result in different environmental outcomes. Although not all species being introduced to new environments can survive outside their native range, a species introduction may result in establishment, spread and impact on the new ecosystem. Some species introductions may lead to positive effects such as enhanced fisheries, but many others have significant negative impacts on the new ecosystem and are termed invasive species. Invasions also affect human health and economy. In Europe alone the economic impacts of invasive species are estimated to be at least 12 billion EUR per year (Keller et al., 2011).

Covering a broad taxonomic range (Keller et al., 2011), invasive species are considered a leading cause of loss of biodiversity (Albins & Hixon, 2008), only triumphed by habitat destruction (Almqvist et al., 2010). One example of such invasions is the introduction of Nile perch, *Lates niloticus*, to Lake Victoria in 1954. The purpose of this was to counteract the depletion of native fish stocks due to overfishing, leading to the extinction of over 200 native fish species due to predation and resource competition (Lowe et al., 2000).

A non-native species is not guaranteed to succeed in its new environment. The success of an invasion depends on several ecological factors such as interspecific competition, presence of predators and parasites and suitability of the new habitat. The ability to adapt to a new environment depends on the genetic variation of the introduced individuals and the ability to colonize sites big enough to prevent problems with inbreeding or demographic stochasticity (Björklund & Almqvist, 2009).

The round goby, *Neogobius melanostomus*, is an introduced fish species in the Baltic Sea. It displays several characters of an effective colonizer such as high tolerance to varying temperature and salinity, short population turnover time and multiple spawning events per season (Kornis et al., 2012)

Round goby originates from the Black and Caspian seas (Charlebois et al., 2001) and was probably transported to the Baltic Sea in ballast water. Round goby larvae are nocturnal and pelagic, therefore spending time in surface waters during night foraging on zooplankton. Nocturnal filling of ballast tanks could thus easily result in ships taking in thousands of larvae at once. The nocturnal foraging behaviour in round goby also suggest that spending, even an extended amount of time, in a dark ballast tank would do no significant harm to the larvae (Kornis et al., 2012).

The first specimens of round goby in the Baltic Sea were found in 1990 in Puck Bay, Gulf of Gdansk (Kornis et al., 2012) where it is now one of the dominating fish species in both number and biomass (Skóra & Sapota, 2005). Round goby is now found along a majority of the coasts surrounding the Baltic Sea (Christoffersen et al., 2019; Kornis et al., 2012; Kotta et al., 2016) (Fig. 1). The first Swedish specimens were discovered in Karlskrona in 2008 (Florin, 2017) where the

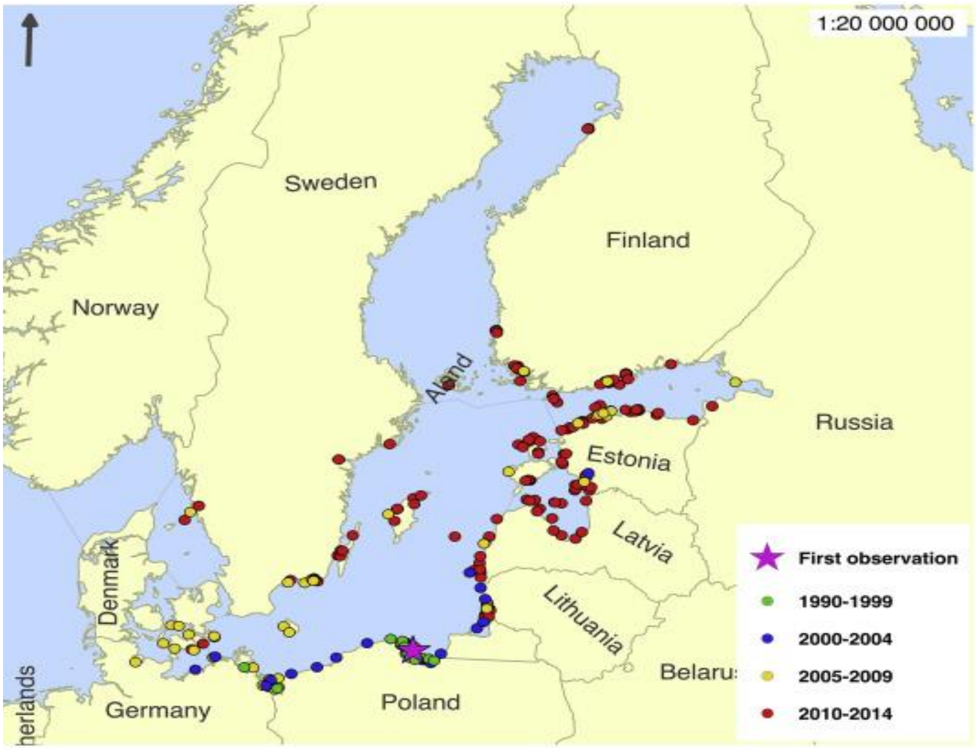

Figure 1. Observations of round goby 1990-2014 in the Baltic Sea (Kotta et al., 2016).

species is now well established. Other established populations are also found in Gothenburg, Kalmar sound, Visby and in the southern part of Stockholm archipelago (Florin, 2019).

At the event of an invasion, the predator-prey interaction is a key-process in understanding the development of population abundances and dynamics of the native community due to the invasion. How non-native prey and native predators interact with each other is therefore a useful framework for studying and predicting the success of an invasion. Unfortunately the main focus for most studies regarding non-native species is the invader's role as a predator in contrast to its role as prey. This leads to a one sided view of the invasion effects and limits the comprehensive understanding of the impacts on a community level due to a partial understanding of the long term consequences of non-native prey on native predators on a population level (Pintor & Byers, 2015). Considering an isolated predator-prey interaction between two species the predator is, by definition, expected to have a negative effect on the prey. In turn the prey has a positive effect on the predator. Can the same assumptions then be made for a non-native prey? Because non-native prey and native predators most often do not share a common evolutionary history, an invasion may result in three possible outcomes for the predator-prey interaction. A non-native prey may lack the appropriate behavioural, chemical or morphological defences needed to escape the native predator, resulting in a pronounced positive effect on the predator population. Conversely, the predator may lack characters needed to be efficient in capturing and consuming non-native prey compared to native prey species, or the prey value of non-native prey is not as high as the prey value of native prey (Pintor & Byers, 2015). The latter scenario does not necessarily have a negative effect on the predator if the new prey of lower value is simply a substitution to prey of higher value, but could theoretically have a negative effect if the non-native prey is an effective competitor to native prey. If the competition leads to a displacement of higher value native prey, leading to decreasing availabilities of these species, the predator would suffer negatively (Pintor & Byers, 2015).

Because of the low biodiversity in the Baltic Sea, predators living there face greater limitations in prey option compared to other cod-inhibited areas, thus potentially increasing the importance of predator-prey interactions (Kulatska et al., 2019).

Round goby has been shown to affect the Baltic ecosystem in both positive and negative ways by serving as both competitor, predator and prey. It competes with several native fish species through resource competition, for example the commercially important European flounder, *Platichthys flesus* (Kornis et al., 2012) and probably also the newly discovered Baltic flounder, *Platichtys solemdali*, that was previously associated with European flounder (Momigliano et al., 2018). According to Kornis et al. (2012) diet overlap and negative correlation in abundance between the species are evidence for this competition. Round goby also limits habitat utilization, thus limiting food availability for flounder (Kornis et al., 2012).

Because of its widespread success and abundance round goby has also become an important prey for a number of piscivorous predators such as cod, *Gadus morhua,* perch, *Perca fluviatilis,* and great cormorant *Phalacrocorax carbo* (Kornis et al., 2012).

Cod is a top predator feeding on a wide range of fish- and invertebrate species, thus acting as a key-species in north Atlantic ecosystems. Most of the areas inhibited by cod, including the Baltic Sea, show trophic cascades where prey populations are increasing as a response to collapsed cod populations. (Kulatska et al., 2019).

During its lifecycle, cod goes through several changes in diet preferences. As a juvenile, it mostly preys on small benthic organisms. The diet then changes with growth. When reaching medium size (Kulatska et al., 2019), 26-55cm according to Almqvist et al. (2010) small fishes like sprat *Sprattus sprattus* and herring *Clupea harengus* are included in the diet and when growing even larger, bigger fish like flounder and smaller cod serve as prey (Kulatska et al., 2019).

Cod diet also varies spatially and seasonally (Ljungberg et al., 2019). In the Baltic Sea, Almqvist et al. (2010) found that together with the shrimp *Crangon crangon*, round goby was the most common prey for cod caught in the Gulf of Gdansk whereas the crustacean *Saduria entomon*, three-spined stickleback, *Gasterosteus aculeatus*, and clupeids were the most common prey for cod caught outside Öland in the central Baltic Sea. Based on weight proportions round goby was the predominant prey during spring and summer whereas eelpout *Zoarces viviparous* was the predominant prey during winter in the Gulf of Gdansk. Outside Öland, cod was instead the predominant prey during winter and spring whereas eelpout was the predominant prey during summer. Except for seasonal differences in prey based on weight proportions, Almqvist et al. (2010) also found that there was size dependent seasonal differences in diet in the Gulf of Gdansk. For cod smaller than 55 cm round goby was the dominating prey during winter whereas larger cods predominantly fed on shorthorn sculpin, *Myoxocephalus scorpius,* and eelpout. In spring the diet for cod smaller than 55 cm instead consisted mostly of clupeids and larger cod fed on round goby and eelpout.

The aim of the present study was to investigate to which extent cod predate on round goby in the Karlskrona archipelago where round goby is well established (Florin, 2019). Is the round goby a common prey for cod in this region and if so, does it make up a part of the diet all year round or just certain parts of the year? Further, to shine more light on the predator-prey interaction between cod and round goby in the Karlskrona region, cod condition over the seasons was investigated to get an idea of the quality of round goby as prey.

2 Material and methods

2.1 Study of bone structures in round goby and black goby

To be able to distinguish round goby from the similar looking native black goby, *Gobius niger,* in cod stomach content an anatomy study was carried out. Two individuals of each species were used to create the reference material. The specimens were in good condition, meaning they could be identified by outer morphological characters that separate the two species. Round goby has a black spot in the rear end of the first dorsal fin close to the back (Fig. 2 & 4). Black goby has a similar black spot but it is placed in the front end and on the top of the first dorsal fin (Fig. 3 & 5). The first dorsal fin of black goby is slightly bigger and has a more plume-like shape compared to the first dorsal fin of round goby. Round goby has a robust body shape, short head and can grow up to 25 cm in length. Black goby has a robust body shape, a wide head and can grow up to 18 cm (Kullander & Delling, 2012). In the Baltic Sea black goby never grow longer than 10 cm (Artdatabanken, 2012). The present anatomical study was therefore needed to be able to separate individuals up to 10 cm.

Figure 2. Round goby (male).
Illustration: Karl Jilg/ArtDatabanken, SLU

Figure 3. Black goby (male).
Illustration: Karl Jilg/ArtDatabanken, SLU

Figure 4. Round goby (female).
Illustration: Karl Jilg/ArtDatabanken, SLU

Figure 5. Black goby (female).
Illustration: Karl Jilg/ArtDatabanken, SLU

The gut and gonads were removed and as much soft tissue as possible were cut away without cutting away any bone. The specimens were then boiled to loosen up the flesh and make it easier to remove from the skeleton. The fishes were boiled for 30 minutes and later another 15-20 minutes if necessary. The skeleton were then dissected out under a stereo microscope.

To remove the flesh from fragile skeletal parts such as fins, one of the black goby specimens was put in protease enzyme. Unfortunately that did not work as expected, probably due to exposure to too high temperature. The enzyme turned into a more solid state instead of evaporating, leaving the bones encapsulated in the enzyme. Since there were only two specimens of each species it was not worth the risk to damage another skeleton to try the enzyme process once more so the other three were only boiled.

Skeletal differences big enough to be considered useful in separating round goby from black goby in stomach content were found in *praemaxillare, dentale, parasphaenoid* and *cleithra* (Fig. 6-9.). Fig. 10 shows the anatomical location of these bone structures. Also, round goby has pharyngeal bones which are lacking in black goby, although the branchial bones in black goby slightly resembles pharyngeal bones (Fig. 11). According to Kornis et al. (2012) the pharyngeal bones function as a crushing device, enabling the fish to break through the shells of mussels which make up a big part of the round goby diet.

The skeletal parts in which differences were found were photographed and used as reference material in the stomach analysis. The skeletons were stored as reference material for future projects.

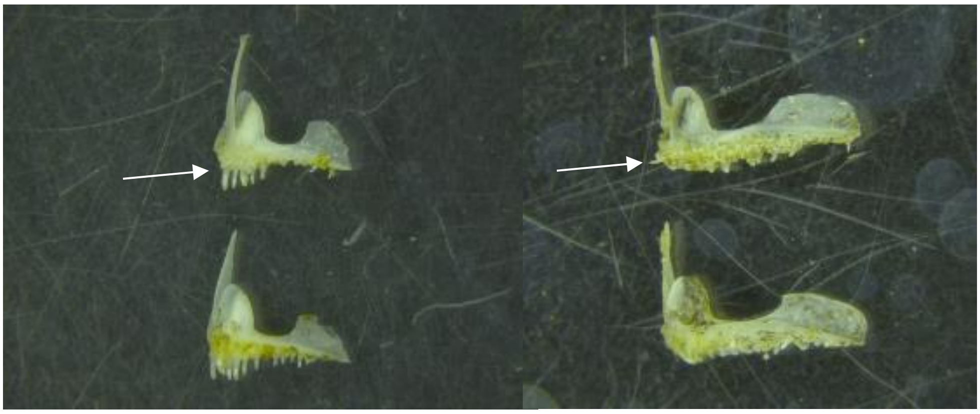

Figure 6. Praemaxillare. Left: round goby, teeth of different sizes, longer in the front and shorter in the back. Right: black goby, teeth of similar size.

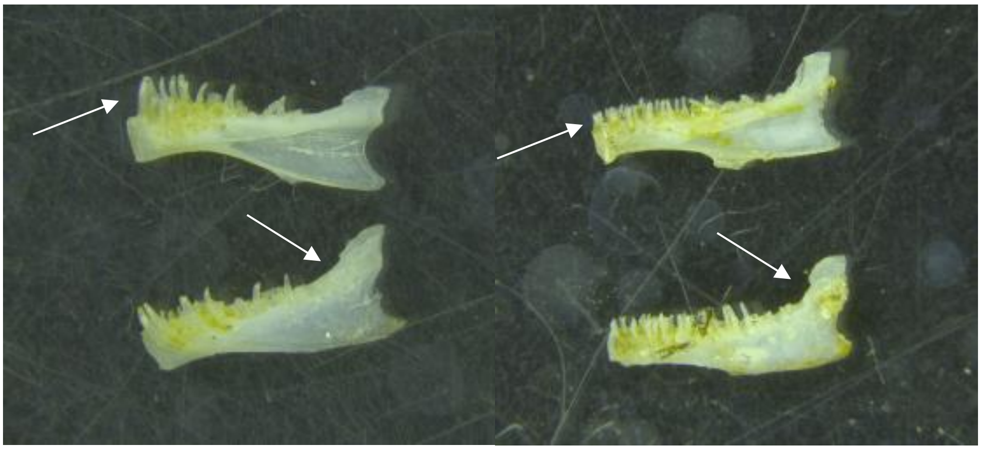

Figure 7. Dentale. Left: round goby, teeth of different sizes, longer in the front and shorter in the back. Smooth angle of jaw. Right: black goby, teeth of similar size, sharp angle of jaw.

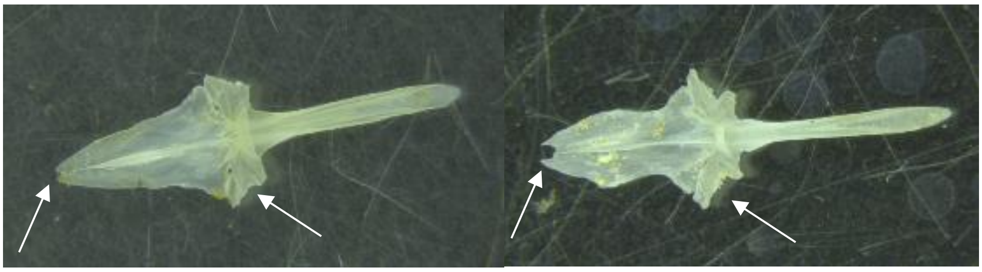

Figure 8. Parasphaenoid. Left: round goby, pointy tip with small slit, smoother edge on lateral extensions. Right: black goby, dull tip with big slit, lateral extensions with serrated edges and slit at the base.

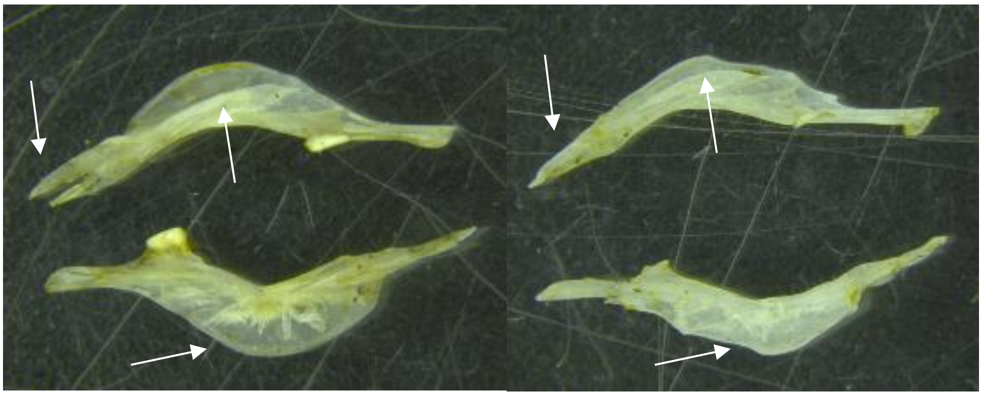

Figure 9. Cleithrum. Left: round goby, slit in upper end wide, bigger outer wing, smaller inner wing. Right: black goby, slit in upper end more narrow, smaller outer wing, bigger inner wing.

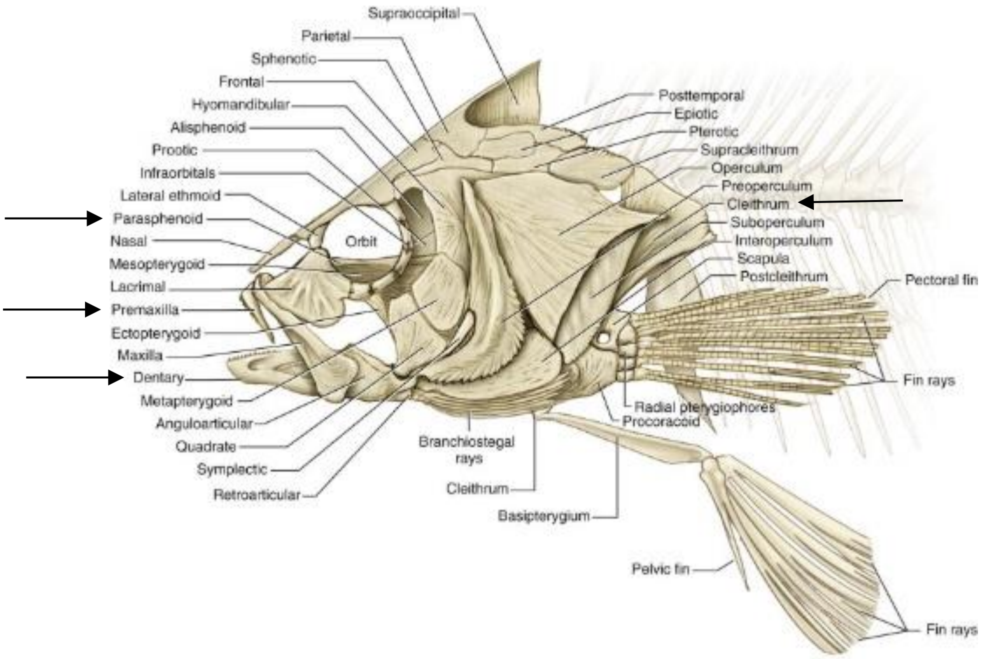

Figure 10. Fish skull anatomy. Arrows the location of the bone structures where differences were found except pharyngeal- and branchial bones (De Iuliis & Pulerà, 2011).

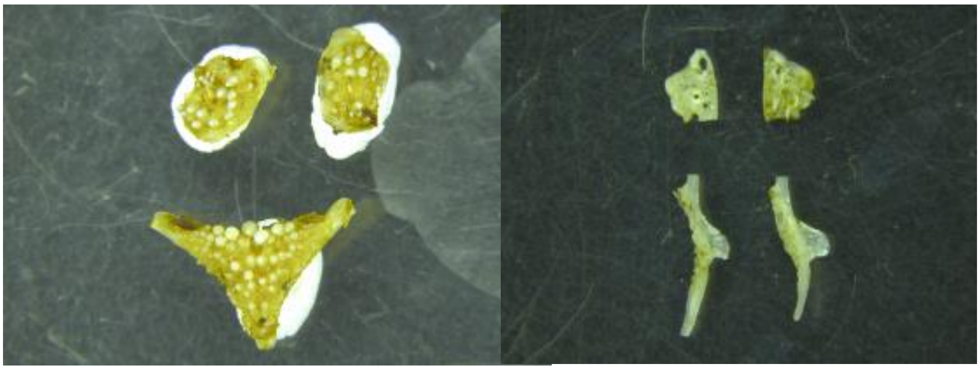

Figure 11. Left: pharyngeal bones in round goby. Right: branchial bones in black goby.

2.2 Stomach analysis

Frozen cod stomachs were bought from a commercial fisherman in Karlskrona in south-east Sweden. The cod were caught in May 2018, November 2018 and January-June 2019 in fishing nets at Söderstjärna (fishing depth 6-13 m), Försänkningen (fishing depth 20 m) and Gärskullen (fishing depth 10-12 m). Weighting, measuring and gutting was carried out by the fisherman. 116 stomachs were analysed in total, out of which 30 were analysed in 2018.

The stomachs were defrosted either in the refrigerator over night or in a bucket of water in the morning. Stomach, intestines and liver were dissected out. Eventual parasite infection on the liver was noted. The stomach content, intestinal content and liver were placed in separate petri dishes and weighed separately.

Gut fullness was estimated according to a scale from 1-6, where 1 represents an empty stomach and 6 represents a full stomach. How digested the stomach content was (digestion state) was also estimated according to a scale from 1-6. 1 represents an empty stomach and thus no stomach content to measure digestion state for and 6 represents prey items that are completely intact and in immaculate condition. For full reference table, see Appendix 1.

Prey from stomach- and intestinal content were sorted under stereo microscope and identified to the lowest taxonomic level. The previous mentioned anatomy study (see 2.1), the database Bonebase (Busekist, 2008), the article *Shapes of otoliths in some Baltic fish and their proportions* (Sapota & Dąbrowska, 2019) and the book *Havets djur* (Köie & Svedberg, 2004) was used as reference material.

Number of prey, prey lengths and estimated percentage of prey out of total stomach content were noted. Parasites and other observations were noted in a comment section.

Solid parts from the stomach content were then put in zip-lock bags marked with stomach ID and date of capture and were stored in the freezer for future reference.

Because no cod fishing is carried out during summer there is no data included for the summer season. The seasons were divided into spring 2018 (May), winter 2018-2019 (November-March) and spring 2019 (April-June). For these seasons 30, 41 and 45 stomachs were analysed respectively. Because there were only few stomachs from the autumn and winter seasons November, January, February and March were merged together and form a winter season to prevent type II error in the statistical analysis. See table 1 for a full description of the cod used in the study.

2.3 Calculations

Total weight and length were used to calculate Fulton's condition factor (FCF) for each cod individual. FCF is a value of condition for an individual calculated from the relationship between length and weight (Nash et al., 2006). A Fulton's condition value of 1 indicates "normal" fish condition. Lower values indicate more meagre individuals (Ljungberg et al., 2019). Fulton's condition factor (FCF) was calculated as followed:

$$FCF = 100 \text{ x } Weight_{cod}/Length^3_{cod}$$

Stomach content weight was used to calculate gut-fullness index (GFI). GFI data can be used to estimate feeding intensity, foraging patterns and environmental limitation on feeding (Herbold, 1986). In the present study GFI was used to get an idea about the feeding intensity for cods in Karlskrona over seasons. Gut fullness index, GFI, was calculated as followed:

$$GFI = 100 \text{ x } Weight_{total}/Weight_{Stomach\ content}$$

Frequency of occurrence (FO) defines as the number of times or with which regularity something happens (Encyclopedia.com, 2019). In the present study FO was used to calculate how frequent a specific prey was present in cod stomachs. FO was calculated as followed:

FO = Number of stomachs where a specific prey is present/total number of stomachs

2.4 Statistical analyses of cod parameters

A Kolmogorov-Smirnov test of normality were carried out for cod length and cod weight to test whether the parameters were normally distributed. Normal distribution was tested to define which statistical test needed to be used for these parameters in the statistical analysis.

A one-way ANOVA test and a Tukey test were conducted for cod length and cod weight as a control test to examine potential differences between cod length and cod weight between the seasons. Significant differences for cod length and cod weight between the seasons would generate questionable results in the analysis of cod condition.

2.5 Statistical analyses of cod condition and stomach content

A Kolmogorov-Smirnov test of normality were conducted to test the normal distribution for cod condition (FCF), gut fullness index (GFI), number of prey in cod stomachs and number of round goby in cod stomachs to test whether these parameters were normally distributed. Normal distribution was tested to define which statistical test needed to be used for these parameters in the statistical analysis.

A one-way ANOVA test and a Tukey test were conducted for Fulton's condition factor (FCF) to test if there were significant differences in cod condition between the seasons.

A Kruskal-Wallis test and a post hoc test were conducted for gut fullness index (GFI) to test if there were significant differences in gull fullness index (GFI) between the seasons.

A Kruskal-Wallis test and a post hoc test were conducted for number of prey found in stomachs to test if there were significant differences in the number of prey present in cod stomachs between the seasons.

A Kruskal-Wallis test and a post hoc test were conducted for number of round goby found in the stomachs to test if there were significant differences in the number of round goby present in cod stomachs between the seasons.

The statistical analyses were conducted using SPSS version 26.

3 Results

3.1 Cod data

Data for the cods used in the study are listed in Table 1.

Table 1. *Cod data for each season.*

Cod data	Spring 2018	Winter 2018-2019	Spring 2019
Individuals	30	41	45
Max length (cm)	57	60	60
Min length (cm)	39	32	32
Mean length ± SD (cm)	47.37 ± 4.63	47.46 ± 6.48	44.16 ± 6.60
Max weight (g)	1845	2245	2175
Min weight (g)	565	295	310
Mean weight ± SD (cm)	1124.67 ± 334.48	1057.34 ± 395.35	930.51 ± 425.31
Max condition (FCF)	1.21	1.18	1.30
Min condition (FCF)	0.78	0.80	0.86
Mean condition ± SD (FCF)	1.03 ± 0.10	0.95 ± 0.09	1.01 ± 0.12

Cod lengths were normally distributed (Kolmogorov-Smirnov test: 0.067, df = 116, p = 0.200). There were significant differences between mean values for cod length over the seasons (One-way ANOVA test: $F_{2, 115} = 3.939$, p = 0.022). Cod caught during winter 2018-2019 were significantly longer compared to cod caught in spring 2019 (Tukey test: p = 0.036). No significant differences between mean values for cod length were found for cod caught in spring 2018 and winter 2018-2019 (Tukey test: p = 0.998) or for cod caught in spring 2018 and spring 2019 (Tukey test: p = 0.071) (Fig.12).

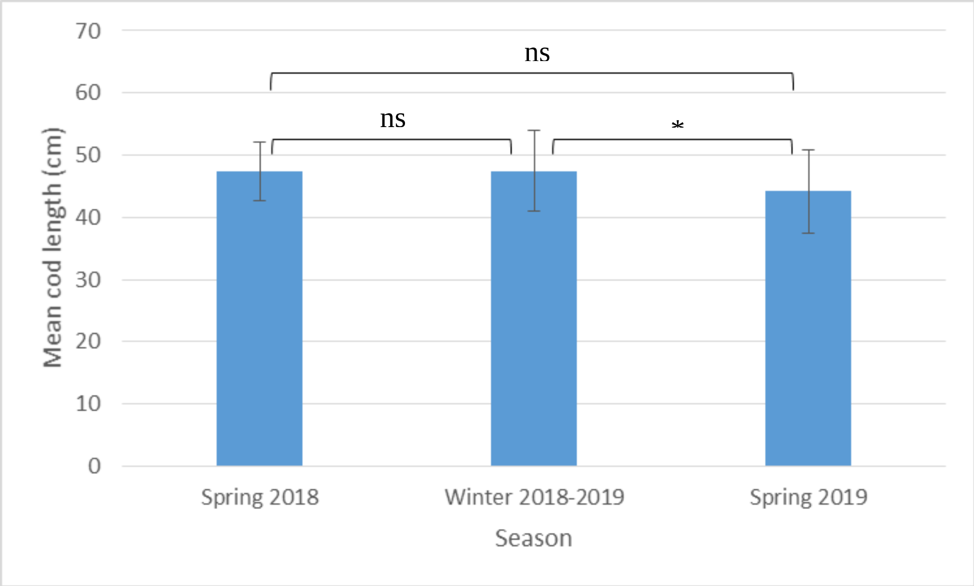

Figure 12. Mean cod length over seasons. Whiskers show standard deviation: ns = not significant, * = significant.

Cod weights were not normally distributed (Kolmogorov-Smirnov test: 0.090, df = 116, p = 0.023). Log-transformation of data generated normal distribution (Kolmogorov-Smirnov test: 0.078, df = 116, p = 0.078). There were significant differences between the mean values for cod weight over the seasons (One-way ANOVA test: $F_{2, 115} = 3.691$, p = 0.028). Cod caught in spring 2018 were heavier compared to cod caught in spring 2019 (Tukey test: p = 0.027). No significant differences between mean values for cod weight were found for cod caught in spring 2018 and winter 2018-2019 (Tukey: p = 0.613) or cod caught during winter 2018-2019 and spring 2019 (Tukey: p = 0.173) (Fig. 13). The relationship between cod length and cod weight is plotted in Fig. 14.

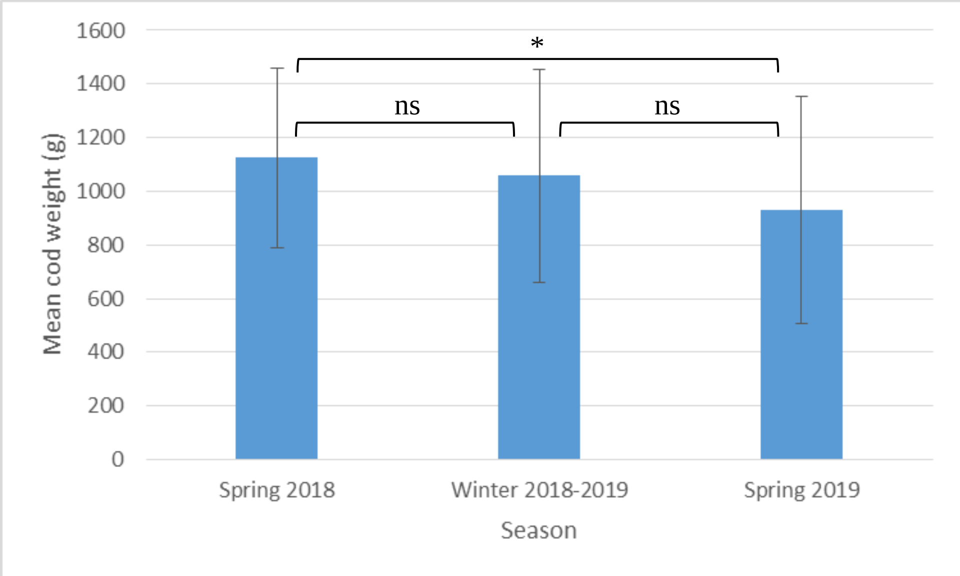

Figure 13. Mean cod weight over seasons. Whiskers show standard deviation: ns = not significant, * = significant.

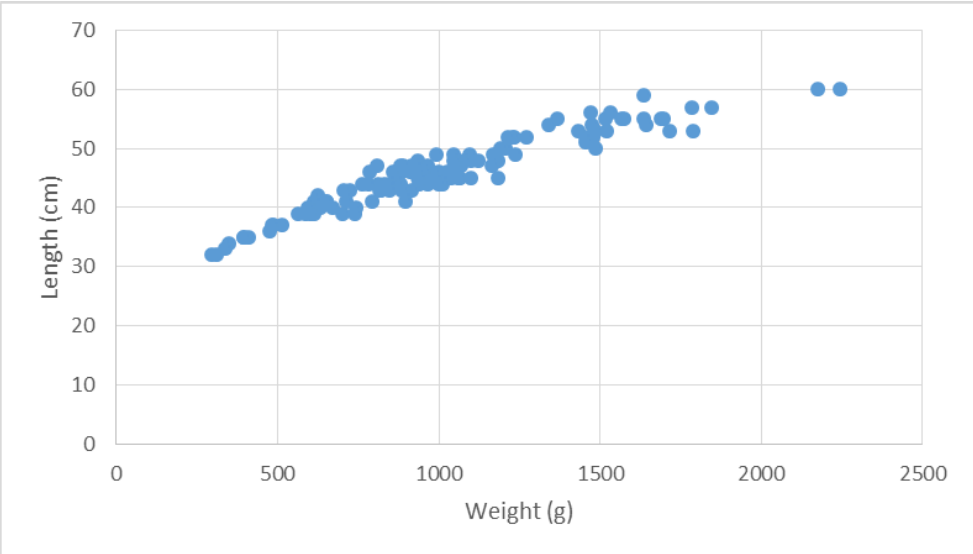

Figure 14. Length and weight relationship for the cod used in the study.

3.2 Cod condition and stomach content

Cod condition was not normally distributed (Kolmogorov-Smirnov test: 0.087, df = 116 p = 0.033). Log-transformation of data generated normal distribution (Kolmogorov-Smirnov test: 0.068, df = 116, p = 0.200). There were significant differences between mean values for cod condition over the seasons (One-way ANOVA test: $F_{2, 115} = 6.828$, $p = 0.002$) (Fig. 15 & 16). Cod caught in spring 2018 had higher condition compared to cod caught in winter 2018-2019 (Tukey test: p = 0.002). Cod caught in spring 2019 had also higher condition compared to cod caught in winter 2018-2019 (Tukey test: p = 0.015). No significant differences in condition were found for cod caught in spring 2018 and spring 2019 (Tukey test: p = 0.652).

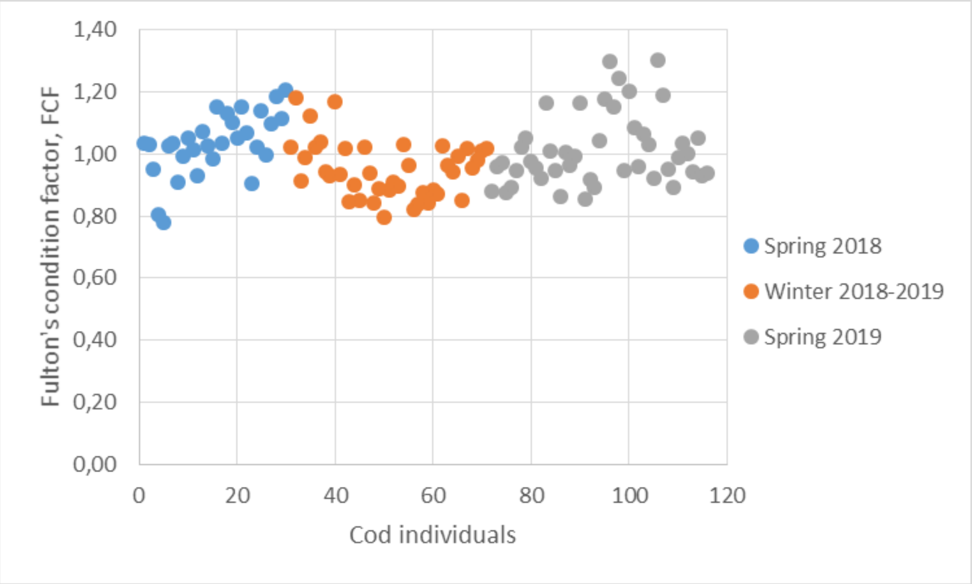

Figure 15. Condition for the cods used in the study per season.

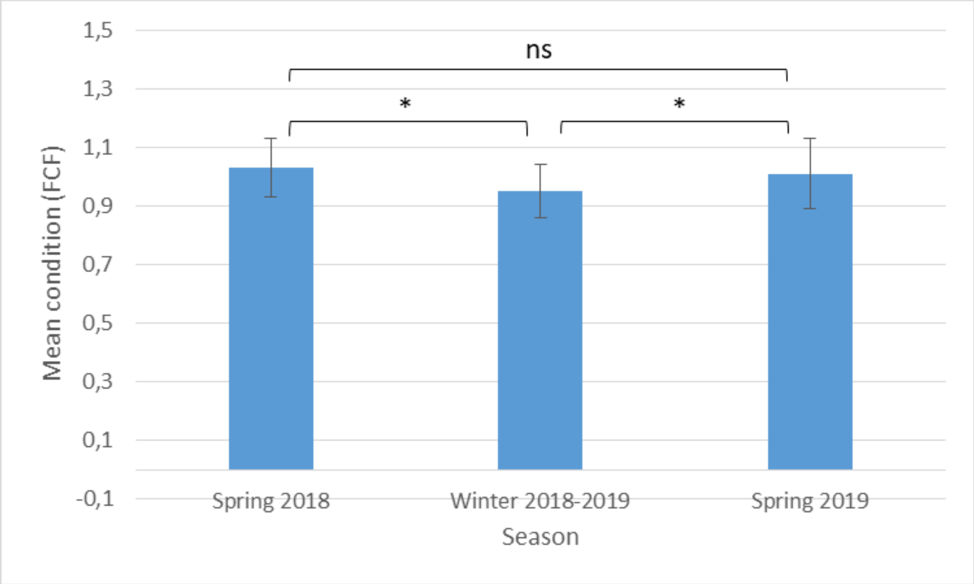

Figure 16. Mean cod condition over seasons. Whiskers show standard deviation: ns = not significant, * = significant.

Gut fullness index was not normally distributed (Kolmogorov-Smirnov test: 0.252, df = 116, p = 0.000). Mean gut fullness index (GFI) was 1.34 ± 1.29 SD for cod caught spring 2018, 1.49 ± 2.55 SD for cod caught in winter 2018-2019 and 1.90 ± 2.85 SD for cod caught in spring 2019 (Fig. 17). There were no significant differences between the median scores for gut fullness index (GFI) between the seasons (Kruskal-Wallis test: 0.828, df = 2, p = 0.661)

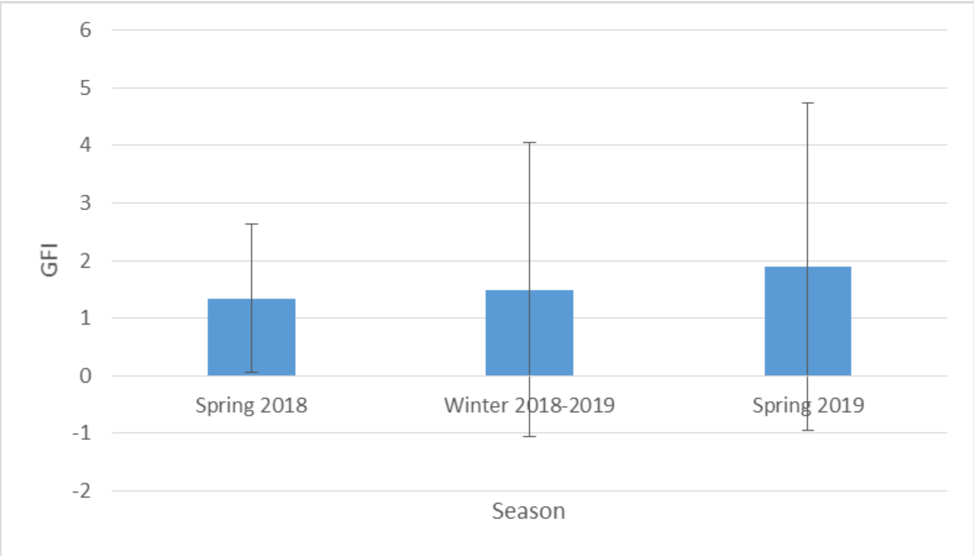

Figure 17. Mean gut fullness index (GFI) for the cod per season. Whiskers show standard deviation.

Number of prey in stomachs were not normally distributed (Kolmogorov-Smirnov test: 0.337, df = 116, p = 0.000). There were no significant differences between median scores for number of prey in stomachs over seasons (Kruskal-Wallis test: 3.126, df = 2, p = 0.210) (Fig. 18).

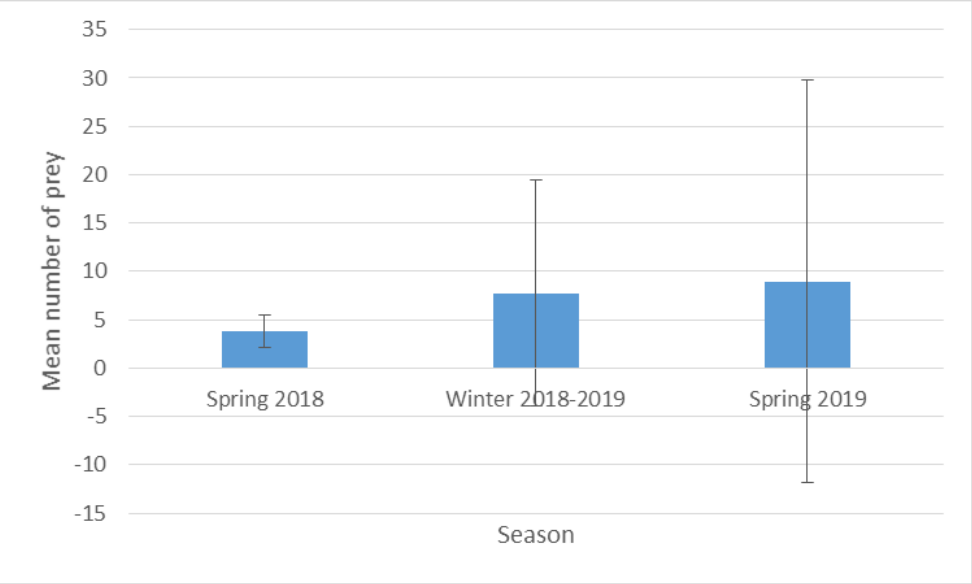

Figure 18. Mean number of prey found in the stomachs per season. Whiskers show standard deviation.

In the stomach analysis 5 fish genera, 3 crustacean genera and 1 mollusc genera were identified. On top of that a total of 17 different prey species were identified, 11 fish species and 6 crustacean species. For a complete list of genera and species, number of individuals and frequency of occurrence for all species found in the stomachs for all seasons pooled, see Table 2. Species lists of number of individuals and frequency of occurrence per season are shown in Appendix 2.

Round goby was the dominant prey in the spring seasons, based on both number of individuals and frequency of occurrence. During winter, three-spined stickleback was the dominant prey followed by perch, *Perca fluviatilis*, based on frequency of occurrence, whereas amphipods, *Gammarus sp.*, was the dominant prey based on number of individuals (Appendix 3).

Gut fullness of the stomachs varied from 1 to 6. Mean gut fullness was 3.87 (spring 2018), 3.83 (winter 2018-2019) and 3.58 (spring 2019). This means that the stomachs were approximately 25% full for all seasons.

Digestion state of stomach content varied from 1 to 6. Mean digestion state was 3.13 (spring 2018), 3.59 (winter 2018-2019), 3.38 (spring 2019). This means that the stomach content were approximately half way through the digestion process for all seasons.

Table 2. *Total numbers of individuals and frequency of occurrences (FO) for all species found in the stomachs for all seasons pooled.*

Species	Amount (total)	FO (total)
Osteichthyes		
Neogobius melanostomus	143	0.57
Gasterosteus aculeatus	66	0.28
Perca fluviatilis	36	0.22
Clupea harengus	34	0.16
Gobiidae sp.	8	0.06
Pomatochistus minutus	7	0.03
Zoarces viviparus	2	0.02
Clupeidae sp.	2	0.02
Pungitius pungitius	2	0.02
Gobius niger	2	0.02
Hyperoplus lanceolatus	1	0.01
Syngnathus typhle	1	0.01
Syngnathiidae sp.	1	0.01
Sprattus sprattus	1	0.01
Cottidae sp.	1	0.01
Pleuronectidae sp.	1	0.01
Osteichthyes unidentified	18	0.11
Crustacea		
Gammarus sp.	323	0.26
Saduria entomon	37	0.17
Palaemon elegans	26	0.04
Crangon crangon	14	0.09
Palaemon sp	14	0.01
Palaemon adspersus	11	0.04
Idothea baltica	11	0.03
Mysidae sp.	7	0.06
Crustacea unidentified	25	0.16
Mollusca		
Mytilus sp.	39	0.09
Unidentified prey	5	0.04

Number of round goby in the stomachs were not normally distributed (Kolmogorov-Smirnov test: 0.241, df = 116, p = 0.000). There were significant differences between the median scores for number of round goby in cod stomachs over seasons (Kruskal-Wallis test: 61.271, df = 2, p = 0.000) (Fig. 19). The number of round goby in stomachs for cod caught in spring 2018 was significantly higher compared to cod caught in winter 2018-2019 (Pairwise comparison test: p = 0.000). The number of round goby in stomachs for cod caught in spring 2019 was significantly higher compared to cod caught in winter 2018-2019 (Pairwise comparison test: p = 0.002). The number of round goby in stomachs was significantly higher for cod caught in spring 2018 compared to cod caught in sprung 2019 (Pairwise comparison test: p = 0.000).

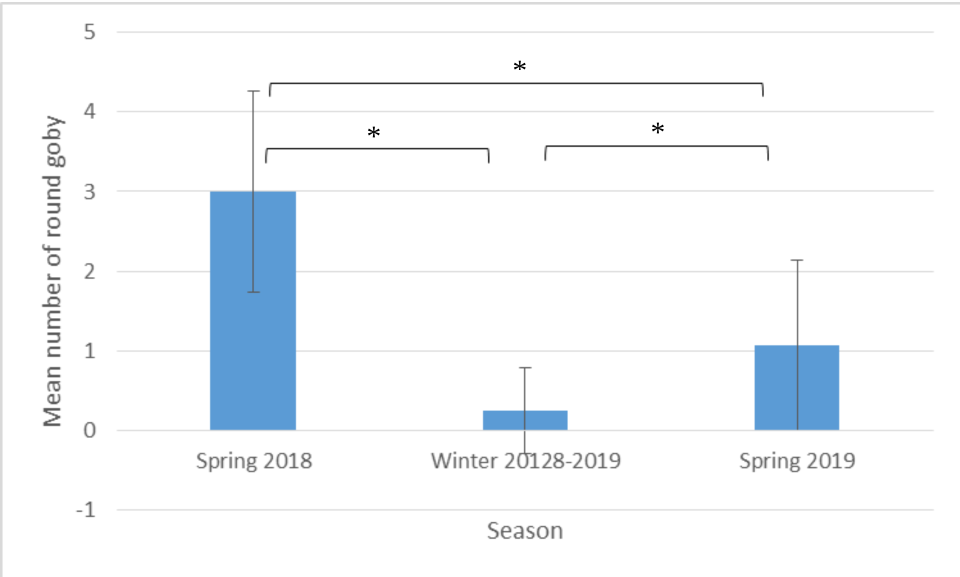

Figure 19. Mean number of round goby found in the stomachs per season. Whiskers show standard deviation: * = significant.

Round goby were found in all stomachs from cod caught spring 2018 (FO = 1), in 20% of the stomachs from cod caught in winter 2018-2019 (FO = 0.2) and in 64% of the stomachs from cod caught in spring 2019 (FO = 0.64) (Fig. 20).

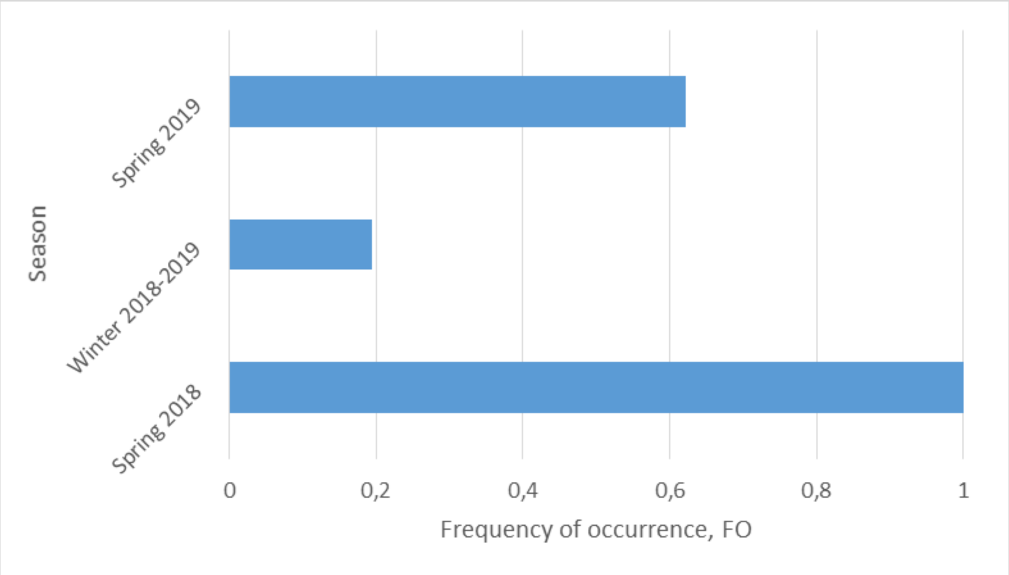

Figure 20. Frequency of occurrence of round goby in stomachs per season.

Of all prey found in the stomachs, round goby accounted for 79% of stomach content for cods caught in spring 2018, 3% of stomach content for cods caught winter 2018-2019 and 12% of stomach content for cods caught spring 2019 (Fig. 21).

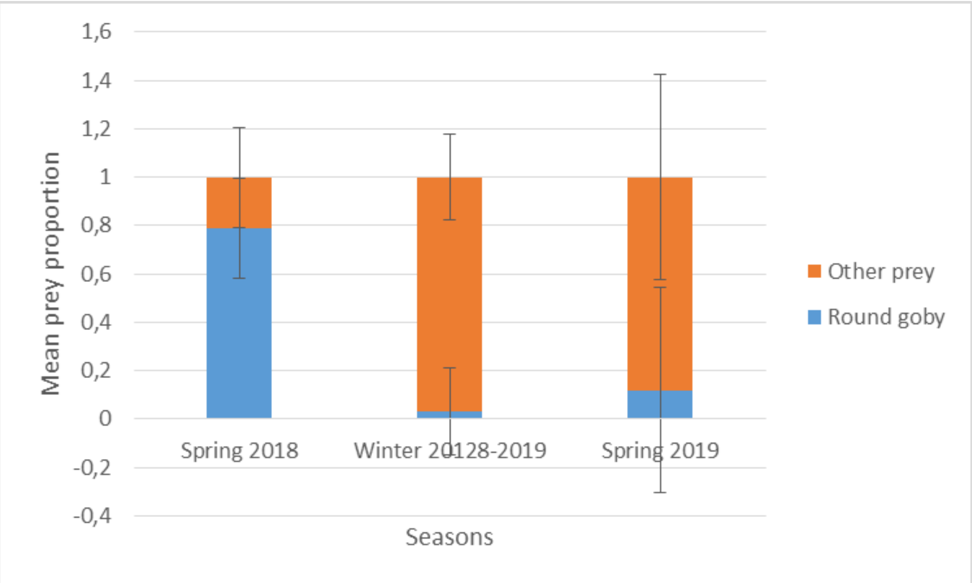

Figure 21. Mean proportion of round goby compared to other prey found in the stomachs per season.

4 Discussion

Prey items were found in almost all stomachs and there were no significant differences between the seasons for either the number of prey found in stomachs or gut fullness index (GFI), thus indicating that the feeding intensity for cod does not vary between seasons. There were significant differences for round goby presence in the stomachs, showing a higher presence during spring and lower presence during winter. According to Kornis et al. (2012) round goby prefers shallow water (0.7-3m) in summer during spawning season and then migrate offshore during winter. Christoffersen et al. (2019) studied migrating behaviour for round goby in the Karrebaek and Dybsoe estuary located in the western Baltic Sea. Round gobies were caught, tagged with acoustic transmitters and released again. Receivers were then placed in the three outlets leading to the open sea. Their study showed diverse migrating behaviours for round goby in the winter season. Some individuals left the estuary in autumn for the open sea and returned in spring. A few migrated to upstream fresh water areas during winter whereas others never left the estuary at all (Christoffersen et al., 2019).

Atlantic cod is also known to migrate between seasons. According to Comeau et al. (2002) cod generally migrate inshore during summer and return to offshore areas in winter, perhaps to avoid near-freezing water temperatures inshore. Other possible triggering factors in cod migration might be food availability, photoperiod and concentrations of dissolved oxygen (Comeau et al., 2002). Schaber et al. (2012) studied seasonal vertical migration of cod in the Bornholm Basin in the southern Baltic Sea. They found a migratory behaviour from deep water in winter to more shallow in summer. This upward shift was strongly believed to be the result of oxygen depletion in the deeper parts of the basin because when oxygen levels were favourable the cod would stay in the deep (Schaber et al., 2012). These migrating behaviours for both predator and prey speak for a potential lack of a spatial overlap during winter. I conclude, based on the previous mentioned migrating behaviours for the two species, that predator and prey probably do not coexist to the same extent during winter, therefore resulting in a decreased consumption of round goby and a shift to other prey.

The cod used in the present study had a mean length below 55 cm and are therefore classified as medium sized cod (Almqvist et al., 2010). When compared to the results from Almqvist et al. (2010) for cod in the Gulf of Gdansk, cod in this study seem to have a different prey preference. In the Gulf of Gdansk, round goby was the predominant prey in spring, but not during winter based on weight proportions, which match the results from the present study. But when comparing the diet for medium sized cod the results do not match. Almqvist et al. (2010) found that medium sized cod predated predominantly on round goby during winter and on clupeids in the spring. In the Karlskrona region, this study showed that three-spined stickleback and perch were the dominating prey during winter whereas round goby was the dominating prey in spring. These completely contradictory results indicate that cod diet shifts spatially and seasonally, as stated by Ljungberg et al. (2019).

Almqvist et al. (2010) compared diet for cod in the Gulf of Gdansk with cod from Öland and found differences in diet between the two locations. Cod from Öland did not feed on round goby at all, most probably because it was not as well established then as it is now. However, the seasonal differences in diet between Gulf of Gdansk and Karlskrona could be the result of a potential intraspecific competition among cod in the Gulf of Gdansk. Because large cod were feeding predominantly on round goby during spring, smaller individuals might be pushed up in the pelagic, thus feeding on pelagic prey. In Karlskrona medium sized cod seem to be, according to the size records of the cod used in the present study, more common than large cod and therefore I conclude that there is no such competition in that region. Medium sized cod therefore feed on bottom dwelling prey such as round goby during spring. There is a possibility that large cod does exist in the Karlskrona region, but perhaps more offshore from the fishing locations. It is also possible that large cod are not found there because of a high fishing pressure. A lack of spatial overlap between medium sized and large cod could therefore also be a potential reason for the seasonal diet differences seen for medium sized cod from Karlskrona and Gulf of Gdansk. However, ten years have passed since Almqvist et al. (2010) carried out their study and a lot could happen to the ecosystem during that time. Thus, the results from that study might not necessarily represent the present diet for cod in the Gulf of Gdansk.

Though round goby was the predominant prey during spring, the amount of round goby found in stomachs from spring 2019 was significantly lower compared to in spring 2018. A reason for this difference might be the extremely hot summer in 2018. An unexpected decrease in round goby along the Swedish coast was found in autumn 2018 and the reason was believed to be death due to the heat or migration to deeper and colder water (Eiderbrant, 2019). I conclude that the differences between the spring periods seen in the present study is most likely the result of a mass death causing a decrease of the round goby population. If the decrease in 2018 was the result of migration to deeper water I would expect the amount of round goby for spring 2019 to be similar to spring 2018 because of a potential, but likely, return of round gobies when the water reaches a favourable temperature. But because of the significantly lower amounts of round goby in stomachs from spring 2019, considering that round goby seems to be a favourable prey and foraging intensity for cod does not seem to shift over the year, I think this difference is likely to be the result of a decreased population size. The lower amount of round goby consumed could then be explained by fewer individuals and if round gobies are not abundant enough, the cod shifts to other prey.

Round goby was the predominate prey for cod in the present study but there is a possibility that these results are affected by potential failure in separating round goby from black goby. Limited time and availability of reference specimens only allowed us to study differences in bone structures for two specimens from each species. Based on these specimens, clear differences in bone structure were found between round goby and black goby, but due to a small sample there is still a possibility for intraspecific variation. Only two specimens of black goby were identified in the stomach content analysis and one of them were used in the anatomy study.

The bone structures identified as round goby matched the reference material with no clear intraspecific variation detected. Although, because of limited amount of reference specimens, it is not possible to exclude intraspecific variation in these bone structures for black goby. There was a small difference in length of the teeth in *dentale* between the reference specimens, but they were still not as long as the teeth in the round goby *dentale*. Despite teeth variation the angle difference in the rear end of *dentale* is still present and can be used for identification (Fig. 7). In other words, I consider it still unlikely to confuse a black goby *dentale* for a round goby *dentale*. Though it is a subjective assessment, I find it quite unlikely that there would be a huge intraspecific variation for black goby, considering that a majority of the bone structures identified as round goby did not deviate markedly from the reference material. I therefore conclude that the risk of misidentification is small and the results are probably reliable, but until more specimens have been analysed potential intraspecific variation cannot be eliminated.

Other gobiids were found in the stomach content but could not be identified, except for the sand goby *Pomatochistus minutus.* The same bone structures could be used to identify sand goby as well. Sand goby bone structures looked like a mixture of characters from both round goby and black goby bone structures but did deviate enough to enable identification. Except for differences in bone structures, differences in size are also a good way to separate round goby from the other two gobiids. Because black goby never grow bigger than 10 cm in the Baltic Sea and sand goby can grow to a max length of 9.5 cm, (Kullander & Delling, 2012) all gobies longer than that are thereby round goby.

There seems to be a strong predator-prey interaction for the cod population in Karlskrona, because of the high appearance of round goby in the stomachs. Round goby seems to be a valuable substitution or complement to native prey but is it a high quality prey? There were significant differences in cod condition between the seasons, showing that cod caught in winter had lower condition compared to the cod caught in the spring periods. Significant seasonal differences were also found for the presence of round goby. During winter the presence of round goby in the stomachs was low while it was the dominating prey during both spring periods. However, there was a significantly lower presence of round goby in spring 2019 compared to spring 2018. Despite this, there were no significant differences in cod condition for these seasons. If round goby was a high quality prey to cod I would have expected a lower cod condition in spring 2019 due of the lower amount of round goby present in the stomachs. These results indicate that round goby might not affect cod condition markedly and perhaps not be a prey of higher quality compared to other prey. However, significant differences were also found in length and weight for cod between the seasons. This means that the differences in condition might depend on cod size rather than ingested prey. A possible way to further analyse this could be to divide the cod individuals into different size classes within each season. In that case, individuals of similar size would be grouped together, thus decreasing the size variation between the individuals. The condition analysis could then be conducted for the different size classes instead and probably generate more reliable results. If no significant differences between the seasons are found for cod length and cod weight but significant differences are still found for condition using this method, it can be estimated that the condition for these cod individuals does depend on prey ingestion rather than cod size. Unfortunately time did not allow to include this analysis within this study.

Because Fulton’s condition factor is based on total weight (Nash et al., 2006), condition values might be overestimated in the spawning season. Baltic cod is known to spawn between February and November with the main spawning season between May and August (Hinrichsen et al., 2016). During spawning season the gonads mature and grow larger, thus adding extra weight to the body. Using total weight to calculate condition can therefore generate biased results because cod in spring and summer might be heavier due to mature gonads and not due to good health. Using somatic weight, which is body weight minus gut and gonad weight, when analysing condition would probably generate more reliable results. But because the fisherman weighted the cod and prepared the stomachs before they were bought, it was not possible to use somatic weight in this study. Hence, total weight was used.

The cod stomachs were bought from a commercial fisherman and therefore the sampling for this study depended on his fishing occasions and catch amount. In this study there is a data gap between June 2018 and October 2018, either because no fishing was carried out during that period or due to a lack of catch. As a consequence this study only treats the presence of round goby in stomach content over two seasons, winter and spring, which gives a biased view of the predation on round goby over the year. From an ecological perspective the predator-prey interaction between two seasons is however still valuable information. Perhaps for future studies the sampling could be carried out within the university to prevent the sampling being dependent on an external person. In that case the sampling probably could be more consistent during the whole year and with good catches generate good data for all seasons.

In conclusion, this study shows an indication of a strong predator-prey interaction and cod seems to benefit from a round goby invasion. But further research is required to fully understand the advantages and disadvantages from this invasion.

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Appendix 1.

Table 3. *Guidelines for estimation of gut fullness.*

Stomach Fullness Codes:	Description	Digestion State Codes:	Explanations from AFSC, 2015*
1	empty	1 - stomach empty	No items found in stomach
2	trace of prey	2 - traces of prey items	Only a few parts left of the prey item because most of the item has been completely digested away, fish bones with no flesh remaining
3	trace- 25 % full	3 - < 50% intact	Extensive digestion is evident but there may be several parts and perhaps some well digested chunks remaining. Fish would have some flesh remaining, large crustaceans may be missing parts due to digestion, and it may be impossible to distinguish individual small crustaceans in a slurry of parts
4	25 – 50 % full	4 - 50-75% intact	Prey items that are still partially intact, but remaining portions may be softened due to digestion. For example, fishes would have no exposed skin remaining and parts of the head or tail may be disarticulated, but a majority of the flesh would still be present; large and small crustaceans may have most of the carapace and appendages intact, but have the carapace and internal flesh softened due to digestion
5	50 – 75 % full	5 - 75-100% intact	Prey items that are in good to almost perfect condition, but often with some damage due to digestion. For example fish are mostly intact, but may be missing some skin or fin rays (usually the first parts of the fish to be digested away).
6	75 – 100 % full	6 - no digestion	prey items which are in immaculate condition

* https://www.afsc.noaa.gov/REFM/REEM/Manuals/LabManual.pdf

Appendix 2.

Tabell 4. *Total number of individuals and frequency of occurrence (FO) for prey found in stomachs of cods caught in spring 2018.*

Species	Number of individuals	FO
<u>*Osteichthyes*</u>		
Neogobius melanostomus	85	1
Gasterosteus aculeatus	1	0.03
Zoarces viviparus	1	0.03
Gobius niger	1	0.03
Hyperoplus lanceolatus	1	0.03
Syngnathiidae sp.	1	0.03
Pomatochistus minutus	0	0
Clupea harengus	0	0
Clupeidae sp.	0	0
Pungitius pungitius	0	0
Perca fluviatilis	0	0
Gobiidae sp.	0	0
Syngnathus typhle	0	0
Sprattus sprattus	0	0
Cottidae sp.	0	0
Pleuronectidae sp.	0	0
Osteichthyes unidentified	3	0.1
<u>*Crustacea*</u>		
Mysidae sp.	5	0.17
Gammarus sp.	2	0.07
Crangon crangon	2	0.07
Saduria entomon	2	0.03
Palaemon adspersus	0	0
Palaemon elegans	0	0
Palaemon sp	0	0
Idothea baltica	0	0
Crustacea unidentified	5	0.17
<u>*Mollusca*</u>		
Mytilus sp.	1	0.03
<u>*Unidentified prey*</u>	4	0.13

Table 5. *Number of individuals and frequency of occurrence (FO) for prey found in stomachs of cod caught in winter 2018-2019.*

Species	Number of individuals	FO
Osteichthyes		
Gasterosteus aculeatus	35	0.49
Clupea harengus	29	0.37
Perca fluviatilis	25	0.41
Neogobius melanostomus	10	0.2
Pomatochistus minutus	7	0.1
Gobiidae sp.	6	0.12
Pungitius pungitius	2	0.05
Zoarces viviparus	1	0.02
Syngnathus typhle	1	0.02
Cottidae sp.	1	0.02
Pleuronectidae sp.	1	0.02
Gobius niger	0	0
Hyperoplus lanceolatus	0	0
Syngnathiidae	0	0
Sprattus sprattus	0	0
Clupeidae sp.	0	0
Osteichtyes unidentified	6	0.1
Crustacea		
Gammarus sp.	105	0.39
Saduria entomon	29	0.34
Palaemon elegans	20	0.1
Palaemon adspersus	9	0.07
Idothea baltica	9	0.05
Crangon crangon	7	0.12
Palaemon sp	0	0
Mysidae sp.	0	0
Crustacea unidentified	15	0.22
Mollusca		
Mytilus sp.	2	0.02
Unidentified prey	0	0

Table 6. *Number of individuals and frequency of occurrence (FO) for prey found in stomachs of cods caught in spring 2019.*

Species	Number of individuals	FO
Osteichthyes		
Neogobius melanostomus	48	0.62
Gasterosteus aculeatus	30	0.27
Perca fluviatilis	11	0.24
Clupea harengus	4	0.07
Gobiidae sp.	2	0.04
Clupeidae sp.	2	0.04
Sprattus sprattus	1	0.02
Gobius niger	1	0.02
Zoarces viviparus	0	0
Pungitius pungitius	0	0
Hyperoplus lanceolatus	0	0
Syngnathus typhle	0	0
Syngnathiidae sp.	0	0
Pomatochistus minutus	0	0
Cottidae sp.	0	0
Pleuronectidae	0	0
Osteichthyes unidentified	9	0.13
Crustacea		
Gammarus sp.	216	0.27
Palaemon sp	14	0.02
Saduria entomon	6	0.11
Palaemon elegans	6	0.02
Crangon crangon	5	0.07
Palaemon adspersus	2	0.04
Idothea baltica	2	0.04
Mysidae sp.	2	0.04
Crustacea unidentified	5	0.09
Mollusca		
Mytilus sp.	36	0.20
Unidentified prey	1	0.02

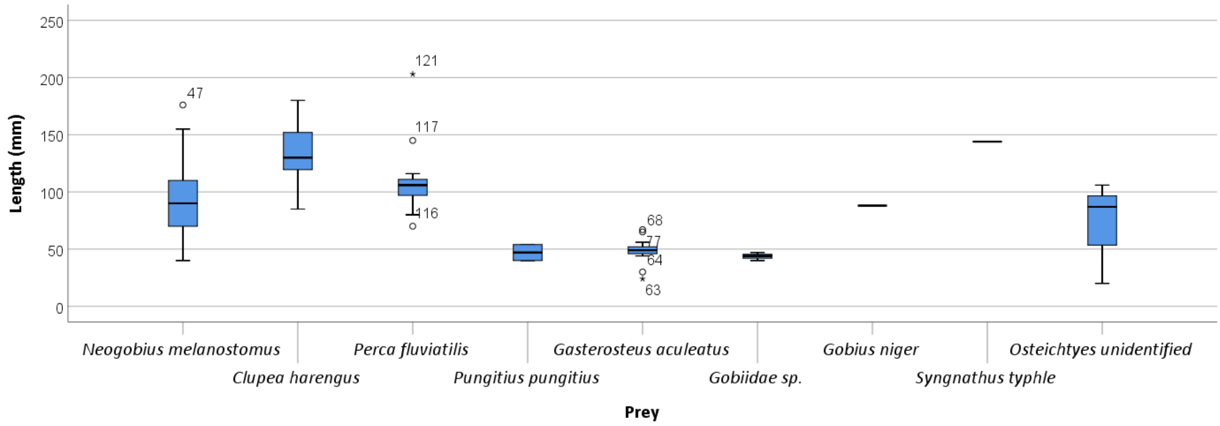

Figure 22. Length range of fish prey found in the stomachs.

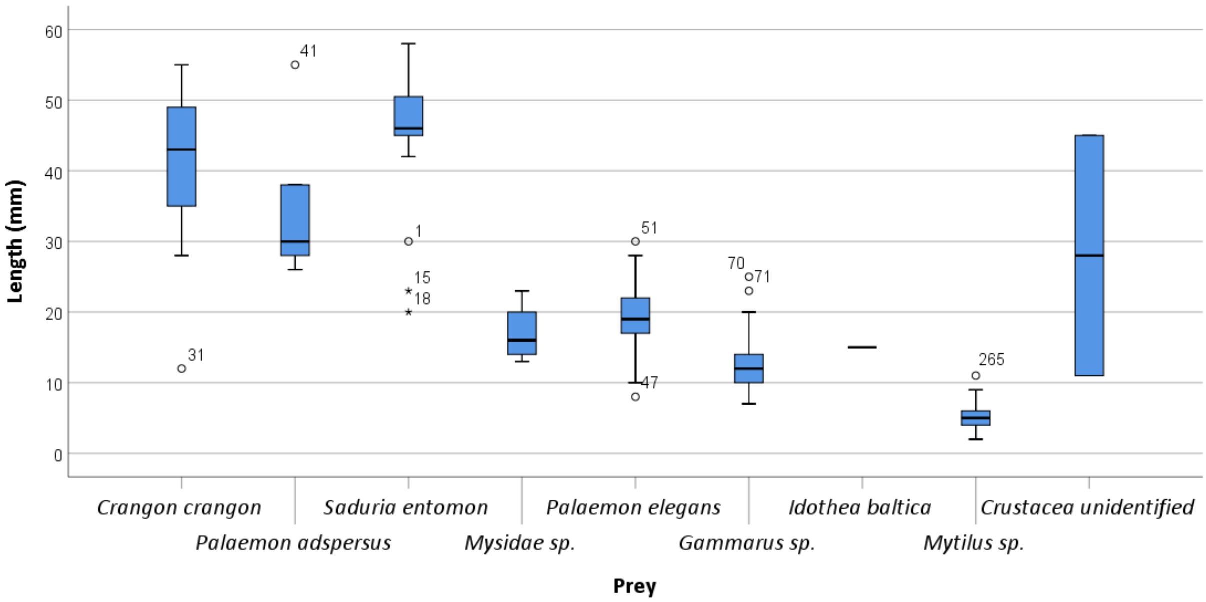

Figure 23. Length range of crustacean and mollusc prey items found in the stomachs.

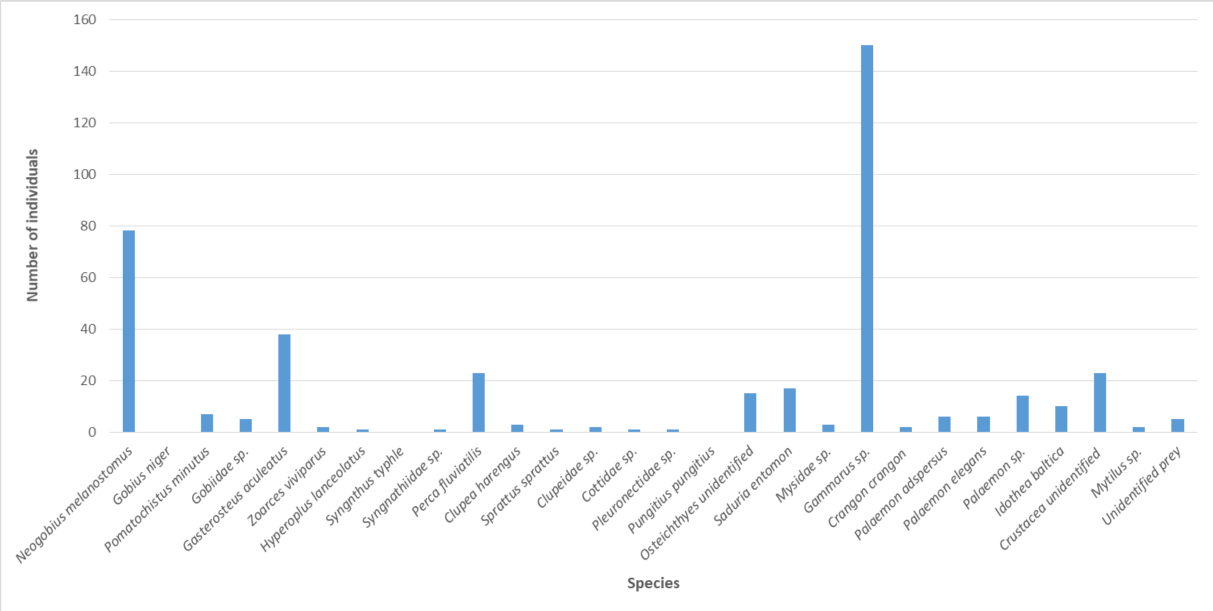

Figure 24. Immeasurable prey found in the stomachs.