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# Round goby (Neogobius melanostomus) - a potential threat to northern pike (Esox lucius) recruitment in the Swedish Baltic Sea coastline?

Master's thesis in Biology Supervisor: Gunilla Rosenqvist, Irja Ida Ratikainen, Isa Wallin May 2019



Master's thesis

Norwegian University of Science and Technology Faculty of Natural Sciences Department of Biology

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## Abstract

Invasive species are a major threat to aquatic biodiversity worldwide, triggering strong ecological impacts on aquatic ecosystems. A highly successful aquatic invader is the round goby (Neogobius melanostomus), which has spread to both freshwater and brackish habitats worldwide. The present study focused on round goby in Sweden, and if it could impact the local ecosystem through predation of early life stages of northern pike (Esox lucius), a native predator which has experienced a population decline in Sweden's coastal waters. I hypothesized that round goby could act as a potential threat to pike recruitment and at the same time successfully outcompete a native predator on pike in early life stages - the three-spined stickleback (Gasterosteus aculeatus). This was tested by combining a laboratory experiment and a literature review. The experiment was performed at Ar Research Station in Gotland, Sweden, where predation on pike juveniles by round goby and three-spined stickleback and competition over this prey item was studied. To be able to predict how the round goby could impact pike recruitment in the Baltic Sea, a literature review of the current knowledge of round goby predation on eggs and juveniles of native fish around the world was performed. The experiment demonstrated that round goby predated on pike juveniles, but it was not possible to confirm any competition between round goby and three-spined stickleback. The literature review showed that experimental studies on round goby consumption of fish eggs and/or juveniles demonstrate that round goby could negatively impact recruitment of native fish. The results from the experiment and the literature review conclude that the round goby could act as a threat to pike recruitment in Sweden by predating on eggs and juveniles, but the extent and importance of this threat is not clear. Still, an important hindrance to restoration of threatened fish species is high mortality rates in the early life stages, e.g. from predation. Since round goby has not yet established in freshwater in Sweden, prevention of round goby invasion to such environments should be prioritized by stakeholders.

# Sammendrag

Invasive arter er en stor trussel mot akvatisk biodiversitet verden over, da de fører til sterke økologiske virkninger i akvatiske økosystemer. En svært suksessfull invasiv fiskeart er svartmunnet kutling (Neogobius melanostomus), som har spredt seg til både brakkvann og ferskvann over store deler av verden. Den nåværende studien fokuserte på svartmunnet kutling i Sverige, og om den kan påvirke det lokale økosystemet gjennom predasjon på tidlige livsstadier av gjedde (Esox lucius), en stedegen art som har opplevd en populasjonsnedgang langs kysten av Sverige. Min hypotese var at svartmunnet kutling kan fungere som en trussel mot rekruttering av gjedde, og samtidig utkonkurrere en stedegen predator på gjedde - trepigget stingsild (Gasterosteus aculeatus). Dette ble testet ved å kombinere et laboratorieeksperiment og en litteraturstudie. Eksperimentet ble utført ved Ar forskningsstasjon på Gotland i Sverige, hvor predasjon på gjeddeyngel fra svartmunnet kutling og trepigget stingsild og konkurranse over dette byttet ble undersøkt. For å kunne forutsi hvordan svartmunnet kutling kan påvirke rekruttering av gjedde i Østersjøen, ble det utført en litteraturstudie om nåværende kunnskap om svartmunnet kutling som predator på fiskeegg og -yngel rundt om i verden. Eksperimentet viste at svartmunnet kutling spiste gjeddeyngel, men det var ikke mulig å bekrefte konkurranse mellom svartmunnet kutling og trepigget stingsild. Litteraturstudien viste at eksperimentelle studier om predasjon fra svartmunnet kutling på fiskeegg og/eller -yngel demonstrerer at svartmunnet kutling kan påvirke rekruttering hos stedegne fiskearter negativt. Resultatene fra eksperimentet og litteraturstudien konkluderer med at svartmunnet kutling kan utgjøre en trussel mot rekruttering av gjedde i Sverige ved å spise egg og yngel, men omfanget og betydningen av denne trusselen er ikke tydelig. Likevel, et viktig hinder for restaurering av truede fiskearter er høy dødelighet i de tidlige livsstadiene, for eksempel grunnet predasjon. Siden svartmunnet kutling enda ikke har etablert seg i ferskvann i Sverige, bør aktører prioritere å forhindre en invasjon av svartmunnet kutling til slike områder.

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# Table of contents

Abstract	V
Sammendrag	vii
Acknowledgements	ix
List of Figures	xiii
List of Tables	xiii
1 Introduction	1
1.1 Invasive species – an overview	1
1.2 Study organisms	2
1.2.1 Round goby	
1.2.2 Northern pike	5
1.3 Current status of pike and round goby populations in Sweden	5
1.4 Study aims	6
2 Methods	9
2.1 Experiment	9
2.1.1 Ethics statement	9
2.1.2 Fish collection and husbandry	
2.1.3 Experimental design	
2.1.4 Observational protocol	
2.1.5       Statistical analysis         2.2       Literature review	
2.2 Literature review	15
3 Results	15
3.1 Experiment	
3.2 Literature review	17
4 Discussion	21
4.1 General findings	
4.2 Experiment	
4.3 Literature review	22
5 Conclusion	27
6 References	

# List of Figures

Figure 1. Distribution of round goby in Europe and North America.	
Figure 2. Round goby	
Figure 3. Fårösund harbor.	
Figure 4. Setup of the experiment.	11
Figure 5. Predation on pike juveniles in each treatment.	15
Figure 6. The distribution of eaten pike juveniles in each treatment.	16
Figure 7. Numbers of screened and included papers for the literature review	17
Figure 8. The number of reviewed cases showing high, low or no impact on native fish, and w	what type of study
was performed	18

# List of Tables

Table 1. Experimental design.	_10
Table 2. Average number of pike juveniles consumed in each treatment.	_16
Table 3. Model output of the logistic regression.	_17
Table 4. Studies investigating round goby as a predator on eggs or juveniles of native fish.	_19

# 1 Introduction

### 1.1 Invasive species – an overview

In today's globalized world, the issue of alien species has increased in magnitude and diversity (Hulme, 2009). An alien species is a species introduced by humans, either accidentally or intentionally outside of its natural past or present distribution (IUCN, 2019). About 5 - 20 % of these species become problematic and are hence termed invasive. An invasive species is defined as: "a species that is established outside of its natural past or present distribution, whose introduction and/or spread threaten biological diversity" (Convention on Biological Diversity).

Biological invasions are divided into the following stages: 1) transport, 2) introduction, 3) establishment and 4) spread (Blackburn *et al.*, 2011). The different stages are separated by certain abiotic and biotic barriers that the species must overcome to pass on to the next stage, like geographical barriers, and being able to survive, reproduce and disperse. Different management efforts can be made to either prevent (before introduction), eradicate (after introduction) or control (after establishment and spread) invasive species (Hulme, 2006). The earlier in the invasion process, the more cost-effective the management measures are. However, it is difficult to predict if an alien species will become invasive. Together with the fact that the process of an invasion often includes a time lag between introduction and spread, it is clear that the nature of invasions can be highly unpredictable (Kowarik, 1995; Hulme, 2006).

Invasive species have certain characteristics that distinguish them from other species: they are often generalists, good disperses, reproduce rapidly, have a short growth period and tolerate a wide range of environmental conditions (Kolar and Lodge, 2001). The higher the propagule pressure (the number of individuals introduced in an area), the more likely it is that the species will become established (Simberloff, 2006). When being introduced to a new habitat, these species could outcompete native species for limited resources, modify the habitat so that the native species no longer can thrive there, directly prey on the native species to the point of extinction or transmit previously absent and/or unknown diseases (Holitzki *et al.*, 2013). The newly invaded habitat may lack specialized natural predators and parasites that would otherwise control the invasive species in its native habitat (Davis, 2009). Disturbed habitats – habitats

altered by humans – may also benefit the invaders, with the native species not being able to adapt to these new conditions (Dukes *et al.*, 2011). This is important in the context of humaninduced climate change, which is expected to cause higher sea temperatures, altered salinization, reduced ice cover and altered flow regimes (Winsor *et al.*, 2001; Poff *et al.*, 2002). This means that previous abiotic barriers that prevented a species from becoming invasive could be removed. For example, most fish are ectothermic, which means that water temperature is an important abiotic variable (Magnuson *et al.*, 1990). Warmer temperatures could cause stress to cold water-adapted fish species, while allowing warm water species or species with a wide temperature tolerance to thrive in new places (Sharma *et al.*, 2007).

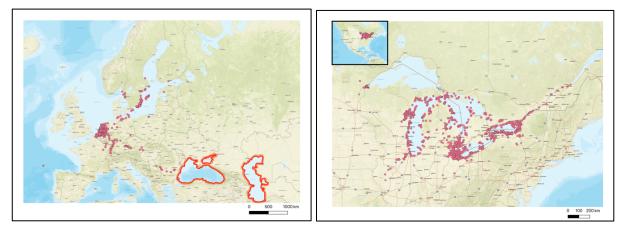
Invasive species are seen as a major threat to aquatic biodiversity worldwide, triggering strong ecological impacts on aquatic ecosystems (Gallardo *et al.*, 2016). A highly successful aquatic invader is the round goby (*Neogobius melanostomus*), which has spread to both freshwater and brackish habitats worldwide. The species possesses characteristics typical for a successful invader: it exhibits a generalist feeding strategy with high consumption rates, can rapidly spread to and establish in new areas and tolerates a wide range of environmental conditions. It can impact native species through direct predation or competition for prey, habitat and/or shelter (Poos *et al.*, 2010; Kornis *et al.*, 2012; Hirsch *et al.*, 2016). In my master's thesis I will focus on round goby in Sweden, and how it may impact the recruitment of northern pike (*Esox lucius*), a native predator that has experienced a population decline in Sweden's coastal waters (Nilsson *et al.*, 2004).

## 1.2 Study organisms

#### 1.2.1 Round goby

The round goby (*Neogobius melanostomus*; Pallas 1814) is a member of the diverse Gobiidae family. The species is native to the Ponto-Caspian region, including the Black, Caspian and Azov Seas (Kornis *et al.*, 2012). Today, the round goby can be found in the Great Lakes in North America, as well as the Baltic Sea in Europe and several major European rivers (Figure 1). The species was discovered for the first time outside native waters in 1990, in both the Baltic Sea and the Great Lakes. There is strong evidence that the round goby was transferred via ballast water in transoceanic vessels. After being introduced in a novel ecosystem, further propagation

may occur through natural dispersal and commercial shipping, or even human activities (Kornis *et al.*, 2012).



*Figure 1.* Distribution of round goby in Europe and North America. Areas marked in red indicate natural range, pink points indicate occurrences outside natural range. Maps were created in QGIS (QGIS Development Team, 2019). Occurrence data is from GBIF (GBIF.org).

Round goby individuals at invasion fronts differ from individuals in established populations: these pioneering individuals have an increased exploratory behavior, high phenotypic plasticity and increased competitive ability (Groen *et al.*, 2012; Brandner *et al.*, 2013; Cerwenka *et al.*, 2014). Adults also seem to have a larger body size (Brandner *et al.*, 2018). Different studies draw different conclusions on whether the sex-ratio at invasion fronts is skewed towards females (Brownscombe and Fox, 2012; Brandner *et al.*, 2013) or males (Corkum *et al.*, 2004; Gutowsky and Fox, 2011). Round goby invasions appear to be a rapid process, with a minimum duration of only one year from introduction to establishment (Brandner *et al.*, 2018).

The round goby exhibits a pigmented body, usually grey, brown or yellow-green, with dark brown spots (Figure 2) (Kornis *et al.*, 2012). They can reach a size of 25 cm and a lifespan of 6 years (Sokołowska and Fey, 2011). The species is sometimes confused with the native black goby (*Gobius niger*), but the round goby has a characteristic black spot on the first dorsal fin (Forsgren and Florin, 2018). Sexual dimorphism is evident in the round goby: the males are generally larger, darker and exhibit enlarged cheeks (Miller, 1984). Breeding males may even turn completely black. Like many other gobies, their pelvic fins are merged into a suctorial disc, which is used to attach to surfaces in streaming water (Kornis *et al.*, 2012). Spawning occurs at water temperatures in the range  $9 - 26 \,^{\circ}$ C (MacInnis and Corkum, 2000). In its native range the spawning season lasts from April to September, with spawning events every 3 - 4 weeks (Charlebois, 1997). The males guard nests in which females lay eggs, after which the male continues guarding the nest until the eggs hatch (Meunier *et al.*, 2009). The species prefers hard

substrates, but is versatile and also utilizes softer substrates like mud and sand (Kornis *et al.*, 2012). It is a benthic species, inhabiting shallower waters rather than deeper depths during the spawning season. The species is known for its aggressive and territorial behavior (Dubs and Corkum, 1996; Balshine *et al.*, 2005).



Figure 2. Round goby. Photograph by Peter van der Sluijs, distributed under a CC BY-SA 3.0 license.

The round goby exhibits a generalist feeding strategy, with a broad diet and high consumption rates (Kornis *et al.*, 2012). Prey taxa include zooplankton, benthic invertebrates, small fishes and fish egg and larvae. It is an opportunistic feeder, exhibiting the ability to adapt to which prey is the most available in an area (Skora and Rzeznik, 2001; Kornis *et al.*, 2012). The diet seems to be determined by body size and age, with gape height and width being limiting factors (Ray and Corkum, 1997). The gape limited juveniles seem to prefer soft-bodied prey like crustaceans and polychaetes, while adults prefer molluscs, as they are able to crush the mollusc shells (Skora and Rzeznik, 2001; Skabeikis and Lesutienė, 2015). Adults exhibit a more stationary behavior than the juveniles, which could also explain the difference in diet (Skora and Rzeznik, 2001). Habitat can also influence diet: in lakes and seas, molluscs are shown to be the primary diet component, while in streams diets are often dominated by non-mollusc benthic invertebrates (Kornis *et al.*, 2012). It is suggested that they feed on abundant prey at daytime and switch to less abundant, but more active prey at night (Carman *et al.*, 2006).

The round goby is a salinity tolerant species, as it inhabits fresh, brackish and marine waters (Kornis *et al.*, 2012). However, there is no evidence of reproducing populations in a full ocean habitat (Charlebois, 1997). The species also shows a wide tolerance in water temperature,

ranging from -1 - 30 °C (Moskal'kova, 1996). In Lake Erie (one of the five Great Lakes), the energetic optimum of round goby was estimated to be 26 °C (Lee and Johnson, 2005). In the Great Lakes the species is most abundant in the warmest lake rather than the coldest lake, which indicates that higher temperatures are more beneficial (Kornis *et al.*, 2012). They can also tolerate low oxygen levels, but will try to escape hypoxic levels (Kornis *et al.*, 2012).

#### 1.2.2 Northern pike

The northern pike (*Esox lucius*; hereafter pike) is a large (<130 cm) and long-lived (>10 years) predatory fish species, which can be found in lakes, rivers and brackish waters throughout the Northern Hemisphere (Forsman et al., 2015). It is considered a keystone species as it can exert top-down control in a variety of ecosystems (Spens et al., 2007; Larsson et al., 2015). Pike populations in the Baltic Sea have two different reproductive strategies: they either spawn in shallow brackish waters or in freshwater habitats (e.g. coastal streams or wetlands) (Nilsson, 2006; Nilsson et al., 2014). They prefer areas of flooded vegetation as these provide essential shelter for larvae and juveniles (Lappalainen et al., 2008; Nilsson et al., 2014). The adults may arrive to the breeding grounds before spawning commences and remain there until after spawning (Frost and Kipling, 1967). During spawning the adults do not eat and can display an aggressive behavior (L. Vallin, The Swedish Anglers Association, personal communication). One female may lay  $8000 - 100\ 000$  eggs over a period of 2 - 5 days, depending on her size and health (Nilsson, 2006). After spawning, the eggs are left unattended and are extremely vulnerable. There is limited knowledge of egg survival in natural spawning grounds in the Baltic Sea, but Nilsson (2006) reported low egg survival in three spawning areas along the Swedish coast of the Baltic. In one of the areas (Kalmar Sound) the entire spawn disappeared possibly due to predation.

## 1.3 Current status of pike and round goby populations in Sweden

The Baltic Sea and its coastal areas have suffered from eutrophication and pollution since the 1960s, due to drainage of land, channelization and loss of wetland habitats (Jansson and Dahlberg, 1999; Elmgren, 2001). These changes in the environment led to a severe change in fish community structure in the 1990s (Nilsson *et al.*, 2004). Predatory fish species like pike declined abruptly, while zooplanktivorous fish species like three-spined stickleback (*Gasterosteus aculeatus*) increased heavily in abundance. The decline in pike populations was most likely caused by poor recruitment due to loss of suitable spawning and nursery grounds

(Andersson *et al.*, 2000; Nilsson *et al.*, 2004). In addition, recruitment could be further suppressed by egg predation by three-spined stickleback or other predators (Andersson *et al.*, 2000; Nilsson, 2006; Bergström *et al.*, 2015; Nilsson *et al.*, 2019). Measures have been taken to restore wetlands in Sweden and other countries around the Baltic Sea, some with the goal of reducing agricultural nutrient loads and others to increase biodiversity (Paludan *et al.*, 2002; Hansson *et al.*, 2005). Pike recruitment in freshwater has been shown to be important for their abundance in the Baltic Sea, as nearly half of the pike in the Baltic is of freshwater origin (Engstedt *et al.*, 2010). Restored wetlands with the aim of increasing pike populations have been termed "pike factories", and in Sweden more than 20 of these exist today (Sportfiskarna, 2017).

Round goby was first discovered in Karlskrona, Sweden in 2008 and had established a reproducing population by 2010 (Artdatabanken, 2015). It was probably spread via ballast water, as there is shipping traffic between Karlskrona and Gdansk, Poland where the round goby is abundant (Florin and Karlsson, 2011). The round goby is now established on the western, southern and eastern coasts of Sweden. In the end of May 2016, it was discovered for the first time in Swedish freshwater in Hauån, Gotland (R. Gydemo, County Administration Board, Gotland, personal communication). A year later it was found further upstream in Vägumeån, Gotland (P. Landergren, County Administration Board, Gotland, personal communication). Since round goby can be expected to expand to freshwater bodies around the Baltic Sea in the future and establish permanent populations, it will likely encounter pike of different life stages on a regular basis. Thus, it is important to investigate potential ecological consequences of such encounters.

### 1.4 Study aims

With the recent observations of round goby in freshwater in Sweden and the Baltic Sea pike populations being restored, a relevant question to ask is: could round goby impact the local ecosystem through predation on early life stages of pike? As of March 2019, there are no studies on round goby predation on pike eggs and juveniles. However, there are studies on round goby predation on eggs and juveniles of other native fish species around the world, as documented in an extensive literature review by Hirsch *et al.* (2016) that quantified the knowledge of round goby impacts on native fish species. I hypothesize that the round goby can act as a potential

threat to pike recruitment by predating on pike juveniles, and at the same time successfully outcompete a native predator on pike in early life stages. This was tested by combining a laboratory experiment and a literature review. First, in the laboratory experiment I wanted to see if round goby would eat pike juveniles, a novel prey item, and if it would outcompete a native species, the three-spined stickleback in competition for this prey. Based on the fact that round goby is a generalist and opportunistic feeder. I predicted that round goby would predate on pike juveniles. Three-spined stickleback is known to predate on pike in early life stages, but I predicted that round goby would outcompete three-spined stickleback as the round goby is bigger and more aggressive. To test this, pike juveniles were added to aquaria with round goby, aquaria with three-spined stickleback and aquaria with both round goby and three-spined stickleback. Their stomach contents were examined to see how many pike juveniles had been consumed by each species. If three-spined stickleback consumed more in aquaria alone than when together with round goby, this could be an indication of round goby outcompeting threespined stickleback. Second, I wanted to review the current knowledge of round goby predation on eggs and juveniles of native fish around the world, to be able to predict how the round goby could impact pike recruitment in the Baltic Sea. This was done by extracting relevant studies already reviewed in Hirsch et al. (2016) and reviewing new studies on the topic.

## 2 Methods

## 2.1 Experiment

The experiment was conducted at Ar Research Station, Campus Gotland, Uppsala University, Sweden (57°55'01.1"N, 18°56'15.4"E).

#### 2.1.1 Ethics statement

The experiment was carried out under the ethical permit S27-15 by the Swedish Board of Agriculture. When the experiment was finished, the remaining pike juveniles were released back to Österby myr. The round gobies were euthanized by cutting over their spinal cord. The other fish were released back where they originally were caught.

#### 2.1.2 Fish collection and husbandry

Fish collection took place in Fårösund harbor (57°51'59.0"N 19°03'30.2"E). A total of 11 fishing net traps were placed at 3 different locations on the  $24^{th}$  of May 2018 in the harbor and left overnight (Figure 3). Cooked shrimp (*Pandalus borealis*) was used as bait, with 2 - 4 shrimps in each trap. A total of 58 round gobies and 61 three-spined sticklebacks were caught.

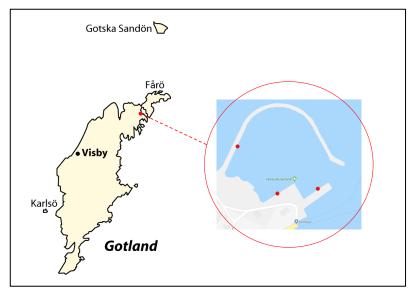


Figure 3. Fårösund harbor. Red points indicate the locations of the different traps (Gullichsen, 2019).

Only the round gobies were measured (total length, to the nearest mm), and sex was determined (Kornis *et al.*, 2012). From the day they were caught and until the experiment started the 29<sup>th</sup>

of May 2018, the round gobies and three-spined sticklebacks were kept in tanks measuring 90 cm x 90 cm x 50 cm (405 L) at Ar Research Station. The round gobies were categorized in different tanks in the following categories: female 7-14 cm, female > 14 cm, male 7-14 cm, male >14 cm. To mimic natural conditions, the tanks were filled with artificial eelgrass, and terracotta tubes and bricks to use as hiding spots. The tanks were aerated with airstones and supplied with water from the Baltic Sea, which held a temperature of 5.4 - 15.5 °C and salinity of 6.7 - 7.2 ‰. All fish were fed twice a day with brine shrimp (*Artemia* sp.), bloodworms and mysid shrimp (Mysidae).

Pike eggs were collected the 9<sup>th</sup> of April 2018 by stripping of females and artificial fertilization in Österby myr, a pike factory in Kräklingbo on eastern Gotland ( $57^{\circ}26'42''N 18^{\circ}42'41''E$ ) and incubated in streaming freshwater in tanks at the research station. Post-hatching and until start of experiment, the juveniles were in tanks (22 L each) with water supply from Lake Bästeträsk which held a temperature of 15.5 - 17.7 °C. They were fed with brine shrimps three times a day. As a food supplement, they were fed freshwater plankton from a pond outside the field station once a day.

#### 2.1.3 Experimental design

There were four different treatments: 1) Round goby with pike juveniles ('RG'), 2) three-spined sticklebacks with pike juveniles ('ST'), 3) both round goby and three-spined sticklebacks with pike juveniles ('RG-ST') and 4) a control group with only pike juveniles ('Control'). An overview of the experimental design is seen in Table 1.

Treatment	Number of individuals per species in each treatment	Replicates
RG	1 round goby, 4 pike juveniles	12*1
ST	3 three-spined sticklebacks, 4 pike juveniles	5
RG-ST	1 round goby, 3 three-spined sticklebacks, 4 pike juveniles	5
Control	4 pike juveniles	5

Table 1. Experimental design.

<sup>&</sup>lt;sup>1</sup> \*Originally the number of replicates for treatment 'RG' was 13, but a replicate was removed from further analysis after the experiment due to an individual being injured.

The experiment was conducted in 40 cm x 20 cm x 25 cm (20 L) plastic aquaria. The aquaria were separated from each other with white screens to avoid the fishes observing each other. A black cover was hung behind the aquaria to ensure the background was similar for all aquaria. Transparent plates were put on top of each aquarium, to prevent any fish from jumping out. Oxygen pumps were provided to each aquarium. In the 'RG-ST'-treatments, a dividing plate was put in the middle to prevent the round gobies and three-spined sticklebacks to interact with each other before onset of the experiment. This was removed when the experiment started. Two grey plastic tubes (5 cm inner diameter) and two pots of artificial eelgrass were put into each aquarium. Figure 4 shows the setup of the experiment.



Figure 4. Setup of the experiment.

Male round gobies of size 7-14 cm were used. We did not know the sex of the three-spined sticklebacks. The day before the experiment, food was withheld for the three-spined sticklebacks and round gobies that would be used in the experiment to ensure similar hunger levels for all individuals during the experiment. The experiment was conducted in freshwater, as the pike juveniles originated from freshwater. The round gobies and three-spined sticklebacks were put into the aquaria the day before the experiment started, as both species needed to acclimatize from brackish water to freshwater. This was carried out by gradually replacing the brackish water with freshwater every hour for 10 hours. During this process the salinity went down from 6.5 ‰ to 0.7 ‰, and temperature went up from 6.2 °C to 16.9 °C.

#### 2.1.4 Observational protocol

The experiment was conducted in two sets. The first set was carried out on the 29<sup>th</sup> of May 2018. Five replicates of each treatment were arranged in total of 20 aquaria. The round gobies

and three-spined sticklebacks were already present in the aquaria from the night before. The experiment started at 07.00 AM when the pike juveniles were added to each aquarium, and ran for 4 hours. After this, the number of pike juveniles left in each aquarium was counted. One replicate in the 'RG'-treatment was removed from further analysis, as the round goby seemed fatigued and we observed blood in the water which could indicate that the individual was injured. It was euthanized shortly after discovering this. The remaining pike juveniles were put back into the storage tanks. In aquaria where all pike juveniles were retrieved, i.e. where the round gobies and/or three-spined sticklebacks had not eaten any pike juveniles, the fish were acclimatized back to brackish water using the method previously described and put into a new tank. The rest of the fish were euthanized by cutting over their spinal cord. To preserve the stomach content, the fish were put in a refrigerator. The fish were dissected shortly after this, and the stomach content was examined under a stereo microscope.

The second set was carried out on the 2<sup>nd</sup> of June 2018. This time, only the 'RG'-treatment was used because we wanted to get more data from this treatment. There were eight replicates, where every replicate consisted of one round goby and four pike juveniles. The round gobies were already present in the aquaria from the night before. The experiment started at 07.00 AM when the pike juveniles were added to each aquarium and ran for 4 hours. After this, the number of pike juveniles left in each aquarium was counted. The remaining pike juveniles were put back into the storage tank. The round gobies used in the second set were not euthanized as we did not need to look at the stomach content, but rather used the number of remaining pike juveniles to find out how many had been eaten. They were acclimatized from freshwater to brackish water using the same method explained earlier, before they were put back into a separate tank.

#### 2.1.5 Statistical analysis

To visualize how many replicates of each treatment showed pike predation, the data was plotted in R 3.5.1 (R Core Team, 2018) using the *ggplot2* package (Wickham, 2016). Categories were set as 'Pike predation' and 'No pike predation', depending on if the replicates showed predation or not. The number of pike juveniles eaten in each replicate was therefore not taken into account here, but was used when calculating the average amount of pike juveniles eaten for each treatment. To investigate the predation in 'RG' and 'ST' in relation to 'RG-ST', a logistic regression was conducted in R 3.5.1. A generalized linear model with a binomial distribution was chosen, as it is count data. The response variable was the number of eaten pike juveniles in each replicate, and the predictor variable was treatment. Results were considered significant at  $\alpha = 0.05$ .

#### 2.2 Literature review

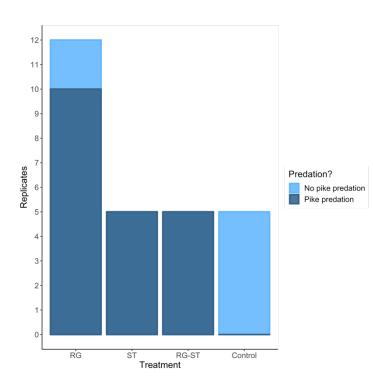
To review the current knowledge of round goby predation on eggs and juveniles of native fish, I conducted a systematic literature review. This was performed in two steps. First, to review literature from 2015 and earlier, the literature review by (Hirsch et al., 2016) was accessed. It contained a table with all reviewed studies. Studies where the native species functioned as prey to round goby and their life stage being eggs or juveniles were extracted and the full text was reviewed. Studies on round goby predation on conspecific eggs and juveniles were excluded, as I only was interested in round goby impacts on other species. Second, to review newer papers not included in Hirsch et al. (2016), a literature search was carried out in the Web of Knowledge database (http://webofknowledge.com). The search terms used were 'round goby,' 'neogobius melanostomus,' 'predation,' 'predator,' 'diet,' 'eggs,' 'YOY,' 'juvenile,' separated by Boolean operators 'AND' or 'OR': ('round goby' OR 'neogobius melanostomus') AND ('predation' OR 'predator' OR 'diet') AND ('eggs' OR 'YOY' OR 'juvenile'). The last search was conducted on the 14<sup>th</sup> of March 2019. The resulting list of publications was refined to only include peerreviewed studies from 2015 - 2019 presenting original research. These papers were reviewed, and papers that did not mention round goby nor look at predation on eggs and juveniles were excluded. In the final list of papers, all reference lists were screened for additional papers which were added to the list if they met the same criteria as the papers found in the Web of Knowledge.

The focus when analyzing each study was which species was affected by round goby, if the study was performed in the field or in the lab, if the study was performed in freshwater or brackish water, if round goby used eggs and/or juveniles as a food item and lastly, the level of impact. The latter was classified as either 'high', 'low' or 'none' depending on the conclusions made in each study. 'High' indicated that the round goby readily consumed eggs and/or juveniles, and that predation possibly could affect the native species negatively. 'Low' indicated consumption of eggs and/or juveniles, but that it was not an important part their diet. 'None' indicated no predation of eggs and/or juveniles in the study, even though the round goby had the possibility to consume them.

## 3 Results

## 3.1 Experiment

The number of replicates in each treatment showing predation on pike juveniles is visualized in Figure 5.



*Figure 5.* Predation on pike juveniles in each treatment. 'RG' refers to the treatment with round goby and pike juveniles, 'ST' refers to the treatment with three-spined sticklebacks and pike juveniles, 'RG-ST' refers to the treatment with round goby, three-spined sticklebacks and pike juveniles, and 'Control' refers to the control group with only pike juveniles.

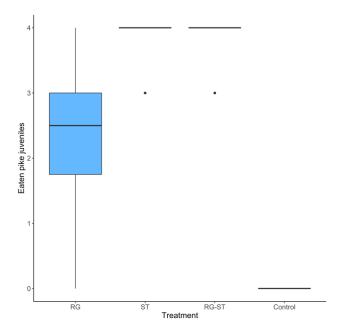
Treatment 'RG' showed predation on pike juveniles in 10 out of 12 replicates. Treatment 'ST' showed predation on pike juveniles in all replicates. Treatment 'RG-ST' also showed predation on pike juveniles in all replicates. However, after examining the stomach content of the fish in this treatment under a stereo microscope, it was clear that the stomach content had dissolved to such a degree that it was impossible to observe pike remains in such detail as to determine how many were eaten by each fish. Therefore, it was not possible to confirm the outcome of any competition between round goby and three-spined stickleback. Still, round goby aggression towards three-spined stickleback in their mouths. One of the three-spined sticklebacks died shortly

after observing this, while the other one was visibly damaged. In the 'Control' treatment all pike juveniles survived, which shows that mortality is due to predation in the other treatments.

In treatment 'RG', an average of 2.2 pike juveniles were consumed (Table 2). On average 3.8 pike juveniles were consumed in treatment 'ST' and in treatment 'RG-ST'. The only pike juvenile that was not consumed in treatment 'RG-ST' was found dead at the end of the experiment, which could explain why there was not 100% consumption as the predators may not want to consume a dead prey item. The distribution of eaten pike juveniles in each treatment is seen in Figure 6.

Table 2. Average number of pike juveniles consumed in each treatment.

Treatment	Average number consumed (out of 4)	Proportion	
RG	2.2	0.54	
ST	3.8	0.95	
RG-ST	3.8	0.95	



*Figure 6*. *The distribution of eaten pike juveniles in each treatment. Note that both treatment 'ST' and 'RG-ST' only have one outlier.* 

Less pike juveniles were consumed in treatment 'RG' than in 'RG-ST' (P = 0.009) (Table 3). Predation in treatment 'ST' was not significantly different from 'RG-ST' (P = 1.000). The control treatment clearly has lower mortality than the other treatments, but this difference is not significant due to problems with estimation of the standard error because there was no variance in this group (all pike juveniles survived).

Treatment	Estimate	P-value		
Intercept	2.944	1.026	0.004	
RG	-2.777	1.066	0.009	
ST	<0.001	1.451	1.000	
Control	-22.31	2175	0.992	

*Table 3.* Model output of the logistic regression with number of pike juveniles eaten as the response variable, and the treatments 'RG', 'ST' and 'Control' compared to 'RG-ST' as explanatory variables.

## 3.2 Literature review

Out of 113 reviewed papers in Hirsch *et al.* (2016), 8 of the papers met the criteria and were added to the personal database (Figure 7). The literature search in Web of Knowledge resulted in a list of 72 papers, which was narrowed down to 4 when following the criteria. In addition, 2 papers were included in the personal database when screening reference lists in the other papers. This resulted in a total of 14 studies that were analyzed. As some of the studies explored multiple species or life stages, they were split into separate rows, which resulted in a total of 24 unique cases (Table 4).

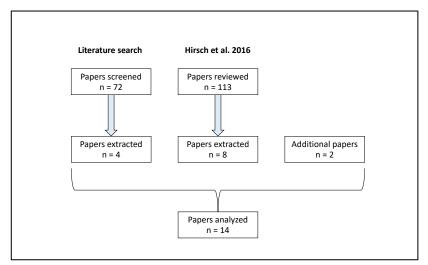
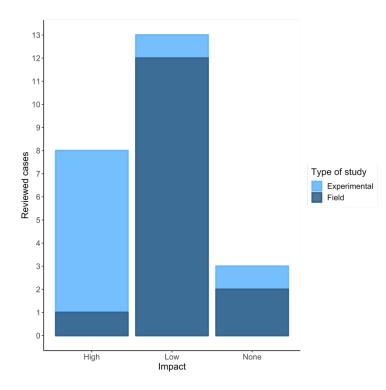


Figure 7. Numbers of screened and included papers for the literature review.

The literature review showed that round goby in 8 out of 24 reviewed cases had high impact on native fish by feeding on eggs and/or juveniles, and in 13 out of 24 cases had low impact on native fish by eggs and/or juveniles not being an important part their diet. 3 out of 24 cases showed no impact on native fish. Of the cases showing high impact, 7 out of 8 are from in-situ or laboratory studies, while for the cases showing low impact, 12 out of 13 are from field studies (Figure 8). 2 out of 3 cases showing no impact are from field studies.



*Figure 8. The number of reviewed cases showing high, low or no impact on native fish, and what type of study was performed.* 

**Table 4.** Studies investigating round goby as a predator on eggs or juveniles of native fish, ranging from 1999-2019. Affected native species are shown with both scientific and common name. 'Egg' refers to the stage before the fish is hatched, and 'juvenile' refers to the stage after hatching and until adulthood. 'Field' refers to a diet study performed by sampling specimens from their natural habitat and analyzing their stomach content, 'lab' refers to an experimental study performed in controlled environment in a laboratory, 'in-situ' refers to an experimental study performed in the natural habitat of the study species. Category 'Impact' refers to impact on native fish, where 'high' indicate consumption of eggs and/or juveniles by round goby and that predation could affect the native fish negatively, 'low' indicates eggs and/or juveniles not to be an important part of round goby diet, 'none' indicates no predation on eggs and/or juveniles even though round goby had the possibility of consuming them.

Scientific name	Common name	Life stage	Type of study	Ecosystem	Region	Food item	Impact	Source
Salvelinus namaycush	Lake trout	Egg	Lab	Freshwater	North America	Yes	High	Chotowski & Marsden (1999)
Salvelinus namaycush	Lake trout	Juvenile	Lab	Freshwater	North America	Yes	High	Chotowski & Marsden (1999)
Micropterus dolomieu	Smallmouth bass	Egg	In-situ	Freshwater	North America	Yes	High	Steinhart et al. (2004)
Salvelinus namaycush	Lake trout	Egg	Lab	Freshwater	North America	Yes	High	Fitzsimons et al. (2006)
Oncorhynchus mykiss	Rainbow trout	Egg	Lab	Freshwater	North America	Yes	High	Fitzsimons et al. (2006)
Platichthys flesus	European flounder	Juvenile	Lab	Brackish water	Europe	Yes	High	Schrandt et al. (2016)
Pimephales promelas	Fathead minnow	Egg	Lab	Freshwater	North America	Yes	High	Almeida et al. (2017)
Clupea harengus	Atlantic herring	Egg	Field	Brackish water	Europe	Yes	High	Wiegleb et al. (2019)
Cottus bairdii	Mottled sculpin	Egg	Field	Freshwater	North America	Yes	Low	French III & Jude (2001)
Cottus bairdii	Mottled sculpin	Juvenile	Field	Freshwater	North America	Yes	Low	French III & Jude (2001)
Sander vitreus	Walleye	Egg	Field	Freshwater	North America	Yes	Low	Roseman et al. (2006)
Pisces	Fish	Egg	Field	Freshwater	Europe	Yes	Low	Števove & Kováč (2013)
Cottus bairdii	Mottled sculpin	Juvenile	Field	Freshwater	North America	Yes	Low	Thompson & Simon (2014)
Micropterus dolomieu	Smallmouth bass	Egg	Field	Freshwater	North America	Yes	Low	Thompson & Simon (2014)
Notropis spp.	Eastern shiners	Juvenile	Field	Freshwater	North America	Yes	Low	Thompson & Simon (2014)
Pisces	Fish	Juvenile	Field	Freshwater	North America	Yes	Low	Thompson & Simon (2014)
Pisces	Fish	Egg	Field	Freshwater	Europe	Yes	Low	Vâsek et al. (2014)
Proterorhinus semilunaris	Western tubenose goby	Juvenile	Field	Freshwater	Europe	Yes	Low	Vâsek et al. (2014)
Osmerus eperlanus	European smelt	Juvenile	Field	Brackish water	Europe	Yes	Low	Skabeikis & Lesutienė (2015)
Pisces	Fish	Egg	Field	Freshwater	North America	Yes	Low	Pothoven (2018)
Clupea harengus	Atlantic herring	Egg	Lab	Brackish water	Europe	Yes	Low	Wiegleb et al. (2019)
Pisces	Fish	Egg	Field	Freshwater	Europe	No	None	Všetičková et al. (2015)
Pisces	Fish	Juvenile	Field	Freshwater	Europe	No	None	Všetičková et al. (2015)
Perca flavescens	Yellow perch	Egg	Lab	Freshwater	North America	No	None	Almeida et al. (2017)

## 4 Discussion

## 4.1 General findings

Based on the findings in the experiment and the literature review, it is clear that round goby could act as a threat to pike recruitment in Sweden by predating on eggs and juveniles. The experiment demonstrated that round goby predated on pike juveniles, a novel prey item. The potential threat is also shown by the literature review, which showed that round goby potentially could have negative effects on native species by directly predating on eggs and juveniles. However, the extent and importance of this threat is still not clear.

## 4.2 Experiment

As predicted round goby predated on pike juveniles, a novel prey item, in the experiment. However, on average only 2.2 out of 4 pike juveniles were consumed in treatment 'RG', and the amount eaten between the replicates varied from 0 - 4. Throughout the experiment period it was observed that the round gobies had a lower feeding activity than the three-spined stickleback. A possible explanation for this is that the round goby usually spawns around this time of year, and its appetite is considerably lower during the breeding season (Skabeikis and Lesutiene, 2015). Although the fish were not fed prior to the experiment to ensure the same hunger levels, the round goby may have had a naturally lower appetite for any prey item, which resulted in a lower average amount of pike juveniles consumed.

The three-spined stickleback predated on pike juveniles in all replicates of treatment 'ST'. On average, the three individuals in each replicate consumed 3.8 out of 4 pike juveniles together. This was expected, as the species has previously shown to be a predator of pike eggs and juveniles (Andersson *et al.*, 2000; Nilsson, 2006; Bergström *et al.*, 2015; Nilsson *et al.*, 2019). Monitoring three-spined stickleback populations in the Baltic Sea is therefore seen as an important measure to predict the negative effects they have on pike (Bergström *et al.*, 2015).

I could not confirm the outcome of any competition between round goby and three-spined stickleback, as it was not possible to determine how many pike juveniles were eaten by each

fish in treatment 'RG-ST'. I still observed aggressive behavior of round goby towards threespined stickleback in two of the replicates. This could be a result of spawning season, as male round gobies defend their nests by being aggressive (Corkum *et al.*, 1998). In a study from the Gulf of Gdansk in the Baltic Sea, Corkum *et al.* (2004) suggest that round goby and threespined stickleback are likely to co-occur, as they have different preferences in diet (three-spined stickleback is omnivorous and round goby is molluscivorous). However, in a scenario where prey is limited it is still reasonable to expect some sort of competition. I suggest the potential for competition is still there, as round goby have a broad diet and can outcompete other native species (Hirsch *et al.*, 2016).

Should this experiment be performed again, some modifications should be considered. The densities of round goby and three-spined stickleback should be changed so that they are similar. With different densities it is difficult to compare averages between the species. The length of the experiment could be shortened, as the stomach content was dissolved after just 4 hours. Another solution could be to film each aquarium and record what happens, and thus avoid the need of performing a stomach analysis.

### 4.3 Literature review

The results from the literature review do not give a clear picture of the threat for native species by round goby. 8 out of 24 cases were classified as 'high' which indicated that round goby readily consumed eggs and/or juveniles, and that predation could possibly affect the native species negatively. Affected native species include lake trout, rainbow trout, smallmouth bass, European flounder, fathead minnow and Atlantic herring. The two studies on lake trout, which is almost extirpated from the Great Lakes, concluded that round goby invasion of lake trout spawning habitats is one of the most important threats to lake trout restoration (Chotkowski and Marsden, 1999; Fitzsimons *et al.*, 2006). Fitzsimons *et al.* (2006) also included another salmonid, rainbow trout, where results were similar to lake trout. Smallmouth bass, which often nests in the same rocky habitats usually preferred by round goby, experienced heavy predation on eggs when the nest guarding male was experimentally removed (Steinhart *et al.*, 2004). As a result of the high densities of round goby, an entire smallmouth bass brood could be consumed within 15 minutes. In one of few studies performed in brackish water, Schrandt *et al.* (2016) observed that round goby readily ate small juvenile European flounders, but not larger individuals, which is consistent with gape size-limited predation. This is the first evidence of direct predation on flounder by round goby, and could negatively affect the commercial fishery of flounder, which is an important species for human consumption. They also observed round goby damaging or killing larger individuals of flounder, possibly because of territorial behavior or as an attempt to eat them. While round goby consumed yellow perch eggs in the study by Almeida *et al.* (2017), these particular eggs had their natural protective casing (skein) experimentally removed. Yellow perch is therefore not included in category 'high', as eggs with skein were not found to be a food item for round goby. However, the same study observed heavy consumption on fathead minnow eggs by round goby. Another case from Europe is the Atlantic herring, where Wiegleb *et al.* (2019) observed smaller round gobies readily consuming herring eggs. They propose that the round goby diet shifts to crustaceans or molluscs when reaching a certain size, but underline the need for further investigation.

13 out of 24 cases were classified as 'low', which indicated consumption of eggs and/or juveniles, but that it was not an important part their diet. Affected species included mottled sculpin, walleye, smallmouth bass, eastern shiners, western tubenose goby, European smelt, Atlantic herring, yellow perch and non-classified fish. In each case round goby were found to consume eggs and/or juveniles, but it was not an important part of their diet. The findings in these studies confirmed what we know about round goby diet: they are generalists whose preferred prey types include dreissenids, chironomids and cladocerans.

3 out of 24 cases were classified as 'none', where one of them is the previously mentioned yellow perch. Yellow perch is one of few freshwater fishes which has a protective egg skein to avoid predation. This rarity could possibly be explained by the energetic cost of producing egg skein (Almeida *et al.*, 2017). Všetičková *et al.* (2015) found no predation pressure on fish eggs and juveniles in a river system in Europe, and highlight the fact that there is little or no evidence that eggs and juveniles are a common part of round goby diet in Europe, compared to studies from North America.

When comparing the studies in category 'high' against category 'low', it becomes clear that the type of study performed greatly affected the conclusion. 7 out of 8 cases categorized as 'high' are from studies performed in a controlled environment; either in-situ or in a laboratory. On the other hand, almost all cases categorized as 'low' are from field studies performing dietary analyses. A possible explanation for this pattern is that the round goby is opportunistic and will

adapt to whatever prey is most available (Skora and Rzeznik, 2001; Kornis et al., 2012). Thus, when presented with only one prey type (fish eggs and/or juveniles) in the experimental studies, it would choose to prey on it. Fitzsimons et al. (2006) tested the effect of zebra mussels (Dreissena polymorpha), a common prey for round goby, on egg consumption, and found that egg consumption was unaffected by the presence of zebra mussels. However, the statistical test used had low power, and by testing more individuals of round goby they may have found that presence of another prey type affected egg consumption. Schrandt et al. (2016) explain that the low amounts of fish consumed in diet studies of round goby could be because of digestion and that the effect of predation on small native fish could be underestimated, as they observed almost complete digestion of flounder within 12 hours. The experiment performed in this thesis supports this, as the stomach content of round goby was dissolved after just 4 hours. In laboratory studies, it is also possible that the consumption rate could be artificially enhanced due to little space in the aquarium. Fitzsimons et al. (2006) tested this, when evaluating the effect of tank size on egg consumption by round goby. With five size classes (0.08 m<sup>2</sup>, 0.25 m<sup>2</sup>, 0.50 m<sup>2</sup>, 1.0 m<sup>2</sup> and 10 m<sup>2</sup>) they observed that egg consumption increased with tank size. However, increasing the tank size beyond  $1.0 \text{ m}^2$  did not yield a significant increase in egg consumption. They suggest that data from 1.0 m<sup>2</sup> tanks are reasonable estimates of egg consumption in the field. If the experiment in this thesis is to be repeated, this should be taken into consideration.

Although most of the studies in the literature review did not find eggs and/or juveniles to be an important part of round goby diet, many of them still highlight the need for further research on round goby predation on eggs and juveniles. For example, in the study by Roseman *et al.* (2006) data indicated that round goby was not a major threat to walleye eggs. Still, they underlined the importance of further research on round goby, as it could have a potential to become important predators on walleye eggs. French III and Jude (2001) did not find mottled sculpin eggs and juveniles to be of great importance in round goby diet, but did observe aggressive round gobies occupying the preferred habitats of mottled sculpin, including spawning areas. This could disrupt spawning and negatively influence mottled sculpin recruitment.

If round goby were to spread to the spawning areas of pike, there are several key points that should be addressed. Intraspecific competition and low availability of prey in an already established area of a species could facilitate a spread to a new area (Brandner *et al.*, 2013). A possible scenario is that heavy population growth of round goby in brackish water habitats in

the Baltic Sea could lead to satiated habitats and force a spread to new areas like freshwater habitats. We would expect a round goby invasion front to consist of pioneering individuals of a greater size and with greater energy reserves (Brandner *et al.*, 2018). These individuals are better fit to overcome certain barriers that would be expected, like water flow, low water levels and floodgates. This pattern is seen in Denmark, where round goby was observed about 10 km upstream in 2015, which marks the first time the species has been observed that far away from the Danish coast (Bjørn and Sivebæk, 2015). To reach this area it had to overcome a relatively strong current and low water levels.

The suitability of wetlands as habitat for round goby have been the topic of several studies from North America. Cooper et al. (2007) hypothesized that coastal wetland habitats would have lower round goby densities than adjoining coastal lakes. Round gobies were found in both habitats, but preferred lake habitats over wetlands, which supports the idea that wetland habitats are more resistant to round goby invasion. They suggest the lack of hard substrates in wetlands to possibly be an explanation. These findings are supported in Cooper et al. (2009) and Young et al. (2010). Another study by Coulter et al. (2012) did not find substrate type to explain use or avoidance of wetlands by round goby. However, they found that some wetlands, especially those with high productivity, could be more resistant to round goby invasion than open water habitats. This was supported in Coulter et al. (2015). They highlighted the importance of conserving coastal wetlands with naturally high productivity in the Great Lakes, to offer native species refuge from round gobies. Looking at the pike factories in Sweden, these studies could be good news. When studying production of pike juveniles in three restored coastal wetlands (pike factories) in Sweden, Nilsson et al. (2014) found that the construction of these could be crucial for pike recruitment. The only one that showed an increase in production was the one with new areas of flooded vegetation. Pike factories should therefore be constructed as shallow vegetated areas, as these have higher productivity, higher water temperature and offer important shelter for pike juveniles. Optimizing the design of a pike factory could therefore be favorable in two ways: to minimize round goby invasion and increase juvenile pike production.

If the spawning seasons for round goby and pike overlap, this could possibly act as a natural damper to predation of pike eggs and juveniles, considering round goby feeding activity is lower when spawning (Skabeikis and Lesutienė, 2015). However, this is probably not the case in Sweden: while the spawning season for round goby in the Baltic Sea hits its peak in July

(Skabeikis and Lesutienė, 2015), the spawning season for pike in Gotland usually takes place in March and April (L. Vallin, The Swedish Anglers Association, personal communication).

## 5 Conclusion

Round goby has been observed to predate on pike juveniles in a laboratory experiment, and reviewed experimental studies on round goby consumption on fish eggs and/or juveniles show that round goby could negatively impact recruitment of native fish. Still, most field studies on the same topic report consumption of fish eggs and juveniles to be of low importance for the round goby. As the round goby currently has not established in freshwater in Sweden, it is uncertain what a possible field study of round goby diet would show. However, the goal should be to not reach this point. We know that an important hindrance to restoration of threatened fish species is high mortality rates in the early life stages (Chotkowski and Marsden, 1999). We also know that round goby invasions appear to be a rapid process, with a minimum duration of only one year from introduction to establishment (Brandner *et al.*, 2018). The most promising management approach is therefore to prevent introduction to freshwater habitats like pike factories. I suggest the following management measures: optimization of the construction of pike factories, to ban the use of round goby as live bait in freshwater and monitor nearby round goby populations. Research on different prevention methods should be prioritized by stakeholders.

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