

# EFFECTS OF SALINITY ON REPRODUCTION - AND BEHAVIOUR OF ROUND GOBY NEOGOBIOUS MELANOSTOMUS IN THE BALTIC SEA

Dissertation

to achieve a doctoral degree

at the Faculty of Mathematics, Informatics und Natural Sciences

Institute of Marine Ecosystem and Fishery Science at the

University of Hamburg

submitted by

Jan Niemax

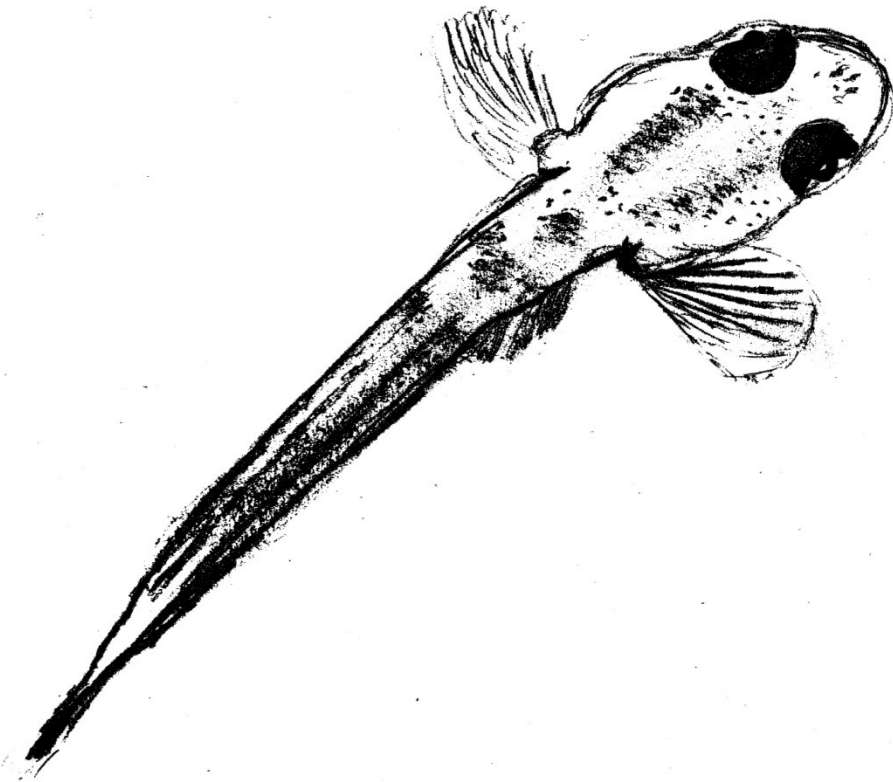
Hamburg 2021

**Reviewer of the dissertation:**

Prof. Dr. Axel Temming

Prof. Dr. Christian Möllmann

Date of Disputation 12.04.2022



***"The Only Constant in Life Is Change."- Heraclitus.***

# Content

Content.....	1
List of Figures.....	4
List of Tables.....	5
Abstract .....	6
Zusammenfassung.....	7
General Introduction.....	10
Round goby <i>Neogobius melanostomus</i> .....	12
Reproduction.....	12
Larval fish.....	13
Larval round goby.....	15
Distribution.....	17
Mechanisms of spreading .....	18
Known consequences invading general .....	19
Known consequences of round goby invasion in the Baltic Sea .....	20
Commercial importance.....	20
Salinity .....	23
Growth of larval fish .....	25
Outline .....	26
How far west can they go? Hatching success of fish egg (round goby <i>Neogobius melanostomus</i> (Pallas, 1814)) affected by temperature and salinity .....	27
Abstract .....	27
Introduction.....	27
Material and Methods.....	30
Field sampling and maintenance.....	30
Experiment .....	30
Statistics .....	33
Results .....	33
Development time.....	33
Hatching success .....	35

Discussion.....	38
Method limitation .....	38
Lessons from the invasion in other areas.....	38
Clutch size.....	39
Temperature effects.....	40
Salinity effects .....	41
Between batch variability.....	41
Implications for round goby spreading .....	42
Salinity a bottleneck? Growth and fitness of post hatched round goby ( <i>Neogobius melanostomus</i> (Pallas, 1814)) affected by salinity .....	44
Abstract .....	44
Introduction.....	44
Material and Methods.....	46
Growth experiment.....	47
Aminoacyl-tRNA synthetase-Measurement.....	47
Survival Experiment.....	48
Statistics .....	49
Results .....	50
Larval length growth.....	51
Growth per day.....	54
Survival Experiment.....	58
Discussion.....	60
Method.....	60
Larval growth.....	60
AARS as growth proxy .....	62
Survival Experiment.....	63
Conclusion .....	64
APPENDIX .....	64
APP. 3.1 Length salinity.....	64
APP. 3.2 ANNOVA spAARS.....	66
Salinity preference of round goby ( <i>Neogobius melanostomus</i> ) an experimental approach with fresh- and brackish water origin fish .....	67
Abstract .....	67
Introduction.....	67

Material and Methods.....	69
Fish .....	69
System .....	69
Results .....	72
Trave River .....	73
Rhine River .....	79
Combined results.....	88
Discussion of Method .....	89
Discussion of Results .....	91
Comparison with other shuttle box experiments .....	92
Conclusion .....	93
General Discussion .....	94
Salinity affects the reproduction of round goby .....	94
Genetic role .....	96
Mechanisms of spreading .....	98
Implications of invasion.....	101
References.....	102
Picture source.....	119
Acknowledgements.....	120
Declaration on oath.....	121

# List of Figures

Fig. 1.1 <i>Neogobius melanostomus</i> at 7.5 mm; upper: dorsal, middle: lateral, lower: ventral view. Length bar = 1 mm (Leslie and Timmins, 2004) .....	15
Fig. 1.2 Observations of the round goby in the Baltic Sea (Kotta <i>et al.</i> , 2016) .....	17
Fig. 1.3 Round goby records showing rapid dispersal (Kalchhauser <i>et al.</i> , 2013) .....	18
Fig. 1.4 Schema of salinity capability (McCormick <i>et al.</i> , 2013) (FW =freshwater, SW=seawater).....	24
Fig. 2.1: Shelter draft.....	31
Fig. 2.2: Breeding box; 1) Exchangeable sieve plate 2) Water inflow 3) Hole grid, hole Ø 2mm (ca. 170mm <sup>2</sup> hole area) 4) Outflow 5) slot for a) glass plates with eggs or b) PVC-plates to separate in different chambers.....	31
Fig. 2.3: Batch sizes number of eggs by the occurrence .....	33
Fig. 2.4: Age of hatching for 3 temperatures .....	34
Fig. 2. 5: Hatching success per salinity observed with “loess smother” .....	36
Fig. 3.6 Shelter: draft.....	46
Fig. 3.2: Breeding box; 1) Exchangeable sieve plate 2) Water inflow 3) Hole grid, hole Ø 2mm (ca. 170mm <sup>2</sup> hole area) 4) Outflow 5) slot for a) glass plates with eggs or b) PVC-plates to separate in different chambers.....	46
Fig. 3.3: Schema of the Experiment.....	49
Fig. 3.4b Length at age (dph) per salinity(indicated right side) and batch(nr. at the top) .....	53
Fig. 3.5 Growth per day [mm] for each batch differentiated by salinities (5, 10, 15, 20 PSU) and days post-hatch (time range from day 1 to 10, 10 – 20 and 1-20) .....	54
Fig. 3.6 a) length-weight, lines nonlinear least squares model fitting, mean length overall salinities at day 10 b) weight at age for different salinities .....	56
Fig. 3.8 larval mortality within 10 days after experiment start.....	58
Fig. 4.1: Shuttle-Box overview.....	71
Fig. 4.3: barrier in the connection pipe, lines within the circle indicate how the foile was gashed to guarantee a pass through for the fishes .....	72
Fig. 4.4 Results of Trave fish no. 1 .....	73
Fig.4.5 Results of Trave fish no. 2 .....	74
Fig. 4.6 Results of Trave fish no. 3 .....	75
Fig. 4.7 Results of Trave fish no. 4 .....	76
Fig. 4.8 Results of Trave fish no. 5 .....	77

Fig. 4.9 Results of Trave fish no. 6 .....	78
Fig. 4.10 Results of Rhine fish no.1 .....	79
Fig. 4.11 Results of Rhine fish no.2 .....	80
Fig. 4.12 Results of Rhine fish no.3 .....	81
Fig. 4.13 Results of Rhine fish no.4 .....	82
Fig. 4.14 Results of Rhine fish no.5 .....	83
Fig. 4.15 Results of Rhine fish no.6 .....	84
Fig. 4.16 Results of Rhine fish no.7 .....	85
Fig. 4.17 Results of Rhine fish no.8 .....	86
Fig. 4.18 Results of Rhine fish no.9 .....	87
Fig.4.19 Combined results, frequency [%]~ salinity .....	88
Fig.4.20 Combined results without 35 PSU, frequency [%]~ salinity .....	88
Fig.4.1 Occurrence of round goby .....	96

## List of Tables

Tab 1.1: Landings (tones product weight) of round goby <i>Neogobius melanostomus</i> in Europe, and total landings (tones product weight) of Latvia and Estonia ( <a href="http://ec.europa.eurostat/web/fisheries/data/database">http://ec.europa.eurostat/web/fisheries/data/database</a> ).....	22
Tab. 2.1: Temperature (T) & salinity (S) measured during the experiment .....	32
Tab. 2.2: Minimum and maximum time (days) arising blackeye stage and hatching .....	35
Tab. 2.3: Hatching success in % per treatment & replicate .....	37
Tab. 2.4: Hatching success in average % per temperature and salinity .....	37
Tab. 3.1. Mean length, weight and spAARS activity per batch / day and salinity .....	50
Tab. 3.2 values of constants for nls model in Fig. 6a, equation $W=aL^b$ .....	57
Tab. 3.3. Measured salinity during experiment mean value & sdv .....	59



## Abstract

Over the last centuries, the conditions and inhabitants in many ecosystems changed. This applies likewise to the relatively young Baltic Sea. It is a not fully occupied ecosystem and therefore vulnerable to disturbance by newly introduced species. One of the most successful invaders of the Baltic Sea in the last decades is the round goby *Neogobius melanostomus*. This bottom-living gobiid species was first observed in the early nineties in Puck Bay Poland and subsequently spread over wide areas within the Baltic Sea. Due to the changing salinity conditions from nearly freshwater in the Bothnian Sea up to nearly full marine in the Kattegat, the Baltic Sea is not uniquely well suited for round goby. For adult round gobies, some studies on salinity tolerance exist. But for a full understanding and forecast of further dispersal also the ability to reproduce in higher salinities needs to be considered. The knowledge in this field is still very limited and will hence be a focus of this thesis. Moreover it is known that there is a difference in the salinity tolerance of adult gobies depending on whether they originate from fresh water or salt water, which was the motivation to conduct a salinity preference experiment with a comparison of round goby from the brackish and freshwater origin.

The first question in this context is about the ability of round goby to breed eggs in different salinities. The ability of round goby eggs to develop in different combinations of salinity and temperature typical for the Baltic Sea, was investigated in a full factorial design. Temperature mainly affected the development time, whereas hatching success was strongly influenced by the salinity. Hatching success was generally low at 10°C but best in 5 PSU and decreasing with rising salinity. At 5 and 10 PSU hatching success declined with increasing temperature from 15°C to 20°C while at 15 and 20 PSU hatching success remained stable with high variability between clutches. The highest hatching success overall was found 5 and 10 PSU at 15°C.

To further clarify which life stage of limits mostly the spread of round goby into areas with higher salinities an experimental approach was used to determine post-hatch growth and mortality rates. An aquarium experiment was conducted to disentangle the salinity influence on length growth, weight gain, and survival of round goby larvae. Larval hatching success was very low at 25 PSU and larvae hatched at 25 PSU did not survive longer than one day. Whereas larvae that were older than 20 days at exposure (hatched at 15 PSU) survived salinities of 29PSU for 10 days. The length growth of larvae was not

significantly affected by salinity, in contrast to the weight at length that was highest at 10 PSU. Moreover we conducted first tests of spAARS measurements as a possible proxy of growth, which is till today mainly used in zooplankton. This method did not deliver the expected results, and no clear correlation between spAARS and growth could be established

Finally, the question of salinity preferences of adult round goby from freshwater and brackish water populations was addressed with a shuttle box experiment. The challenge was to provide a suitable experiment setup that worked for the specific behaviour of this species. Used was the most suitable setup, a shuttle box, in which round gobies from freshwater and a brackish water habitat were exposed to the choice of salinity. Slight differences in salinity preference between the different origins (brackish and freshwater) were found. Both preferred salinities below 20 PSU. Fish from freshwater showed a tendency to lower salinities (below 15 PSU) in comparison to those from brackish water. Moreover, while 1/3 of brackishwater fishes stopped shuttling in high salinities 2/3 of the freshwater fishes did. This stopping of the shuttle behaviour indicates a higher physiological stress for the freshwater fish.

## Zusammenfassung

In den letzten Jahrhunderten haben sich die Bedingungen und Bewohner in vielen Ökosystemen dieser Welt verändert. Auch die relativ junge Ostsee befindet sich in einem ständigen Wandel. Durch ihr Ökosystem mit nicht vollständig besetzten Nischen ist sie anfällig für Störungen durch neu eingeführte Arten. Einer der erfolgreichsten Invasoren der Ostsee in den letzten Jahrzehnten ist die Schwarzmund Grundel *Neogobius melanostomus*. Diese bodenlebende Gobiidenart wurde in den frühen Neunziger Jahren in der Danziger Bucht zum ersten Mal nachgewiesen und breitete sich in der Folge über weite Bereiche der Ostsee aus. Aufgrund der wechselnden Salzgehaltsverhältnisse, von fast Süßwasser im Bottnischen Meer bis zu fast marinem Wasser im Kattegat reichen, ist die Ostsee auch für die euryhaline Schwarzmundgrundel nicht überall leicht zu besiedeln. Für adulte Schwarzmund Grundeln gibt es einige Studien zur Salzgehaltstoleranz, aber für ein vollständiges Verständnis und eine Vorhersage der weiteren Verbreitung muss auch die Fähigkeit zur Fortpflanzung im Salzgehalt berücksichtigt werden. Das Wissen in diesem Bereich ist sehr begrenzt und die Forschung dazu ist Teil der vorliegenden Arbeit. Außerdem ist ein Unterschied in der Salinitätspräferenz adulter Schwarzmundgrundeln zwischen Süß- und

Brackwasserherkunft bekannt, was mich zu einem Salinitätspräferenz-Experiment mit einem Vergleich von Schwarzmundgrundeln aus der Brack- und Süßwasserpopulationen motivierte.

Die erste Frage in diesem Zusammenhang ist die nach der Salzgehaltstoleranz des Eistadiums. Es wurde die Fähigkeit von Schwarzmundgrundel-Eiern, sich in verschiedenen, für die Ostsee typischen Kombinationen von Salzgehalt und Temperatur, zu entwickeln untersucht. Die Temperatur beeinflusste hauptsächlich die Entwicklungszeit, während der Schlupferfolg stark durch den Salzgehalt beeinflusst wurde. Der Schlupferfolg war im Allgemeinen bei 10°C gering, bei 5 PSU am besten und nahm mit steigendem Salzgehalt ab. Bei 5 und 10 PSU nahm der Schlupferfolg mit steigender Temperatur von 15°C auf 20°C ab, während bei 15 und 20 PSU der Schlupferfolg stabil blieb, mit einer hohen Variabilität zwischen den Gelegen. Der höchste Schlupferfolg insgesamt wurde bei niedrigeren Salinitäten bei 15°C gefunden.

Im Folgenden wurde die Salinitätstoleranz der Larven als möglicher Engpass für die Verbreitung untersucht. Dafür wurden im Experiment die Wachstums- und Überlebensraten von Larven untersucht. Es wurde ein Aquariumsexperiment durchgeführt, um den Einfluss von Salzgehalt und Temperatur auf das Längenwachstum, die Gewichtszunahme und das Überleben von Schwarzmundgrundel-Larven zu entschlüsseln. Das Schlüpfen der Larven war bei 25 PSU sehr begrenzt und die Larven überlebten nach dem Schlüpfen nicht länger als einen Tag. Dagegen überlebten Larven die bei exposure über 20 Tage alt waren die höheren Salzgehalte von 29 PSU über 10 Tage. Das Längenwachstum der Larven wurde durch den Salzgehalt nicht signifikant beeinflusst, im Gegensatz zur Kondition, die bei 10 PSU am höchsten war. Außerdem wurden erste Tests von spAARS-Messungen als möglichen Proxy für das Wachstum durchgeführt, der bis heute hauptsächlich im Zooplankton verwendet wird und nicht die erwarteten Ergebnisse lieferte.

Schließlich wurde die Frage nach Unterschieden in der Salinitätspräferenz von adulten Schwarzmundgrundeln aus Süß- und Brackwasserpopulationen aufgeworfen. Die Herausforderung bestand darin, einen geeigneten Versuchsaufbau zu finden, der für das spezielle Verhalten dieser Art funktioniert. Gestestet wurde mit dem am besten geeigneten Aufbau, eine Shuttlebox, Schwarzmundgrundeln aus einem Süßwasser- und einem Brackwasserhabitat wurden der Wahl des Salzgehalts ausgesetzt. Es wurden leichte Unterschiede in der Salinitätspräferenz zwischen den verschiedenen Herkünften (Brack- und Süßwasser) gefunden. Beide bevorzugten Salzgehalte unter 20 PSU. Fische aus dem Süßwasser zeigten eine Tendenz zu geringere Salinitäten (unter 15 PSU) im

Vergleich zu denen aus dem Brackwasser. Außerdem hörte 1/3 der Brackwasserfische bei hohen Salinitäten auf zu pendeln, während 2/3 der Süßwasserfische dies taten - dies deutet auf eine höhere physiologische Belastung für die Süßwasserfische hin.

## General Introduction

Species live in specific habitats. Varying and partly overlapping habitats form a bigger picture, summarized as an ecosystem. An ecosystem is generally defined as all living animals and plants in a certain area that interact in complex relationships with each other and the environment. Ecosystems may be distinguished by the type of environment, e.g. land or water-based, or by the separation due to abiotic factors - like freshwater and saltwater, or moreover by the distance between each other or other barriers. However, also interactions between habitats or ecosystems do occur. Examples are the interaction between a land and a water ecosystem, for instance a cormorant living in a land-air habitat preys on fish that live in a water habitat. In addition to these biotic factors are ecosystems our days under strong pressure of different atmospheric and anthropogenic drivers. Humans are part of ecosystems too, influencing and partly controlling these with high impacts. No marine ecosystem of this planet is left unaffected by humans, and 41% are affected in multiple ways (Halpern *et al.*, 2008). Threats are e.g. habitat loss, eutrophication, hypoxia, pollution, altered sedimentation, overexploitation, disease and so on (Kappel, 2005; VENTER *et al.*, 2006; Ducrotoy and Elliott, 2008; Crain *et al.*, 2009). In recent times a new threat has received increasing attention, namely the introduction of alien species in ecosystems. A study looked among other species, at 213 fish species. Of these, 53% were considered to be imperiled by alien species (Wilcove *et al.*, 1998). Worldwide over 1500 invasive alien species are recorded and for these the global transport routes are considered to be the main pathway of introduction (Turbelin *et al.*, 2017). In the marine case, this is represented by commercial cargo ship traffic (Williams *et al.*, 1988; Carlton and Geller, 1993; Minchin and Gollasch, 2002).

At the coasts of the United States over 400 nonindigenous species are known (Ruiz *et al.*, 2012). This does not include inland waters such as the Great Lakes. Within these, at least 139 nonindigenous aquatic species managed to establish, including 25 fish species (Mills *et al.*, 1993). One of these is the round goby (Jude *et al.*, 1992).

Likewise, the Baltic Sea has to deal with dozens of invading species. Since the last 200 years over 100 non-indigenous species (NIS) were recorded, it is assumed that, despite its very specific hydrographic conditions, about 70 species were able to establish populations (Leppäkoski *et al.*, 2002a, 2002b).

The necessity of protecting ecosystems against the steady transport of NIS reached politics meanwhile.

As an example, for the Great Lakes in the United States of America, with a large number of invasive species (Mills *et al.*, 1993; Ricciardi and Macisaac, 2000) the government established in 1993 regulations for ballast water exchange before ships enter the lake system (United States Coast Guard, 1993). For German territorial waters, similar rules for the ballast water exchange exist (Dobrindt and Hendrick, B. Bundesministerin für Umwelt, Naturschutz, 2014).

New species introduced to a system can cause multiple issues. For example, competition for food, spawning sites, but also the extinction of other species through increased predation. Round goby almost extirpated Mottled sculpins in certain areas in Lake Michigan by means of competition for food of small sized fish, space for intermediate sized fish and spawning grounds for adult fishes (Janssen and Jude, 2001). An additional threat originates from introduced diseases against which the "native" species are not resistant. A pathogen of cyprinid *Pseudorasbora parva* causes spawning inhabitation and increased mortality at European cyprinid *Leucaspis delineatus* (Gozlan *et al.*, 2005).

For decades a species on a rise is the round goby. With its origin in the Ponto- Caspian area it spreads throughout northern Europe and the Great Lakes in northern America. This goby managed to establish a permanent populations in very different environmental conditions. The capability to resist different environmental conditions, notably salinity, is the major subject of the following thesis.

### Round goby *Neogobius melanostomus*

Round goby *Neogobius melanostomus* (Pallas, 1814) is a member of the family Gobiidae, which is one of the most species-rich fish families. The color varies from a slight brown with possible black points to a deep black for mature males. A distinctive character is a large black spot on the first of two separated dorsal fins. It preys mainly on Molluscs and Crustacean, but also Polychaets and larger individuals prey also on other fish (Almqvist *et al.*, 2010; Skabeikis and Lesutienė, 2015). It is a demersal bottom-dwelling species capable of living in freshwater and brackish waters. It grows up to about 24cm (total length) in length, with a sexual size dimorphism, where females are smaller at age. The sex is determinable by the urogenital papilla, which is arrow-shaped in males and nearly quadratic with a cut in at the tail end on females. The sex ratio is varying with the invasion status of the area: in invasion fronts, females are encountered in higher numbers (Brownscombe and Fox, 2012; Groen *et al.*, 2012), whereas areas of longer inhabitation are mostly male-biased (Corkum *et al.*, 2004; Young *et al.*, 2010; Gutowsky and Fox, 2011; Brownscombe *et al.*, 2012). Differences in size are also noticeable between gobies from environments with different salinities where fish grow larger in higher salinities (Corkum *et al.*, 2004). The oldest age is reported as 6 years (Sokołowska and Fey, 2011). Age reading with otolith is possible, but depending on the method the results can vary, the identification of the first annulus is problematic, best results were achieved while reading the whole otolith (Florin *et al.*, 2017).

### Reproduction

Round goby is an iteroparous spawning species. Spawning takes place in multiple events throughout the year from April to September (Skora *et al.*, 1999). It chooses its nest site in a preferred depth range of 0.2-1.5m (Marsden *et al.*, 1996) but nests were also found in Lake Erie as deep as 11m (Wickett and Corkum, 1998). Males start preparing the nest ~ 10 days in advance of the egg deposition. They modify the nest by picking gravel and rubbing secrets along the ceiling of the nest with its genital papilla. Furthermore, they start to fan the nest prior to egg deposition (Meunier *et al.*, 2009). Nests are often constructed underneath rocks but also in any other suitable cave-like structures such as e.g. beer cans or artificial reefs (Marsden *et al.*, 1996). If a high competition for mating opportunities exists, male fish may develop alternative reproductive tactics aiming at reduced energy costs by utilizing the reproductive

investment of other males. Normal males (type 1) are large and invest more in growth than in reproduction. They defend their territory, court females and have increased androgen concentrations, and show secondary sexual characteristics. Whereas the type 2 male is smaller, invests more in reproduction than in growth, shows no secondary sexual characteristics. Instead of courting females, they add ejaculate to eggs that were placed in a cave of another male goby in a rapid attack or by imitating females and entering caves of males undisturbed by cave owner (Oliveira *et al.*, 2008). These two male types were also identified in round goby (Corkum *et al.*, 1998). Male round goby can show a dark black pigmentation (type 1) or a mottled gray color (type 2, sneaker males) similar to females. At the invasion front, round goby is shifting its energy investment towards somatic growth instead of reproduction (Brandner *et al.*, 2013).

### Larval fish

Fish stocks are dependent on their recruitment, that is mainly affected by the survival of the early life stages. Years of research developed a different hypothesis to explain the high variabilities of year class strengths. Hjort postulated that the success of a year class is dependent on the ability to change from internal to external feeding shortly after the yolk sac depletion that lack of food in this phase might cause starvation mortality (Hjort, 1914). Later he added to his theory that a drift of eggs or larvae into unfavorable conditions has a high impact on larval survival (Hjort, 1926). Later Hjort's theories were combined into the "Match/ Mismatch" Hypotheses by Cushing, who assumed that a match of fish spawning and larvae production with sufficient food from a zooplankton bloom implying that larval starvation is not only relevant in the first feeding period (Cushing, 1975). Another mortality factor is predation which is typically decreasing with increasing size (Ware, 1975). Hence larval survival can also be influenced via growth. Since Houde's "Emerging of Hjort's shadow" (Houde, 2008) predation is assumed to be a major factor in the early life of marine fish. However, bigger larvae are not necessarily more likely to survive (Litvak and Leggett, 1992). Litvak and Leggett showed that bigger capelin yolk-sack larvae at the same age as smaller ones experienced higher mortalities by predators. Nevertheless fast-growing larvae pass earlier the threshold at which the susceptibility to predators decreases more rapidly (Cowan *et al.*, 1996). However, the survival of early life stages can also be directly influenced by hydrographic factors, namely in the case of round goby by combinations of salinity and temperature.



Well investigated case studies for such a direct influence are available for Baltic cod (Köster *et al.*, 2008; Hüsey *et al.*, 2012), turbot (Nissling *et al.*, 2006) and sprat (Nissling *et al.*, 2003; Petereit *et al.*, 2008).

---

## Larval round goby

After a successful egg nursery, round goby larvae hatch with a yolk sack as finished fish and do not go through a metamorphosis. The yolk sac is depleted within the first 10 days (Chapter 3), Fig.1.1 shows a larval fish post yolk sac phase.

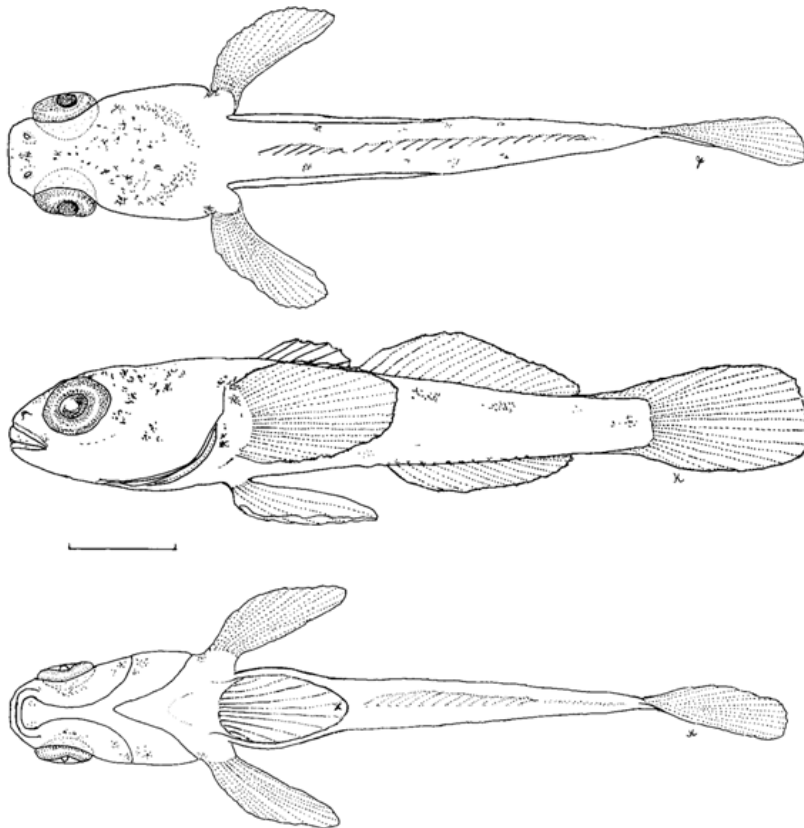


Fig. 1.1 *Neogobius melanostomus* at 7.5 mm; upper: dorsal, middle: lateral, lower: ventral view. Length bar = 1 mm (Leslie and Timmins, 2004)

In freshwater round goby size at hatch is about 5mm (Leslie and Timmins, 2004). Larvae measured one day after hatch in saltwater had a length of 6.2mm – 9.3mm (Chapter 3). Morphometric changes of

round goby growing from larval to adult are generally small, body depth and caudal depth factors are constant (Leslie and Timmins, 2004).

Ichthyoplankton samples within Lake Erie at day and night revealed that round goby larvae perform a vertical migration at night to the surface (Hensler and Jude, 2007; Hayden and Miner, 2009a). The prey of larval round goby is zooplankton. In Lake Erie a larval stomach analysis mainly found dreissenids, *Eubosmina coregoni*, *Daphnia rerocirva* (Hayden and Miner, 2009a).

Many studies reported an influence of salinity on the growth and development of larval fish. For *Inimicus japonicus* salinity influences the hatching and survival rates (Gong *et al.*, 2018). Yolk sac larvae of Atlantic halibut shows tail deformities, necrosis, edema and calculus in early development in high salinities (Bolla and Ottesen, 1998). This shows how early in life salinity already has a decisive influence on developmental processes. As described above fast growth is especially in the early life stage an important factor to escape high predation pressure. The optimum salinity range for larval growth is species depended and can be in lower salinities below 30 PSU (e.g. Tandler *et al.*, 1995; Opstad, 2003; Sampaio *et al.*, 2007) or full marine up to 34 PSU (e.g. Henne and Watanabe, 2003). Therefore it is relevant to study the optimal salinity conditions and the tolerance range also for round goby spreading gradually in more saline areas of the Baltic.

## Distribution

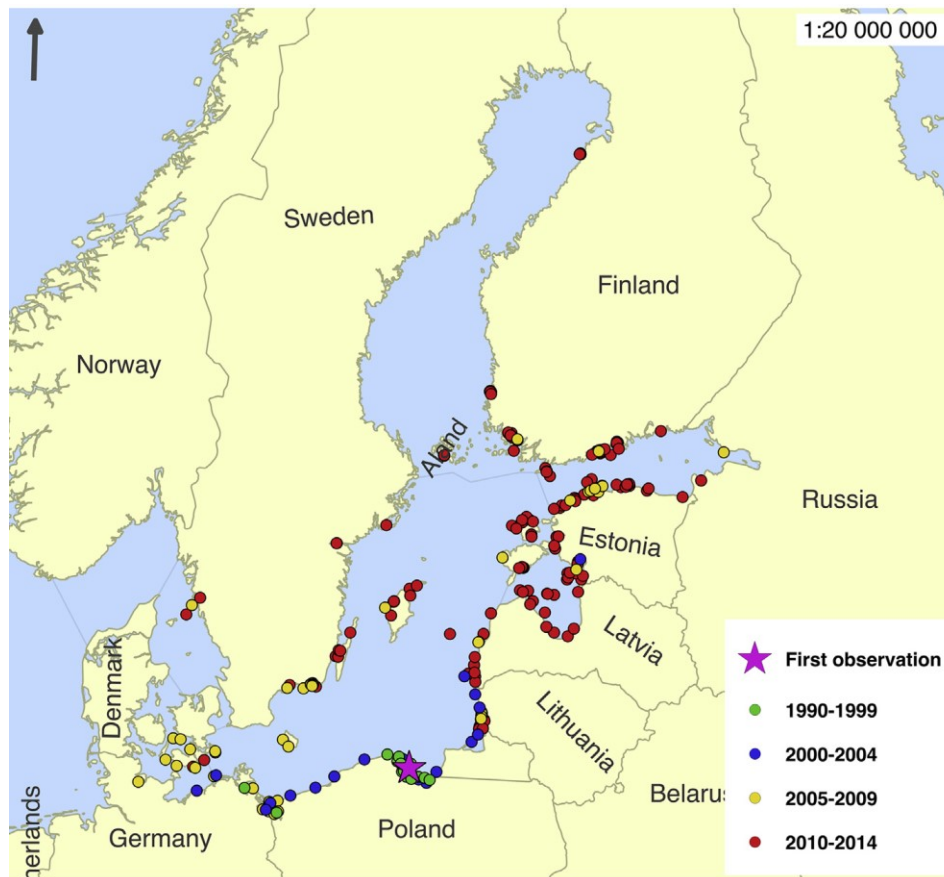


Fig. 1.2 Observations of the round goby in the Baltic Sea (Kotta *et al.*, 2016)

Originally the round goby is distributed at the coasts of the Black, Caspian, Marmara, and Azov Sea. Since early 1990 it started to simultaneously invade the Baltic Sea (Skora and Stolarski, 1993) and the St. Claire River in the USA (Jude *et al.*, 1992), which is connected with the Great Lakes. Subsequently, round goby invaded all Great Lakes (Charlebois *et al.*, 2001). The latest Baltic Sea distribution map, published in 2015 (Fig.1.2) shows that the dispersal includes meanwhile all Baltic countries.

Only for the northern Belt area, the east coast of Jütland and northern Schleswig-Holstein were no observation registered. In freshwater, the dispersal is similarly successful and spacious. Fig.1.3 shows how fast round goby was able to spread over the rivers, starting in 1997 in Danube River and the Rhine in 2004. Whereas the establishment in the Danube possibly has its origin in the Black Sea, the first individuals in the Rhine likely were introduced from downstream (river mouth) (Corkum *et al.*, 2004;

Borcherding *et al.*, 2011). Besides these records shown in the map, there are many recent (Matern *et al.*, 2021) and partly unpublished observations, e.g. in Elbe river they were first reported in 2013 by (Hempel and Thiel, 2013).

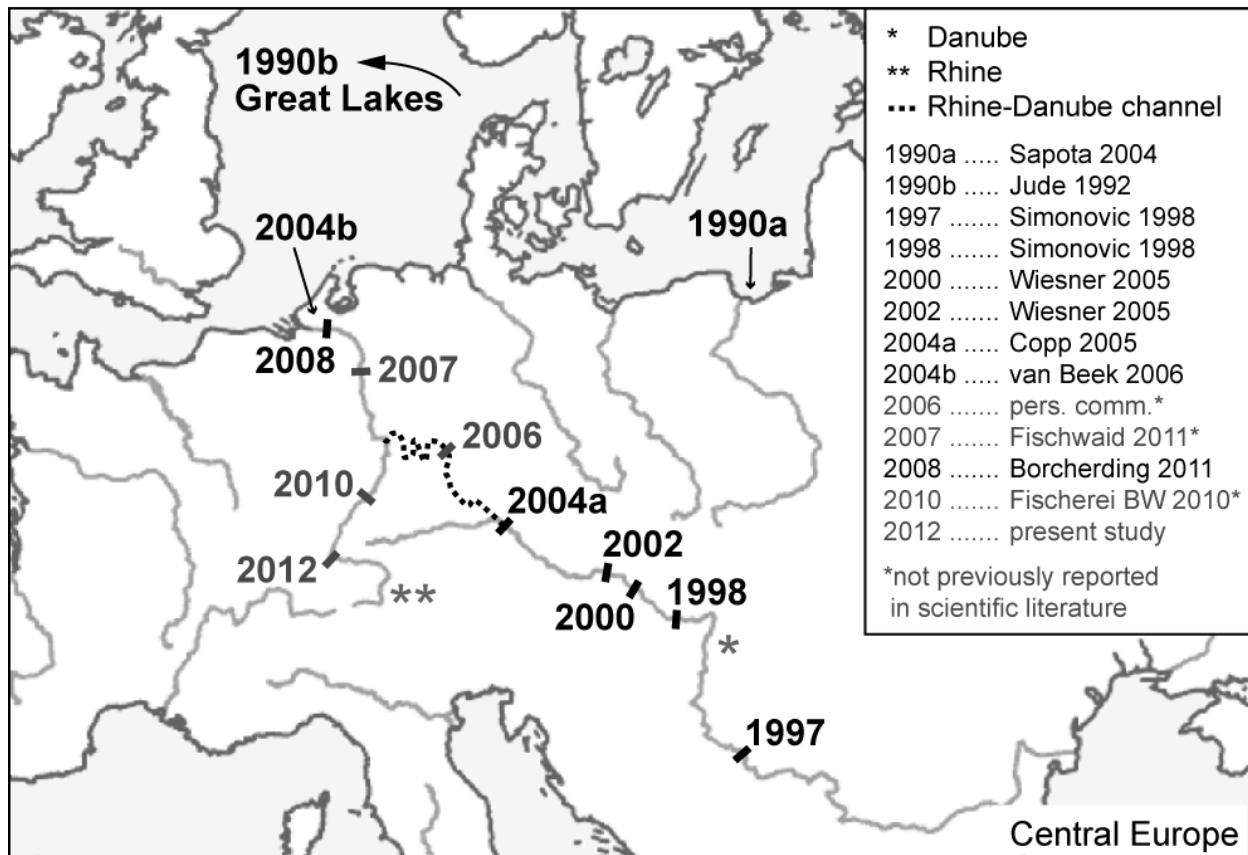


Fig. 1.3 Round goby records showing rapid dispersal (Kalchauer *et al.*, 2013)

### Mechanisms of spreading

While this illustrates how successful the invasion is, the question remains, what makes this fish such a good invader? Spreading of species can take place by regional diffusion, depending on population growth and a diffusion coefficient (Shigesada *et al.*, 1995) or by rapid transport over a long-range (e.g. by ballast water of commercial ships). Due to their short pelagic life, the dispersal potential is assumed to be low for round goby. Along the Danish coast round goby expanded from 2008 to 2013 with a rate of

about 30km per year (Azour *et al.*, 2015). Since eggs are attached to substrate the possibility of dispersal by drifting eggs is compared to pelagic spawners absent. The larval stage may have the highest dispersal potential. One hypothesis of how the round goby cross large distances is that larval gobies get sucked into ballast water tanks and transported over long distances. In Lake Erie (one of the Great Lakes, USA) a diel vertical migration of larvae was observed. At night round goby larva were caught in surface ichthyoplankton net tows, whereas none were present at day (Hensler and Jude, 2007). Therefore a ballast water transport of larval can be a transport vector if ships take ballast water at night. Our findings within the chapter “hatching success” indicate that the transport of eggs spawned in hollows and notches on a ship hull can theoretically lead to successful hatching in different areas.

### Known consequences invading general

Generally, a fully occupied (all niches are occupied) ecosystem is assumed to be more resilient against intruders than a system with many free or insufficient inhabited niches. Effects of invading species are diverse and can be positive or negative or both. Invaders often have negative effects on biodiversity within a trophic level, but can have positive effects on biodiversity of higher trophic levels (Thomsen *et al.*, 2014). An example is given below for the Baltic Sea, where pike perch benefits of round goby populations as a new prey source. Within the Great Lakes knowledge on effects caused by round goby is more advanced compared with the Baltic Sea. For example, round goby have caused most likely a recruitment failure in mottled sculpins by interfering with their breeding (Janssen and Jude, 2001). Another investigation revealed that the round goby was able to gain weight in a direct competition experiment while other native species were only able to maintain their weight or lost significant weight (Bergstrom and Mensinger, 2009). The zebra mussel population within the Great Lakes is subject to another round goby impact. It is suggested that the size structure of the zebra mussel population was altered due to the predation of round goby (Ray and Corkum, 1997). The Smallmouth Bass is a target of catch and release angling in the Great lakes. If the nest guarding males have been removed by anglers, round gobies quickly consume their eggs (Steinhart *et al.*, 2004). This possibly influences the recruitment of the Smallmouth Bass. These examples, as well as many other examples of round goby impacts on invaded ecosystems e.g. (Barton *et al.*, 2005; Johnson *et al.*, 2005; Lederer *et al.*, 2008), emphasize the strength of round goby as an invader. However also effects on predators of fish are possible (Johnson *et*

*al.*, 2010). They observed an alteration in cormorant foraging behavior, that changed from limnetic – demersal based (main prey alewife, threespine stickleback, and yellow perch) to benthic (main prey round goby). Hence the invader possibly reduces the predation on other native fish species. In addition, a change in prey species is possible due to the loss of the original preferred food by the extinction of this by an invasive species. As an enhancement, a replacement of a non-indigenous species in an ecosystem by another invading species is also possible (Lohrer *et al.*, 2008).

### Known consequences of round goby invasion in the Baltic Sea

Within the Baltic Sea, the effects or changes caused by round goby are partly investigated. A comparison of cod and perch stomachs of areas with presence and absence of round goby showed large differences in diet composition (Almqvist *et al.*, 2010). Where round goby was abundant, it became an important prey item. Round goby became additionally an important prey for cormorants (Oesterwind *et al.*, 2017). Effects on the food web were also shown for the Gulf of Gdansk where the European flounder shifted its foraging into deeper waters when round goby became abundant because of a similar prey field (Karlson *et al.*, 2007), further small flounder *Platichthys flesus* is consumed by round goby (Schrandt *et al.*, 2016). Another example was studied in the eastern Baltic Sea where different fish species shifted their prey field resulting in higher resource competition after the arrival of round goby (Ustups *et al.*, 2016). Investigation on pike perch (*Sander lucioperca*) stomachs identified the round goby as the most important fish prey taxon, leading to enhanced growth and condition (Hempel *et al.*, 2016; Oesterwind *et al.*, 2017). A lab experiment revealed that round goby and the North American mud crab *Rhithropanopeus harrisii* have a significant predation pressure on different benthic invertebrates have locally depleted blue mussel banks (Puntila *et al.*, 2018).

### Commercial importance

The commercial importance of round goby is low within the area of the Baltic Sea, especially in comparison with the original Ponto-Caspian habitats, where it supports one of the most important fisheries. Commercial landings of round goby were first recorded in European landing statistics in 2007.

The overall landed product weight in 2007 was about 900kg, caught by Estonia alone. Since 2014 Latvia is also landing round gobies and the overall product weight increased in 2017 up to 139 tons with a decrease in the following years in Estonia and a rise to 1037 tons in Latvia in 2018 (Tab1.1). In comparison to the total landings for both countries of roughly 60,000t, round goby remains a rather unimportant species. Within the area of the Black Sea, it has a much higher importance. In Bulgaria, gobies are the second most important fish species following official statistics. This includes 8 gobiid species, while round goby takes the largest percentage. However, observations on fish markets and interviews with fishers reveal that probably only 10% of catches are declared for the official statistics. Thus gobies unofficially are supposed to be the most fished species. (Zarev *et al.*, 2013)



# General Introduction

Latvia (all species)	Estonia (all species)	Latvia (Neogobius melanostomus)	Estonia (Neogobius melanostomus)	European Union (27 countries)	European Union (28 countries)	Year
80,998.2	75,824.14	-	0.09	0.09	-	2007
85,767.2	81,825.03	-	0.36	0.36	-	2008
71,531.2	87,059.41	-	0.49	0.49	-	2009
67,133.8	84,965.08	-	1.12	1.12	-	2010
59,316.82	68,210.33	-	4.05	4.05	4.05	2011
59,843.85	61,027.66	-	16.91	16.91	16.91	2012
65,357.33	62,117.3	-	9.08	9.08	9.08	2013
61,626.1	60,373.56	109.7	19.18	128.88	128.88	2014
66,010.4	63,250.48	167	30.63	197.63	197.63	2015
60,172.77	59,648.35	581.09	89.41	-	-	2016
67,284.69	62,340.24	1,022.95	139.32	1,162.27	1,162.27	2017
74,384.07	63,54.16	1,307.47	120.06	-	-	2018
73,082.2	80,992.18	649.42	118.95	768.37	-	2019

Tab 1.1:  
Landings (tones product weight) of round goby *Neogobius melanostomus* in Europe, and total landings (tones product weight) of Latvia and Estonia (<http://ec.europa.eu/eurostat/web/fisheries/data/database>)

### Salinity

Generally, fish species can be distinguished by their preferred habitat in the specification of the water type; into freshwater species, brackish water species, and full marine species. Following definitions are given in McCormick *et al.*, 2013 (Fig. 1.4). Hence fish can be split into 3 groups by salinity tolerance and further into 4 groups by their migration behavior.

Amphihaline	- Capable of surviving in freshwater and seawater
Euryhaline	- Capable of surviving in a wide range of salinity
Stenohaline	- Capable of surviving only a narrow range of salinity
Diadromous	- Truly migratory fishes which migrate between the sea and freshwater
Anadromous	- Diadromous fishes spend part of their lives in the sea and migrate to freshwater to breed
Catadromous	- Diadromous fishes which spend part of their lives in freshwater and migrate to the sea to breed
Amphidromous	- Diadromous fishes that reproduce in freshwater, pass to the sea as newly hatched larvae where they feed and grow, then return to freshwater as juveniles for another period of feeding and growth, followed by reproduction.

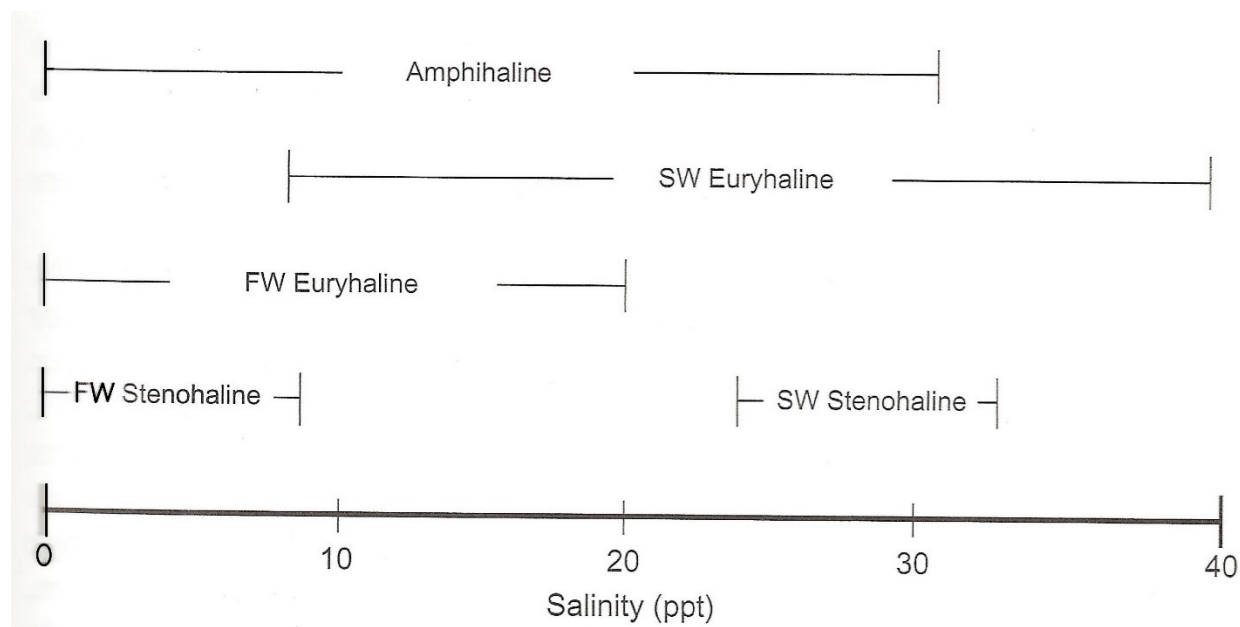


Fig. 1.4 Schema of salinity capability (McCormick *et al.*, 2013) (FW =freshwater, SW=seawater)

Round goby is exposed to varying salinities in the original habitats. Within the Caspian Sea, which has a different salt composition with higher amounts of calcium and sulfates (Nikolai Vasil'evich and Plotnikov, 2012), it varies from 1 PSU in the north at the Volga River to 13.5 PSU in the east, with an average of 12.8 PSU. Furthermore, in the brackish waters of the Black Sea, the salinity is about 19PSU (Marsden *et al.*, 1996). Within its non-indigenous distribution range in the Baltic Sea the salinity range is similar, from about 5 PSU in the eastern areas (Gulf of Finland, Gulf of Riga, etc.) up to around 20 PSU in the Belt area, but with an increasing trend to higher salinities. Therefore following the definitions in Fig.1.4 round goby should be considered as a freshwater euryhaline species. The tolerance of salinities differs between populations. Round goby in the Great Lakes (USA) (Ellis and Macisaac, 2009; Karsiotis *et al.*, 2012) have reduced survival rates at up to 30 PSU compared to those with a brackish water history (Hempel and Thiel, 2015). The influence of salinity on growth of juvenile goby was recently investigated, revealing the highest weight gain in 7.5 PSU, followed by 15 and 0.1 PSU. The lowest weight was gained at 30 PSU (Hempel and Thiel, 2015). But generally, the knowledge on salinity influence, tolerance, and preference of round goby is limited, especially regarding an adaption to higher salinities. Moreover nothing is known about the influence of salinity on larval round goby. Therefore these points are addressed in this thesis.

## Growth of larval fish

Growth is measured as length or weight gain over time. In larval fish, this often is a problem since handling and especially weighing is difficult. Further, the only reliable weight measure for larvae is the dry weight which excludes repeated measurements on the same individual. Therefore large numbers of larvae are needed to follow the growth of a cohort. These high numbers are particularly problematic in the larval stage as this is associated with a high mortality rates (Cushing, 1975; Bailey and Houde, 1989; Leggett and Deblois, 1994). However, mortality in the laboratory is different from the field. Thus, length or weight gain experiments are often still possible in the laboratory. Nevertheless, further methods are required to perform such data collection in situ. An alternative is the analysis of the daily increment width of otoliths allowing conclusions on past growth (e.g. Brothers *et al.*, 1976). Otolith reading was used earlier for adult round gobies as a method to determine the age in a fecundity investigation (Macinnis and Corkum, 2000a). This method would probably provide a practicable way for growth analyses but is comparably time-consuming. Laboratory investigations on larval cod exemplified the correlation between increment width and feeding status, the increment width was likewise correlated with the RNA/DNA ratio (Clemmesen and Doan, 1996).

As an indicator for growth and nutritional status the RNA/DNA ratio was applied numerous times. The RNA/DNA ratio provides information about the protein synthetic capacity of cells (Buckley, 1984; Ferron and Legget, 1994) and shows changes in feeding condition and growth within 1-3 days. Within the last years, another biochemical measure became more popular, the aminoacyl-tRNA synthetases (AARS). This enzyme catalyzes the first step of protein synthesis, the aminoacylation of the tRNA. AARS was recently shown to be applicable as a growth index in different aquatic species (Yebra and Hernández-León, 2004; Yebra *et al.*, 2006, 2011; Herrera *et al.*, 2012). This method was additionally used for herring as a growth index (Herrera *et al.*, 2009). This method will be tested for applicability in round goby larvae in the chapter to analyze potentially different growth rates. Growth rates can vary because of different reasons, e.g. hydrography, food availability or genetics. If salinity also has an effect on the growth of post hatched larval round goby is one topic of the investigations in this thesis.

## Outline

The influence of salinity on the round goby, especially in egg and early live stage, is mostly unknown. Due to its invasive character and the high success in occupation of foreign habitats a better understanding of the salinity influence is needed. Salinity limits of eggs development in brackish or even full marine water and possible interactions with temperature are unknown. The influence of salinity on the post egg stage is also unknown but are important, as larvae can be transported by ballast water to habitats with different salinities. Hence breeding can take place in one area and larval development in another. Investigations in this thesis aim to close this gap of knowledge and help to identify boundary conditions of successful egg development and larval growth depending on salinity and temperature. This knowledge can help understand and possibly predict the future invasion of round goby into western Baltic regions.

Chapter two addresses the question of how temperature and salinity influence the development time and the hatching success of round goby eggs.

The 3rd chapter investigates the capability of round goby larvae to grow and survive in different salinities. The results of aquarium experiments on growth will be described by changes in length and dry weight over time and additionally the possible usability of AARS as a proxy of growth is investigated.

The 4th chapter reports tests of an experimental setup to determine salinity preferences of adult round goby. This chapter will at first show if a shuttle box is a suitable setup to test preferences of round goby, with its pro's and con's. A second aim is to disentangle the differences in salinity preferences of round goby populations originating from brackish and freshwater populations.

## How far west can they go?

### Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by temperature and salinity

#### Abstract

*Round goby *Neogobius melanostomus* successfully invaded the Baltic Sea many years ago. A broad prey field, as well as nest guarding and high flexibility in using different structures and substrates as habitat, are qualifying round goby as a successful reproducing invader. However, the actual distribution within the Baltic Sea suggests that spreading is limited by salinity. This study investigates the ability of round goby eggs to develop in different combinations of salinity and temperature typical for the Baltic Sea. Temperature mainly affected the development time, whereas hatching success was strongly influenced by the salinity. Hatching success was generally low at 10°C but best in 5 PSU and then decreasing with rising salinity. At 5 and 10 PSU hatching success declined with increasing temperature from 15°C to 20°C while at 15 and 20 PSU hatching success remained stable with high variability between clutches. The highest hatching success between 82- 99% was found at lower salinities at 15°C.*

#### Introduction

Non-indigenous species of different taxa like cladoceran *Cercopagis pengoi*, bivalve *Dreissena polymorpha*, the barnacle *Balanus improvisus* (Leppäkoski *et al.*, 2002b), ctenophora *Mnemiopsis leidyi* (Javidpour *et al.*, 2006), decapoda *Eriocheir sinensis* (Ojaveer *et al.*, 2007) invaded the Baltic Sea in the last century. One of the most successful invaders is the round goby *Neogobius melanostomus*. This goby species was first reported in Puck Bay, Poland in the early 1990s (Skora and Stolarski, 1993). Since this first report, it colonized the coastline of nearly all Baltic countries (Kotta *et al.*, 2015; Michalek *et al.*, 2012). With this enormous expansion range round goby is exposed to a wide range of different environmental conditions, especially to different salinities. Salinities ranging from nearly freshwater in eastern regions up to at least 20psu in German, - Danish- and Swedish coastlines. Hempel and Thiel

(2015) observed at 30 PSU no mortality within 84 days in round gobies caught in brackish water at 3.2 PSU. In contrast, Karsiotis *et al.* (2012) showed that round goby caught in freshwater (Lake Erie) survived short-term exposure to 20 PSU with a mortality rate of about 30% if acclimated in a stepwise fashion, while in the long term less than 10% survived. Thus differences in salinity tolerance are potentially dependent on the previous environmental conditions, more precisely on the exposure to the salinity of the previous generation(s). Björklund and Almqvist (2009) found significant genetic differences between populations within a 30 km range, while Azour *et al.* (2015) reported an annual dispersal rate of 10 to 30 km yr<sup>-1</sup>. These findings are somewhat contradicting but are possibly explainable by different behavioral trait expressions in populations of differing establishment histories. Recently populated areas are reported to be characterized by to be more active and less sociable individuals (Thorlacius *et al.*, 2015) than longtime established populations. These differences, however may also point to a high ability to adapt quickly. An alternative explanation is that the salinity tolerance differs between individuals of local populations and needs to be considered individually.

If the geographical spread of round goby in the western parts of the Baltic is analyzed, a shift into higher saline waters becomes obvious, suggesting that the dispersal to nearly marine environment may be only a question of time. In all regions, however, the highest occurrence of round goby was found to be linked to cargo ports (Kotta *et al.*, 2016). This is explained with the assumption, that the most probable vector of distribution for non-indigenous-species, as well as for round goby, is by ship (Sapota and Skóra, 2005; Galil *et al.*, 2014).

The exact pathways from their original habitat, the Black, Caspian, Marmara and Azov Sea are poorly known, but ships appear to be the best explanation since round goby reached nearly simultaneously the Baltic Sea and the Great Lakes in America, where round goby was first found in St Claire River in early 1990 (Jude *et al.*, 1992). Nevertheless, there is still no direct evidence for the exact transport mechanism (Adrian-Kalchhauser *et al.*, 2017). Two possible ways for transportation by ship can be considered. One assumption is based on the behavior of larval round goby, which show a nocturnal feeding in surface waters, where they could easily be sucked into ballast water tanks of ships (Hayden and Miner, 2009a). This possible pathway was already mentioned by Skora *et al.* (1999). Another mechanism may involve round goby attaching their eggs to a ship.

Male round goby prepare their nest 10 days in advance of female spawning (Meunier *et al.*, 2009), which would make a spontaneous spawning event in a ship hull unlikely, if the ship has a short stay in the

harbour. However, if a ship stays a long time in a harbor, nest preparing and spawning in the named period might be possible. A short duration time in the harbor might therefore minimize the number of fertilized eggs on ship hulls. But since there are males with a sneaker behavior that are not stuck to their nests, fertilization of spontaneous spawned eggs is thinkable. If the ship leaves the port after spawning the developing eggs may be exposed to higher saline (marine) conditions during the trip. The hatching may take place in the destination harbor in better conditions, possibly in an estuary. Once a new region is settled successfully, additional spreading may also occur through the migration of adults. In the case of the Baltic, this would expose round goby migrating in the western direction to continuously increasing salinities. Whereas individuals migrating upstream in the estuaries would encounter the opposite, decreasing salinities down to zero. However, currently, the physiological limits of reproduction concerning the salinity of Western Baltic round goby are unknown.

The present investigation is a first step in closing these gaps in knowledge on round goby egg salinity tolerance. Currently occupied habitats suggest a salinity range for adults between 0 to approximately 18 PSU. For adult round goby, 25 PSU appears to be a limit, beyond which a decrease in aerobic scope and perturbations of plasma osmolality occur (Behrens *et al.*, 2016). Most knowledge obtained so far is focused on adult round goby. Only one investigation on juveniles was conducted (Hempel and Thiel, 2015) and nothing is known about constraints for eggs or larvae round goby. Here we investigate the success of round goby egg development in different salinity and temperature conditions experimentally. For this, we incubated round goby eggs from a population of the Trave estuary (Germany) that were spawned at the typical salinity of the region (15 PSU) under a variety of salinity and temperature combinations.



## Material and Methods

### Field sampling and maintenance

Round gobies were caught on 01.10.2014 in Travemünde (Germany) by angling with hook and maggot at the Trave Marina (53°53'46.1"N 10°47'53.9"E) in a water depth of 0.5-3m. The caught fish was transported in an aerated tank to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Here the gobies were kept in aerated tanks (Ø 140cm, water level ~64cm) with an artificial seawater circulation system at 16 °C and 16 PSU.

### Experiment

After an acclimation period of 4 weeks, gobies were exposed to an artificial seasonal cycle of 102 days to initiate spawning. Thereby temperature was ramped down to 6°C in 0.5°C steps within 20 days. The day length was reduced from 16 to 8 hours of light to simulate winter conditions by shortening the light time for half an hour every day within 16 days. Winter conditions were maintained for 46 days. Afterwards, temperatures were increased to 16 °C within 20 days and day length was extended to 16 hours within 16 days to simulate summer conditions. To trigger spawning, fish were split up into groups of three females and one male. The idea of this setup was to prevent competition of males for shelters and minimize aggressive behavior. These groups of four fish were maintained in compartments (0.5m x 0.5m x 0.5m) which resulted from separator panels in larger tanks. Shelters were designed with an exchangeable glass ceiling, which allowed to remove the fertilized egg clutches without damaging them (Fig. 2.1).

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

---

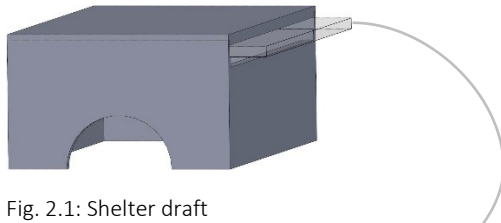


Fig. 2.1: Shelter draft

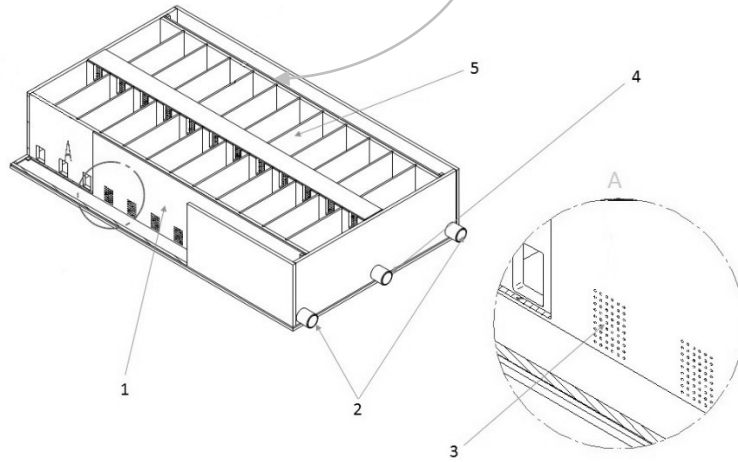


Fig. 2.2: Breeding box; 1) Exchangeable sieve plate 2) Water inflow 3) Hole grid, hole  $\varnothing$  2mm (ca. 170mm<sup>2</sup> hole area) 4) Outflow 5) slot for a) glass plates with eggs or b) PVC-plates to separate in different chambers

These glass ceilings were designed to fit into boxes shown in Fig. 2.2. Shelters were checked daily for new spawning events by carefully extracting the glass plate with minimized disturbance for the inhabiting gobies. If spawning occurred the glass plates containing eggs was transferred to the boxes (Fig. 2.2, No. 5) which were connected to small circulation systems containing approximately 500l of aerated water. Salinities in these systems were set to 5, 10, 15, 20 PSU. Additionally, the temperature was adjusted to 7, 10, 15, 20 °C (Tab. 2.1). In every combination of temperature and salinities (7°C only 5+10 PSU), eggs were - if possible - reared until hatching. For each treatment 3 egg batches were incubated.

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

Tab. 2.1: Temperature (T) & salinity (S) measured during the experiment

	7°C 5PSU		7°C 10PSU		10°C 5PSU		10°C 10PSU		10°C 15PSU		10°C 20PSU		15°C 5PSU	
	T	S	T	S	T	S	T	S	T	S	T	S	T	S
mean	6.8	5.2	6.8	10.2	10.7	5.1	10.2	10.2	10.4	15.2	10.0	20.2	15.5	5.2
Dev.	0.2	0.1	0.8	0.2	0.2	0.6	0.3	0.2	0.5	0.2	0.2	0.2	0.3	0.1

	15°C 10PSU		15°C 15PSU		15°C 20PSU		20°C 5 PSU		20°C 10 PSU		20°C 15PSU		20°C 20PSU	
	T	S	T	S	T	S	T	S	T	S	T	S	T	S
mean	15.5	10.1	15.4	15.2	14.9	20.3	20.5	5.2	21.2	10.2	20.8	15.3	20.2	20.3
Dev.	0.4	0.1	0.3	0.2	0.3	0.2	0.3	0.1	0.3	0.1	0.2	0.2	0.3	0.3

To follow mortality and development of the eggs, batches were photographed every Monday, Wednesday, and Friday. Eggs incubated at 20°C were photographed every day, except for the 15PSU treatment which was monitored only as for the lower temperatures. A repeated incubation at 20°C and 15 PSU with a finer monitoring grid was unfortunately not possible due to a lack of batches. For the digital images, the glass slides were placed inside a water-filled bowl with a black bottom. Photos were taken with a Ricooh Capalio R3 at a resolution of 2592 x 1944 pixels. In the subsequent analysis, eggs were classified into four groups: good, bad, blackeye, and hatched. The classification was based on optical appearance.

- good: an egg is in a good condition, the yolk is ball-shaped, no dissolving or blurriness is noticeable
- bad: an egg is not expected to develop, yolk lost shape and appears blurry and dissolving
- black eye: black eyespots are visible
- hatched: rest of the egg hull is visible in an egg that had successfully passed the blackeye stage

Every single egg development of every batch was tracked individually picture by picture.

## Statistics

All statistical tests were calculated with R 3.2.3 (64-bit). For the differences in hatching age at different temperatures (Fig.4 ) a Kruskal-Wallis Test and a Kruskal-Wallis- Dunn- Post-Hoc test Bonferroni adjusted was performed (R Package PMCMR\_4.1). The fitted lines in Fig. 2.5 were produced by the “geom\_smooth“-function method = “loess” from the “ggplot2” package.

## Results

The total number of eggs per spawning event was highly variable (Fig. 2.3) with batch sizes ranging from 95 to 1067 egg (mean= 419, sd=214).

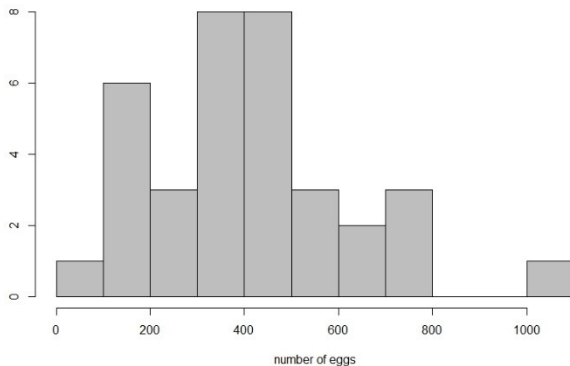


Fig. 2.3: Batch sizes number of eggs by the occurrence

The following results are based on treatments with temperatures  $\geq 10$  °C. Additionally, a temperature of 7°C was tested, but no egg development was observed.

## Development time

The development time was found to be mainly dependent on temperature. Development reached the blackeye stage in all successful treatments. The longest time of 27 days until the first occurrence of blackeye stages was observed at 10°C and 20 PSU. The shortest development time was determined as 4

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

---

days at 20°C in all salinities (Tab. 2.2). First hatching occurred from 11 days at 20 °C and 20 PSU to 65 days at 10°C and 5 PSU. The latest hatching ranged from 19 at 20°C and 20 PSU up to 145 days at 10°C and 10 PSU. The shortest development times were observed in warm water, longest in cold water. Time until hatching decreased significantly ( $\chi^2 = 233.28$ ,  $p = 2.2e-16$ ) with increasing temperature. The following PostHoc test indicated that the differences between all temperatures were significant; 10°C – 15°C ( $p$ -value=  $2e-16$ ), 10°C- 20°C ( $p$ -value=  $2e-16$ ), 15° - 20° ( $p$ -value= 0.0029). The salinity had no obvious influence on the development time (Fig. 4 & Tab.2).

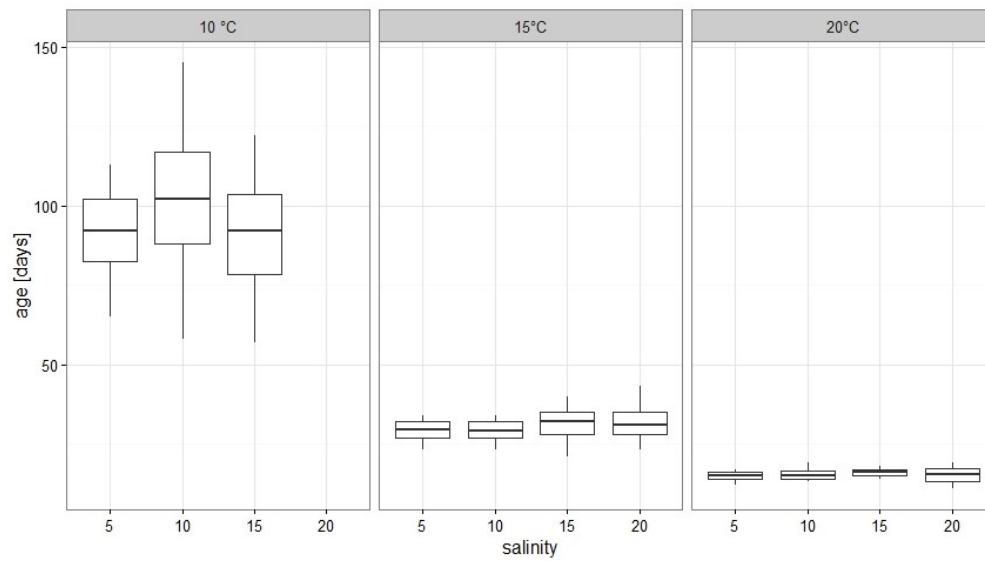


Fig. 2.4: Age of hatching for 3 temperatures

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

Tab. 2.2: Minimum and maximum time (days) arising blackeye stage and hatching

Temp °C	Sal PSU	blackeye_min_age	hatch_min_age	hatch_max_age
10	5	21	65	113
	10	23	58	145
	15	19	57	122
	20	27	-	-
15	5	8	23	34
	10	8	23	34
	15	8	21	40
	20	9	23	43
20	5	4	12	17
	10	4	13	19
	15	4	14	18
	20	4	11	19

### Hatching success

The hatching success appeared to be mostly influenced by salinity, but with additional effects of temperature. The highest observed hatching success was between 94% and 99% (Tab. 2.3, Fig. 2.5) at 15°C and 5 PSU. Higher salinities, as well as lower temperatures, caused a reduced hatching success. At 10°C slightly more than 50% of eggs hatched out of two batches, one at 5 and one at 10 PSU. All other batches had a hatching success below 30%. The lowest hatching rates were observed at 20 PSU regardless of temperature, with one outlier batch that had a success of 81% hatched eggs at 20°C. Hatching failed at 10°C and 20 PSU completely. The best average hatching rates were observed at 15°C for all salinities (Fig. 2.5). The highest success of a single clutch in 20 PSU was found at 20°C, in 15 PSU and 20°C two clutches performed extremely well with 98%, one reached only 37% (Fig. 2.5). Over all (Tab. 2.4) the best hatching success was observed at 5psu and 15°C of 97.33%, decreasing with rising salinity. At 10°C the average hatching success was also decreasing with salinity from 33.43% to 0%. At 20°C this pattern changed, the best average hatching success was recognized at 15psu (78.22%) followed by 5 (64.33%) and 10 PSU (33.5%), worst success was found in 20 PSU (31.12%) (Tab. 2.4).

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

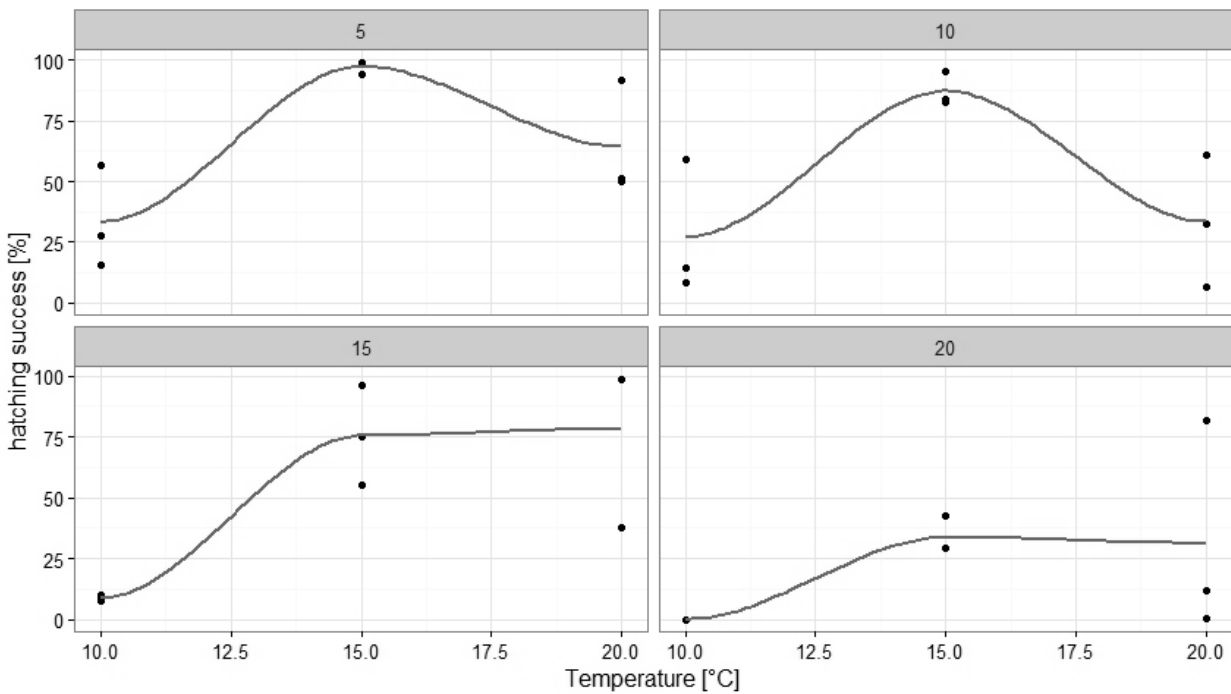


Fig. 2. 5: Hatching success per salinity observed with “loess smother”

Overall, the between batch variability in hatching success was very high. The largest differences were found at 20°C and 20 PSU: one batch performed nearly 70% better than the others. The batch with the lowest success had a hatching rate of 0.4%, the best batch of 81.6% (Tab.3). Similar patterns but with narrower spreads were found at 20°C- 15PSU where the lowest success was about 37.7 % and highest about 98.7%. At 5 PSU the hatching success varied about 40% from 51.3% up to 91.5% at 20°C. At 10°C and 10PSU, the variance was about ~51% from 8.3% to 59.2% and at 5 PSU about 41% from 15.9% to 56.6%. The hatching success variance in the 15°C trials was also increasing with higher salinity from a 5% difference (94%-98.9%) at 5PSU to about 40% (55.1%-96.3%) at 15psu. The 15°C 20psu batches performed worse and had a success variance of about 13% (29.1%- 42.6%).

How far west can they go?  
Hatching success of fish egg (round goby *Neogobius melanostomus* (Pallas, 1814)) affected by  
temperature and salinity

Tab. 2.3: Hatching success in % per treatment & replicate

	5psu	10psu	15psu	20psu
10°C	27.8	59.2	10.3	-
	15.9	14.2	9.0	-
	56.6	8.3	7.4	-
15°C	94.0	95.2	55.1	29.4
	99.1	82.6	75.2	29.1
	98.9	83.7	96.3	42.6
20°C	51.3	61.1	37.7	0.4
	91.5	32.8	98.7	81.6
	50.2	6.6	98.6	11.9

Tab. 2.4: Hatching success in average % per temperature and salinity

	5psu	10psu	15psu	20psu
10 °C	33.43	27.23	8.90	0.00
15 °C	97.33	87.17	75.53	33.70
20 °C	64.33	33.50	78.22	31.12



## Discussion

### Method limitation

The high amount of batches needed for this experiment resulted in a long experiment time. This long time and the fact that many fish spawned multiple times could have affected the results. It can therefore not be excluded that the same individuals contributed with more than one batch to the result and therefore results might not be statistically independent in a strict sense. Furthermore there is a possibility that later produced batches were negatively affected because of a reduced energy reserves of the female. In addition due to the high egg numbers, tracking each egg individually turned out to be difficult. Nevertheless what is done as accurately as possible, hence the error is assumed to be very limited.

### Lessons from the invasion in other areas

Round goby invaded successfully the Great Lakes in northern America in the early 1990s. This freshwater ecosystem allows us to understand the dispersal patterns without any salinity influences. Round goby breed in caves, e.g. between rocks and stones but also on artificial substrates. Nevertheless, the absence of these caves on sandy and muddy substrates areas does not exclude round goby settlements (Young *et al.*, 2010). They found round gobies on all kinds of substrates, but on muddy grounds in lesser numbers. This shows that a lack of breeding opportunities e.g. on sandy and muddy grounds does not act as a barrier for dispersal. Therefore, besides the often named dispersion by ships, round goby can also spread directly over inappropriate areas. A recently published investigation from the rivers Morava and Dyje in the Czech Republic show, that round goby disperse in a non-navigable river with a range of 1.2 to 3.2 km year<sup>-1</sup>. In the case of the Morava river, they extended their range upstream and were able to overcome two weirs that had no fish ladder. Weirs the round goby overcome in the Dyje river had fish ladders (Šlapanský *et al.*, 2017).

After a successful establishment of a population in the Trent-Severn Waterway (Ontario, Canada) the dispersal rate in these areas was determined to be around 9.3 km/year in high-quality habitats, with a probability of 5% that high mobile individuals may disperse 27 km/year (Brownscombe *et al.*, 2012). In the Rhine river with high ship traffic, the yearly upstream expansion was found to be about 67 km

(Manné *et al.*, 2013). Obviously the dispersal of round goby within the freshwater is rather difficult to stop. It is accelerated by ship traffic but also ongoing without. The example in the Czech Republic additionally illustrates that potential barriers like weirs are no guarantee to put an end to the invasion.

### Clutch size

The variability of the batch sizes from less than 100 to over 1000 eggs (Fig.3) is mainly influenced by the size and fitness of the female (Kovtun, 1978; Macinnis and Corkum, 2000b). The amount of spawned eggs is increasing with body size (Kovtun, 1978; Macinnis and Corkum, 2000b). In the original habitats of round goby, the Sea of Azov, spawning in 2-3 batches with fluctuations of individual fecundity between 200 and 3900 eggs are reported (Moskvin, 1940; Gudi-movich, 1946; Il' in, 1949; Trifonov, 1955; Mikhman, 1963; Kostyuchenko, 1964 as cited in Kovtun, 1978). The individual fecundity of females is very variable and differs 15 fold from minimum to maximum amounts of produced eggs (Kovtun, 1978). Even within a narrow size range, the variability in fecundity varies by a factor between 2.6 and 6.1. The weight of the female round goby is more appropriate in predicting fecundity, but fecundity is also varying about 1.3 times in 45-50g fish and 4.5 times in 15-20g fishes (Kovtun, 1978). However, besides these fecundity differences that may explain the variance, there is the possibility that clutches can consist of eggs spawned by different females. In this experimental design, max 3 females were kept in each tank that could have spawned eggs to the same batch and therefore can explain the variability additionally. Charlebois *et al.* (1997) reported that a single clutch can contain up to 10,000 eggs spawned by up to 6 females. Since our experimental setup includes more than one female in each tank/compartment we can neither state safely that all our batches were spawned by one female nor can we allocate them to a certain individual. Tanks with only one female and one male as experiment setup were considered, but would this setup need many more groups to gain an adequate number of batches. This was not possible because of a lack of suitable tanks and laboratory space. Nonetheless of the uncertainties are the variation between the batches sizes observed in our experiments still within a natural reported range.

## Temperature effects

We found that round goby eggs can develop within a broad temperature range down to at least 10°C. The egg development at 10 °C is possible, but the time till hatch is longer than 100 days. Considering a reproduction season from April to September (Skora et al., 1999) there are probably only a few days with water temperatures around 10°C. Hence these results are just indicating the possibility of egg development in cold water and are only relevant by indicating a successful development of early spawned eggs. Round goby spawning has been observed in water temperatures between 9°C and 26°C (Kovtun, 1978). Since at 7°C no development occurred - the lower limit for a successful developing egg is located between 7 and 10°C, probably around 9°C. A batch of eggs spawned in warm waters on a ship hull might be exposed to cold waters during a long-term journey back to favorable waters. This short-term exposure is probably delaying the egg development or cause a stop of development. Further investigations are needed to determine the effect of this short-term exposure. The time until the first hatch at 20° was after 11 days in this investigation and thereby somewhat shorter than the reported 14-15 days at 19-21°C (Moskal'kova 1989 as cited in Marsden et al 1996). The influence of temperature on development time was nearly independent of salinity, a similar picture was found in other Gobiidae species (Fonds and Van Buurt, 1974).

Besides the time till hatch is the hatching success of a batch influenced by the temperature. The optimum temperature was found to be 15°C. The reduced success in cold temperatures was strongest at salinities above 10 psu and beside this reduced success there might be further problems for larval hatched in cold water. Larvae hatching early in the season when zooplankton is less abundant may be stressed by low temperature and in addition experience starvation due to a miss-match with their prey Cushing (1990). Warmer temperatures correlate with reduced hatching success, with the exceptions of three individual batches at 5, 15, and 20 PSU (Fig.5) where the hatching success at 20°C was similar to values obtained at 15°C. At 20°C the most successful batch occurred 20 PSU. This possibly indicates, that if a batch can develop well in high salinities, the chance is best in high temperatures, which is contrasting with other brackish species. For common carp e.g. a decreasing hatching success with increasing temperature was found (Sapkale et al., 2011). For African Catfish a similar pattern as in our experiment was found with a temperature optimum and a decreasing hatching success beyond this (Okunsebor et al., 2015).

## Salinity effects

Salinity did not affect development time, but had a major effect on the hatching success of the round goby eggs. The hatching success at a salinity of 20 PSU is on average low and might even decrease further with increasing salinity. A decreasing hatching success with increasing salinity is also known in *Nile tilapia* where hatching success reduces from 90.3% at 15psu over 66.4% at 20psu to 46.1% at 25psu (Malik *et al.*, 2018). Generally, are teleost fish eggs reported to be isotonic to the blood of the mother while being spawned (Kjørsvik *et al.*, 2004) which is for round goby between 332 and 352 mOsm kg<sup>-1</sup> (Behrens *et al.*, 2016). For comparison, seawater has an osmolality of approximately 1000 mOsm kg<sup>-1</sup> if only NaCl as ions are considered. Therefore if in brackish water the osmolality is approximately half that of seawater, still an osmotic pressure remains for the eggs. The permeability of the egg is changing through the development and in seawater, it is decreasing after fertilization to a minimum within one day (Alderdice, 1988). In our experiment, all eggs were fertilized at the same salinity, with an exposure to the treatment salinity within a time window of 24 hours. This time window depends on the difference between the time when egg batches were spawned and when they were detected afterwards during the daily controls. This variable time delay may have increased the between batch variability of our results if some eggs were already equipped with a mostly semipermeable membrane, whereas others were not when transferred to experimental salinity. For cod eggs, it was found that fertilization in different salinities affects yolk osmolality, whereas a transfer after 24 hours did not (Kjørsvik *et al.*, 1984). Whether round goby eggs fertilized in higher salinities are more vulnerable to salinity and show hatching successa deviating hatching success needs further investigations.

## Between batch variability

The most likely reason for the high degree of variability originates from genetic differences between parent individuals since all experimental fish were caught at the same location. Behrens *et al.*, (2016) also report differences in the ability of individual fish to maintain blood osmolality in high salinities. Since an adaption on the genomic level is commonly supposed to take place over generations, these differences might reflect of phenotypic variation, variances in the gene pool, or epigenetic effects. A study on a *Fundulus* species found significant differences in gene expression within one population for

about 18% of 907 genes (Oleksiak *et al.*, 2002). It has been suggested that there is a mechanism regulating processes next to the genome to facilitate adaption on environmental changes: epigenetics. There are different definitions of epigenetics: Bird (2007) defined these as “the structural adaptation of chromosomal regions such as to register, signal or perpetuate altered activity states”. He further refers to a few studies that suggest the inheritability of epigenetic information. These agree with a recent investigation suggesting the maternal RNA may contain environmental information and can be considered as a pathway for non-genetic inheritance (Adrian-kalchhauser *et al.*, 2018). Therefore it is suggested to consider the differences in salinity tolerance between Grate Lake and Baltic Sea populations to be linked to differences in gene expression. This question should be addressed in further experiments. Recent investigations on sperm motility of round goby showed significantly lower motility of freshwater adapted goby sperm in 8 and 16 PSU water compared to sperm from males of brackish origin (Dietrich, 2017). Green (Green, 2017) tested round goby sperm from two expansion fronts in the Baltic Sea on differences in velocity and motility. One front expanding into higher salinities and one into lower. Sperm of fishes from these two fronts showed a different response in motility and velocity in a variety of salinities. Sperm with a high salinity origin had a peak in velocity at 15-20 PSU, whereas the peak was located at 10 PSU for sperm with a low salinity front origin. The motility was consistently higher between 5 and 25 PSU for sperm from the higher salinity front.

Therefore fertilization by males with a freshwater origin may have reduced success in higher saline waters. This indicates that the origin of the male potentially plays a role in successful reproduction in new habitats.

### Implications for round goby spreading

Besides the Great Lakes, round goby spread over the Baltic Sea since the early 1990s. The invasion started at the Gulf of Gdansk and the coast of East Germany in the 1990s. Round goby increased its distribution till 2004 over nearly the whole Polish and Lithuanian coast. This spreading is going on at the coastlines of Latvia, Estonia, and part of the coastlines of Finland, Sweden, Denmark and Germany till today. A model trying to identify variables influencing the occurrence of round goby within the Baltic Sea was developed by Kotta *et al.* (2016). They found that the exposure of the habitat to waves is the most important explaining variable, salinity is just in 4th place after distance to port and total cargo. The

spreading at the invasion front of Sjaelland (Denmark) is estimated to a disperse of 30km/year (Azour *et al.*, 2015). Hence it is similar to those dispersal rates of a maximum of 27km/year in northern America. The results of our experiment provide the first insight of the ability of round goby to grow eggs in different environmental conditions. We found that habitats with at least 20 PSU are suitable for a successful round goby egg development. Nevertheless, we found high variability not only on the clutch size. One batch still had a hatching success of 81.6% at 20 °C and 20 PSU. A targeted selection and rearing of these successful clutches could lead theretically to a fast genetic selection that also tolerates higher salinity.

As already mentioned spawning on a ship hull is a possible distribution vector for round goby (Adrian-Kalchhauser *et al.*, 2017). But since prevention efforts against invasive species are on a rise many ships are treated with antifouling coats. It is not known how antifouling coats affect the eggs of round goby and if the female round goby are spawning on the coated surface. Nevertheless is there a chance to find uncoated surfaces in hollows and notches that enter into the bow and could be a sufficient place to spawn. This investigation shows that spawned eggs can outlive long transport on a ship hull, especially in cold water with a long time till hatch. Our results indicate that long journeys like an Atlantic crossing are possible. Assuming that a distance of ca. 3100 nautical miles needs to be covered to jump from a populated harbor in Europe towards a suitable place at the coastline of America, which could be every estuary with a sufficient freshwater run of, in combination with an average ship speed of 15kn per hour, the distance can be crossed in less than 10 days. Therefore even the longer distance to Quebec and along the Saint Lawrence River into the Great Lakes is conceivable. Hence round goby could have reached America in an egg stage. Furthermore, the presented results show that a wide range of conditions is suitable for egg development. Even higher saline areas in the Skagerrak with suitable freshwater inflows can be used as habitats. One example of an area with higher salinities is Gothenburg in Sweden where round goby was first reported in 2010 (Puntila *et al.*, 2018). Hence the round goby can successfully breed in a wide area of the Baltic Sea and many river run-offs on the northern hemisphere like the River Rhine, Elbe, Danube.

## Salinity a bottleneck?

### Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

#### Abstract

*Since round gobies first appearance over 25 years ago its distribution covers most parts of the brackish Baltic Sea. Within the area of distribution, the salinity is varying from almost freshwater, around 3.5 PSU in northern Gulf of Bothnia, to approximately 20 PSU in Gothenburg, Sweden. One question to be asked, is in which state of life salinity causes the bottleneck for distribution. Salinity tolerance for adult round goby was found to be depending on origin and differs between freshwater and brackish water habituated round goby. For the hatching success of round goby eggs an optimum range was found at <15 PSU with decreasing hatching success in higher salinities (Chapter 2). However, little is known about the salinity tolerance of larval round goby presently. We conducted an aquarium experiment to disentangle the salinity influence on length growth, weight gain and survival of round goby larvae. Larval hatching was very limited at 25 PSU and post-hatch larvae did not survive 25 PSU longer than a day. Whereas > 20-day old larvae did survive higher salinities of 29 PSU in low numbers within 10 days. The length growth of larvae was not significantly affected by the salinity, in contrast to the weight at length that was highest at 10 PSU. Moreover a first test of spAARS measurements was conducted as a possible proxy of growth, which is till today mainly used in zooplankton. The test however failed.*

#### Introduction

Round goby populations are established in multiple fresh and brackish water systems in the northern hemisphere, Baltic Sea, Danube, Rhein, Elbe, St. Claire River, Great Lakes, and more (Skora and Stolarski, 1993; Charlebois *et al.*, 2001; Clapp *et al.*, 2001; Corkum *et al.*, 2004; Borcharding *et al.*, 2011; Hempel and Thiel, 2013). However, the establishment of a population in marine waters has so far not been observed. The restricting factor in round goby distribution is most probably salinity. Adult round goby show different abilities to cope with various salinities depending on their origin. Freshwater habituated

fish cannot handle higher salinities in contrast to individuals originating from brackish waters (Karsiotis *et al.*, 2012; Hempel and Thiel, 2015). Recent investigations on Baltic Sea round goby showed a combination of the reduced aerobic scope by 30% and increasing blood osmolality at salinities approaching marine conditions suggesting a reduced capacity for osmoregulation (Behrens *et al.*, 2016). These results suggest some physiological limitations in adults to a further spreading in saline habitats, even in fish that were taken from brackish water populations.

Especially the early life stage is vulnerable to different environmental and anthropogenic factors. The successful establishment of populations in close to marine conditions is only possible if the early life stages can survive under increased salinities. In Chapter two the influence of salinity on the development of round goby eggs was investigated and a decreasing success was found in egg hatching success in higher salinities. However the question, if egg hatching or the larval stage forms the bottleneck in the distribution of round goby, remains unanswered. Besides hatching success the larval phase might form the bottleneck. Data about larval round goby mortality caused by salinity do not exist. For mummichog larvae a significant difference in larval mortality is caused by salinity was found for two different populations with differing salinities (Able and Palmer, 1988). Such differences are likewise possible for round goby larvae, especially since differences in salinity tolerance depending on the origin salinity are known for adult round goby, but not part of this investigation

In larvae, survival and growth are to some extent connected according to concepts known as “bigger is better hypothesis”, “growth-mortality hypothesis” or “stage duration-hypothesis”. These are based on the assumption that the vulnerability by predators is decreasing with size (Ware, 1975; Houde, 1987; Cushing, 1990; Cowan *et al.*, 1996; Meekan *et al.*, 2006). Hence fast-growing larvae are leaving earlier the window of high predation risk and thereby can have positive effects on the recruitment.

So far no studies have been conducted on larval round goby growth in different salinities. Therefore this investigation was conducted to find limits and constraints in larval growth of round goby by rearing batches of eggs in different conditions until hatch and monitoring the growth of hatched larvae for 20 days.

Further, was a simple survival experiment conducted with > 20-day old larvae to gain first insights into the salinity tolerance of older larvae. For this larvae were exposed to different salinities from 15 PSU to 29 PSU.



## Material and Methods

Round gobies were caught on 24.08.2016 in Travemünde (Germany) by angling and transported in an aerated tank to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Gobies were kept in aerated tanks (1m x 1m x 0.5m) with an artificial seawater circulation system at 18 °C and 15 PSU.

After an acclimation period of 4 weeks, fish were split up into groups of three females and one male to prevent competition of males for shelters and minimize aggressive behavior. This was realized by splitting tanks (1m x 1m x 0.5m) in 4 compartments (0.5m x 0.5m x 0.5m). Shelters were designed with an exchangeable glass ceiling; therefore removing clutches without damage was possible (Fig. 3.1).

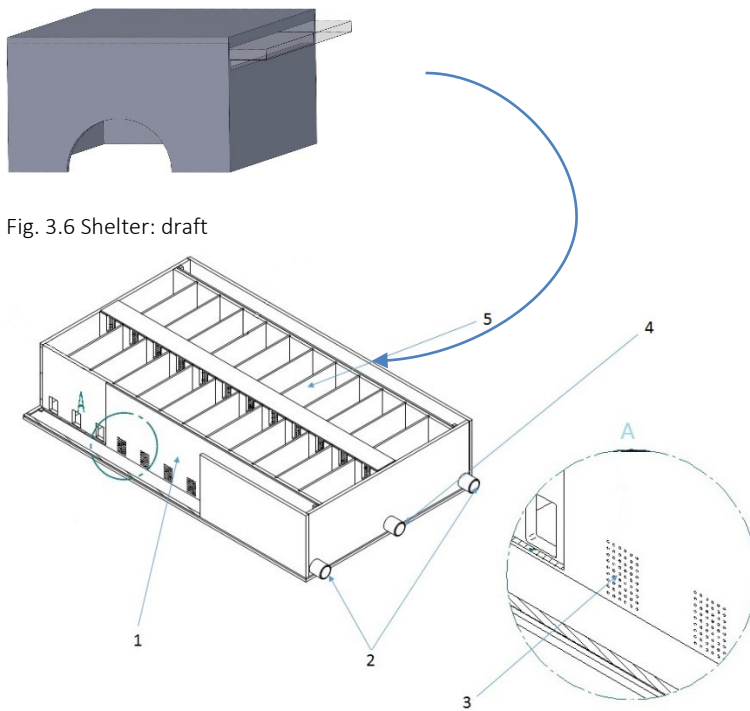


Fig. 3.6 Shelter: draft

Fig. 3.2: Breeding box; 1) Exchangeable sieve plate 2) Water inflow 3) Hole grid, hole  $\varnothing$  2mm (ca. 170mm<sup>2</sup> hole area) 4) Outflow 5) slot for a) glass plates with eggs or b) PVC-plates to separate in different chambers

These glass ceilings were designed to fit into boxes shown in Fig. 3.2. Shelters were checked daily for

new spawning events. To develop eggs under defined conditions, they were moved into different boxes (Fig. 3.2, No. 5) equipped with a small aerated circulation system.

### Growth experiment

Salinities were set to 5, 10, 15, 20, 25 PSU. For each treatment, 3 successful egg batches were incubated at 18°C. For the 20PSU treatment, 5 successful batches were incubated.

Larvae were fed daily with ~ 5000 *Eurytemora affinis* nauplii per compartment. During feeding the water flow was turned off for 2 hours to prevent the flushing out of food. In and outflow was additionally equipped with a fine-meshed net for the same purpose. Post hatch, 10 larvae were sampled on days 0, 10, and 20. Larvae were photographed and afterward frozen at -80°C. Larvae size was measured with help of the calibrated pictures. All larvae except those used for the AARS-measurement were freeze-dried and weighed. These results were used to generate an equation for the length-weight relationship. Larvae weight of AARS measured individuals was estimated from this equation.

### Aminoacyl-tRNA synthetase-Measurement

For measuring the aminoacyl tRNA synthetases (following AARS) activity in the larvae, from each batch the average larval size was determined. Three larvae with the minimum deviation from mean size were chosen. These larvae (frozen at -80°C) were transferred into a 2mL Eppendorf tube. 500µL cooled Tris-HCL buffer 20mM pH7.8 was added as well as a frozen steel bullet and subsequently mixed in a shaker at a frequency of 30 /s. Thereafter the sample was centrifuged at 4°C and 11190 turns per minute for 15 minutes. The supernatant was further analyzed by the method of (Yebra and Hernández-León, 2004) modified and adapted to microplate reading by Yebra *et al.* (2011). 100µl of the sample supernatant was pipetted into 80µl pyrophosphate reagent (PPi, Sigma P7275). The reaction absorbance was measured at 340nm for 60min at 22°C. During the reaction tRNA aminoacylation pyrophosphate (PPi) is released, which is going along with the oxidation of NADH. This process can be measured as a decrease in absorbance. This absorbance rate ( $\Delta\text{Abs} \cdot \text{min}^{-1}$ ) can be converted into pyrophosphate release and

therefore as AARS activity ( $\mu\text{mol PPI} \cdot \text{min}^{-1}$ ) as follows:  $\text{nmol PPI} \cdot \text{h}^{-1} = (\Delta\text{Abs min}^{-1} \cdot \text{Volume reaction (ml)} \cdot 10^3 \cdot 60) / (6.22 \cdot 2 \cdot 0.1 \cdot 0.46) \cdot \text{Volume homogenized (ml)}$

Where  $10^3$  is the conversion from  $\mu\text{mol}$  to  $\text{nmol}$ , 60 is the conversion from min to an hour, 6.22 is the millimolar absorptivity of NADH at 340nm, 2 denotes 2 moles of  $\beta$ -NADH oxidized per mole of pyrophosphate consumed, 0.1 is the Volume of the sample and 0.46 is the correction of path length in cm for the microwell plate.

The protein content of the samples was determined by using a Bicinchoninic acid Protein assay kit (Sigma 9643 and C2284). The protein concentration was measured at 562nm. To generate a calibration line, concentrations of 500 $\mu\text{l}$ , 250 $\mu\text{l}$ , 125 $\mu\text{l}$ , 62.5 $\mu\text{l}$ , 31.25 $\mu\text{l}$ , 15.625 $\mu\text{l}$  and a blank were used. For this calibration line, a protein standard with Bovine Serum Albumin was used (Sigma-Aldrich P0914).

## Survival Experiment

Eggs were incubated and Larvae were reared at 15 PSU and 18°C in boxes as shown in figure 3.2. Larvae were transferred into beaker glasses with 1l volume. On the first day after transfer, all beakers were equipped with water of 15 PSU for short beaker acclimatization. After day two salinities were adjusted to 15 PSU (control), 23, 26 and 29 PSU without any ramping. The day of the salinity adjustment is in the following called start day.

For survival experiments, all larvae were taken from one batch. Larvae D1 were from hatching day 1 and 22 days old at start day. D2 larvae hatched at day two and were therefore 21 days old at start. Larvae D3 hatched on the third day and later and had at the start an age of  $\leq 20$  days. Each beaker contained 10 larvae. These were fed with 3000 *Eurytemora affinis* nauplii each day. Beakers were aerated and 500ml of water was exchanged every day.

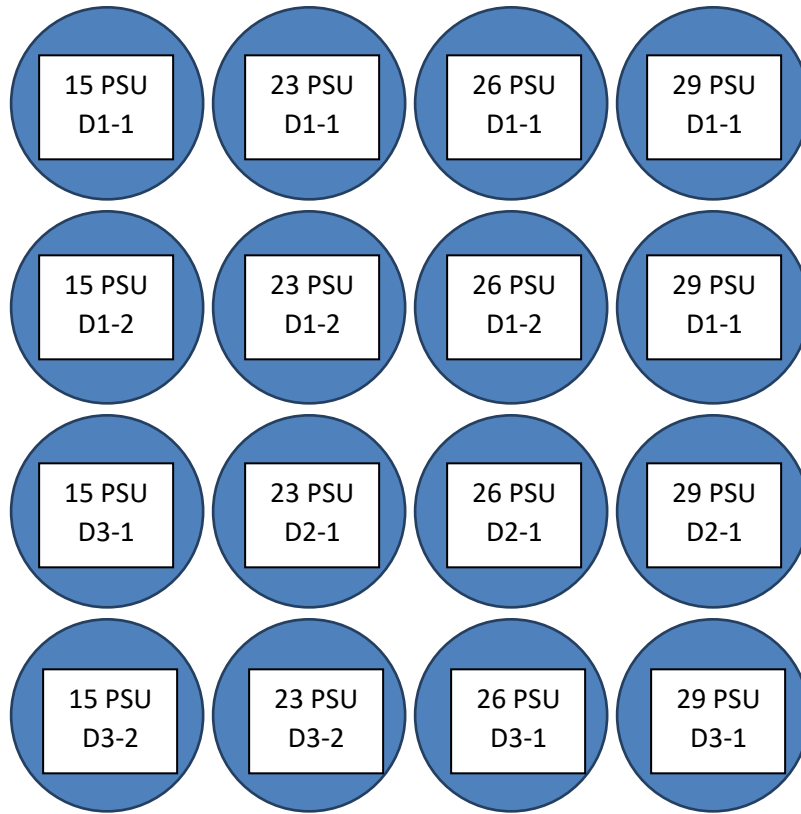


Fig. 3.3: Schema of the Experiment

## Statistics

All statistics were calculated with R 3.2.3 (64-bit). For length/PSU at age differences (Tab. 3.1), a Kruskal-Wallis Test and a Kruskal-Wallis- Dunn- Post-Hoc test Bonferroni adjusted was performed (R Package FSA) similar for the weight/PSU comparison. For loess smoother in Fig. 3.4 the “geom\_smooth”-function from the “ggplot2” package was used, in Fig. 3.4 the equations are calculated with the basic R function “lm”. For the nonlinear models in fig. 6, the non-linear least squares “nls” function was used with the equation  $W=aL^b$  ( $W$ =weight,  $L$ =length,  $a+b$  = constants). For the spAARS comparison, an Anova was calculated by the “aov” function.

Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

## Results

Out of 4 batches incubated in 25 PSU, only one batch ended up in hatching, all others stopped in the development and the eggs run to seed. All larvae that hatched at 25 PSU died within the first 24 hours. Therefore no data for 25 PSU are available.

Tab. 3.1. Mean length, weight and spAARS activity per batch / day and salinity

PSU	Day	Batch	mean length [mm]	mean length all [mm]	mean weight[mg]	mean weight all [mg]	mean spAARS	
5	1	1	7,64		1,01		16,93	
	1	2	7,92	7,85	0,92	1,00	13,95	15,84
	1	3	7,98		1,07		16,65	
	10	1	-		-		-	
	10	2	8,89	9,28	0,87	0,92	22,49	20,60
	10	3	9,66		0,97		18,71	
	20	1	10,96		1,42		15,48	
	20	2	8,93	9,67	0,75	1,17	15,14	15,49
10	20	3	9,12		1,34		15,84	
	1	1	7,72		1,08		15,50	
	1	2	7,84	8,29	1,19	1,05	21,50	16,69
	1	3	9,32		0,89		13,09	
	10	1	10,27		1,62		28,58	
	10	2	9,28	9,65	1,12	1,32	21,38	22,86
	10	3	9,42		1,23		18,63	
	20	1	13,81		3,73		18,27	
15	20	2	9,20	11,88	1,00	2,48	19,71	16,98
	20	3	12,62		2,70		12,97	
	1	1	7,33		1,01		30,92	
15	1	2	6,76	7,23	1,19	1,09	25,06	26,74
	1	3	7,61		1,07		24,26	

Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

	10	1	8,93		1,08		19,86	
	10	2	8,93	8,89	1,23	1,06	20,56	19,45
	10	3	8,81		0,88		17,92	
	20	1	9,07		0,78		22,41	
	20	2	14,10	10,83	1,46	0,98	23,73	22,54
	20	3	9,32		0,72		21,49	
	1	1	6,25		0,67		16,45	
	1	2	8,05		1,24		20,61	
	1	3	7,24	7,35	1,08	1,08	22,80	22,27
	1	4	7,69		1,15		24,94	
	1	5	7,54		1,25		26,54	
	10	1	7,98		0,69		17,40	
	10	2	9,07		1,01		18,48	
20	10	3	8,83	9,04	0,84	0,97	17,36	17,87
	10	4	9,79		1,14		16,75	
	10	5	9,53		1,16		19,34	
	20	1	9,27		1,00		14,25	
	20	2	13,95		1,18		17,88	
	20	3	8,83	10,31	0,81	0,99	15,22	16,20
	20	4	9,55		0,91		16,67	
	20	5	9,96		1,06		16,96	

### Larval length growth

The length measurement on day 1 shows significant differences (APP. 3.1) in length between the different salinities. Differences in size were found to be significant except of the combination 05-10 (p.adj=1.00) and 15-20PSU (p.adj=1.00) (APP.1). At hatch, the 10 PSU larvae were on average the biggest ones (8.29mm), and larvae at 25 PSU the smallest ones (6-75mm) (Fig. 3.4).

At an age of 10 days post-hatch (dph) the differences in length were not significant except for 15-10 PSU, where 15 PSU larvae were significant (p.adj =0.006714) smaller. After 20 dph this picture changed. The

### Salinity a bottleneck?

#### Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

only significant difference ( $p_{\text{adj}}=0.034934$ ) was found between the larva at 10 PSU (9.65 mm) and those at 20 PSU (9.04 mm). The size at hatch varied through the batches and ranged in average size per patch from 6.25mm to 8.05mm.

With increasing age, the variance in length was increasing. In figure 3.4a the different colored points represent the larva of different batches. These show that the increasing variance in length mainly occurred on day 20

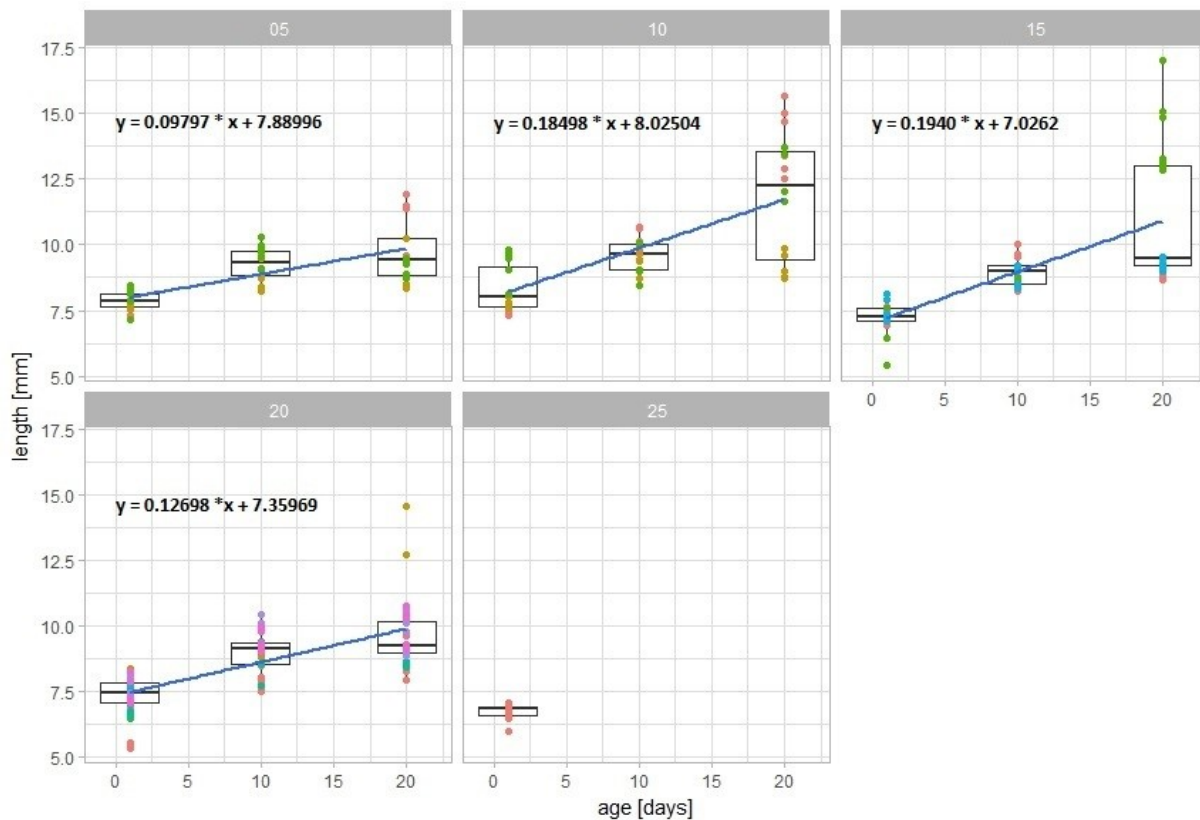


Fig.3.4a length at dph per salinity (5-25) , color = different batches, regression with given equation

### Salinity a bottleneck?

Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

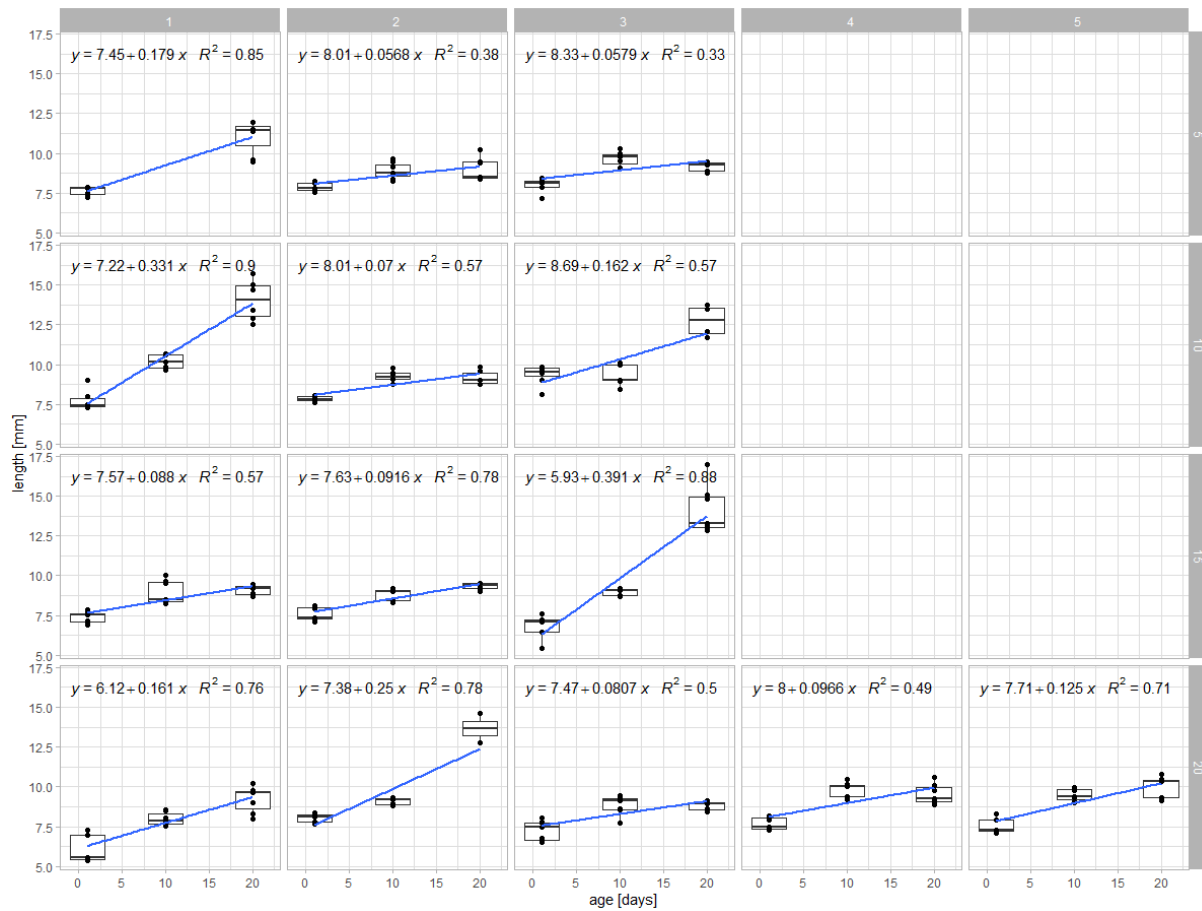


Fig. 3.4b Length at age (dph) per salinity(indicated right side) and batch(nr. at the top)

Despite the not significant differences in average length between the salinity groups, the slopes of the linear model in Fig. 3.4a show slightly different values.

Figure 3.4b reveals the differences between the different batches in length at age. In all salinities, batches were found with larvae longer at age than the “average”. In 5 PSU larvae of the first batch were bigger in length at day 20 than larvae of batch two and three. At 10 PSU larvae of batch 2 were shorter in length at day 20 than larvae of batch one and three. In 15 and 20 PSU again each one batch had bigger larvae at day 20 than all the others.



Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

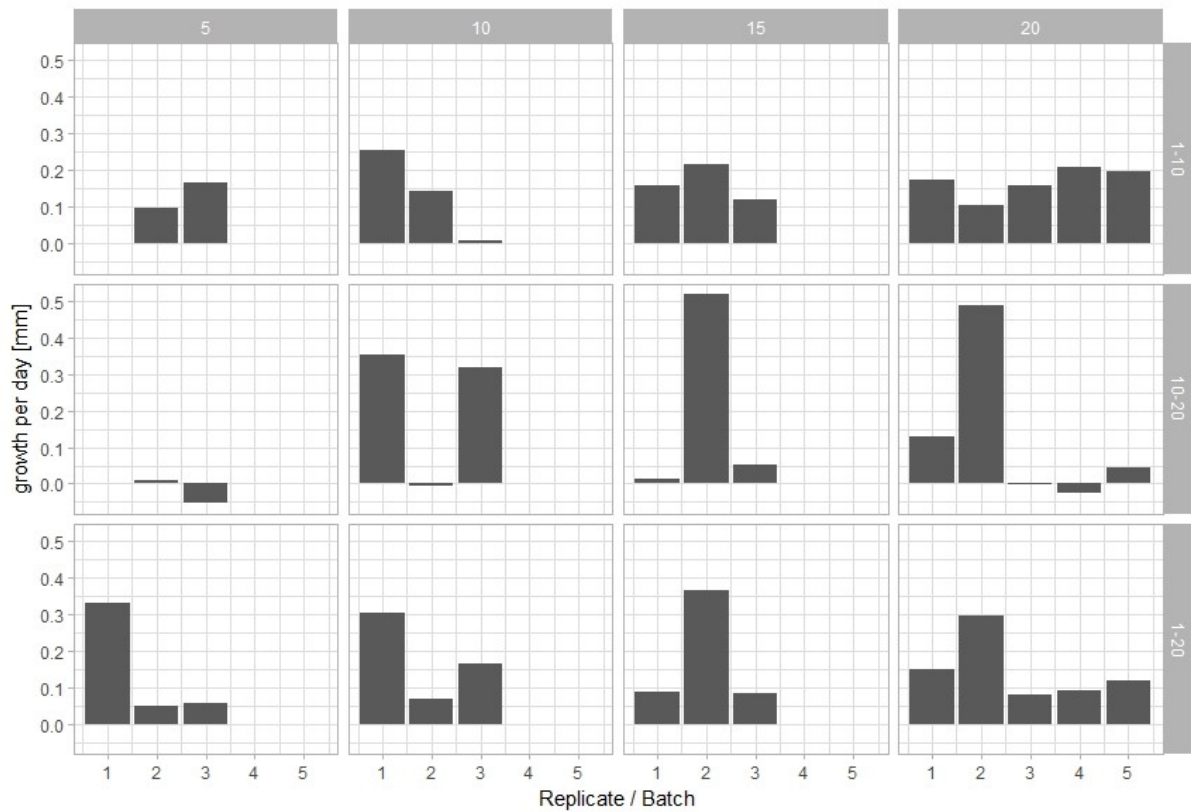


Fig. 3.5 Growth per day [mm] for each batch differentiated by salinities (5, 10, 15, 20 PSU) and days post-hatch (time range from day 1 to 10, 10 – 20 and 1-20)

### Growth per day

Within the first 10 days, the growth per day did show differences between the batches. Growth per day varied from 0.1 to max 0.25mm day<sup>-1</sup> highest and lowest values were both measured in 10 PSU. With one exception at 10 PSU that did grow with <0.001mm day<sup>-1</sup>.

This picture changed after 10 days. Growth per day measured from day 10 to day 20 showed high variation. At 5 PSU one larvae group did not grow and one only with <0.001mm day<sup>-1</sup>. Also two groups at 20 and one group at 10 PSU did not grow. But besides these groups that did not or only slowly grow other groups were growing rather fast. The highest growth rates were observed in one group at 15 PSU with over 0.5mm day<sup>-1</sup>. One group at 20 PSU reached growth rates of ~0.49 mm day<sup>-1</sup>. At 10 PSU the larvae did grow somewhat slower but two out of three groups increased with 0.3 to 0.35 mm day<sup>-1</sup>.

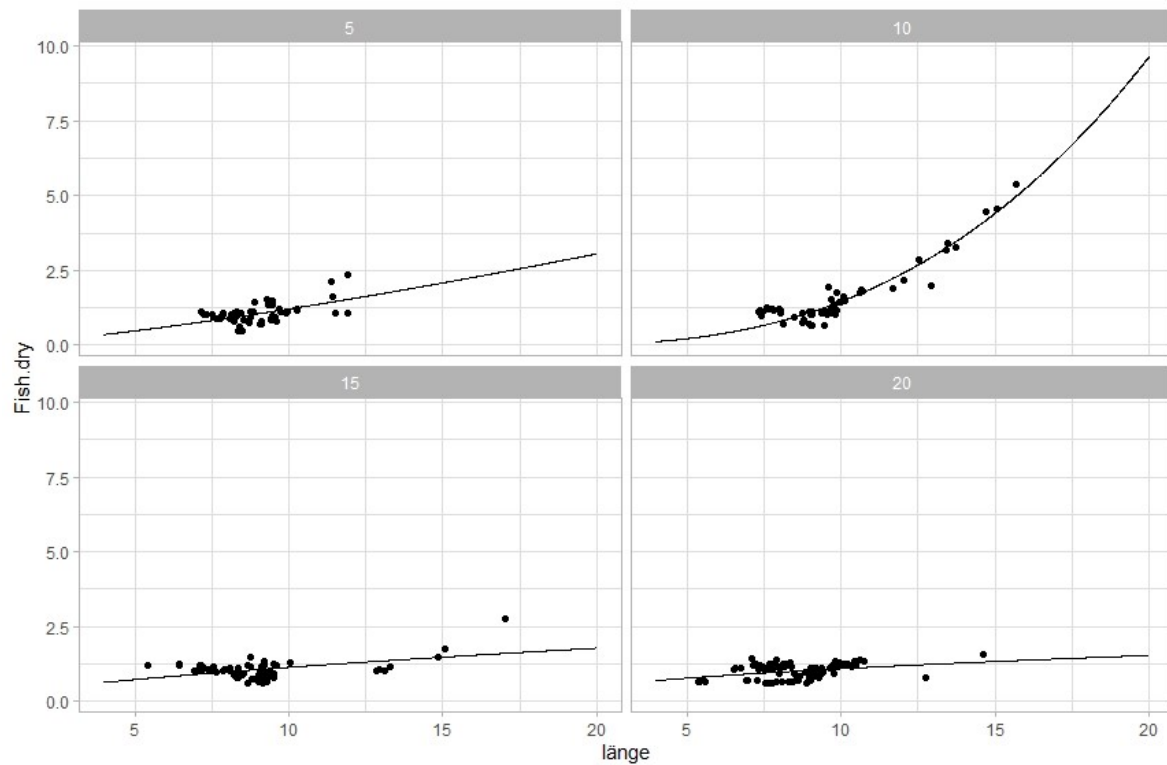
### Salinity a bottleneck?

#### Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

The growth per day over 20 days shows similar variations, always one batch performed better than the rest per treatment, while the other batches grew somehow similarly slow. The slowest growth per day on average over 20 days was found at 5 PSU with  $\sim 0.05$  mm day<sup>-1</sup> fastest growth was measured at 15 PSU with  $\sim 0.37$  mm day<sup>-1</sup> (Fig. 3.5).

Nevertheless, the growth per day over the whole period was never significantly different between the salinities, when all batches were included.



Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

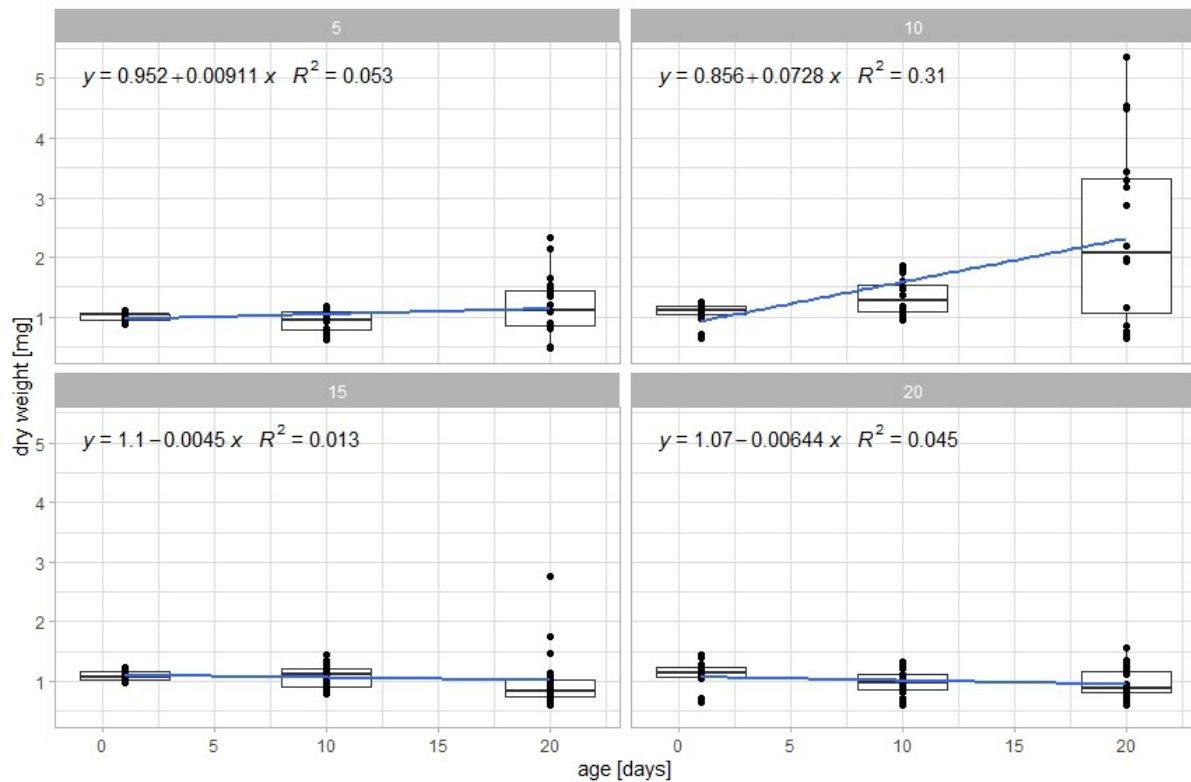


Fig. 3.6 a) length-weight, lines nonlinear least squares model fitting, mean length overall salinities at day 10 b) weight at age for different salinities

The length~ dry weight (Fig. 3.6a) relationships illustrate, that larval weight gain varies depending on salinity. Larvae reared at 10 PSU were heaviest at length, followed by larvae raised at 5 PSU. The lowest condition was observed for larva at 20 PSU while larvae at 15PSU performed slightly better. The slopes (Tab. 3.2, b) of the different models in Fig. 3.7 illustrate how the weight–length relation varies. Significant differences between different weights at given salinities were only found for 10 PSU larvae, all other groups are not significantly different in weight (APP. 3.2). The weight at age plot (Fig. 3.6b) illustrates the influence of salinity on weight gain and shows the best weight gain at 10 PSU.

Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

Tab. 3.2 values of constants for nls model in Fig. 6a, equation  $W=aL^b$

PSU	a	b
5	0.0564	1.3328
10	0.0027	2.7190
15	0.2562	0.6465
20	0.5384	0.3060

### Aminoacyl-tRNA synthetase

The AARS activity measurement results in figure 3.7b illustrate a high variance within the data. The amount of spAARS activity was found to be lowest in the 5PSU. With increasing individual biomass [ $\mu\text{g}$  protein ind<sup>-1</sup>] a decreasing spAARS activity was measured Fig. 3.8a. An ANOVA revealed that the only factor significantly influencing the spAARS is age in days. Between the different salinities and batches, no significant differences in variance were found (APP. 3.2).

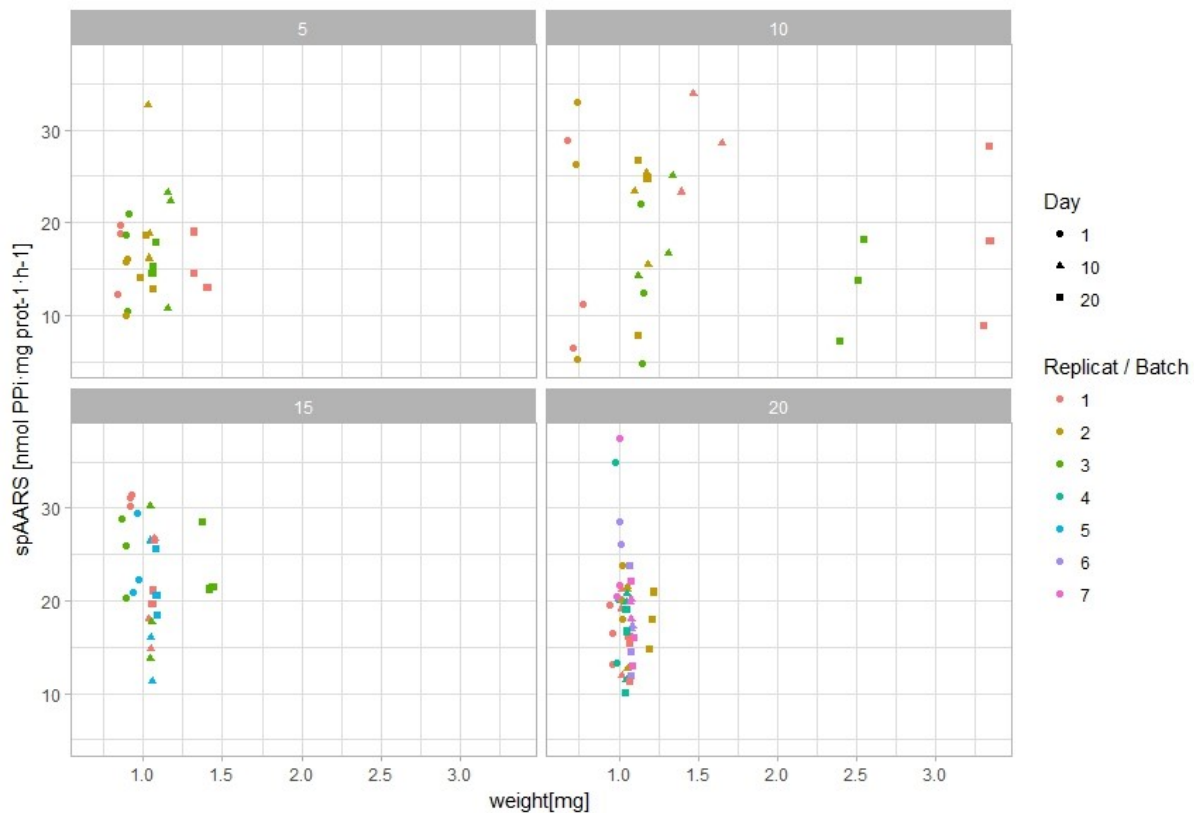


Fig.3.7 relationship  $\text{spAARS [nmol PPI mg prot-1 h-1]} \sim \text{calculated weight[mg]}$ , weight calculated with equations shown in fig. 6 and given tab. 3

## Survival Experiment

The survival experiment showed mortality in the control group at 15 PSU with a maximum of 2 out of 10 larvae dying within the 10 days. In 23 PSU the larvae from hatching day 1 (D1) showed a slightly increased mortality with 3 to 4 larvae dying, whereas one (D3) and two (D2) larvae died from the other hatch days. In 26 PSU between 3 and 6 larval died. Larvae from D3 in 29PSU showed a mortality of 6 to 8 individuals while from the other hatch days 7 (D2) and 8 (D1) larvae died. In all treatments, the mortality was observed throughout the 10 day period. Variance in salinity was varying very limited Tab. 3.2.

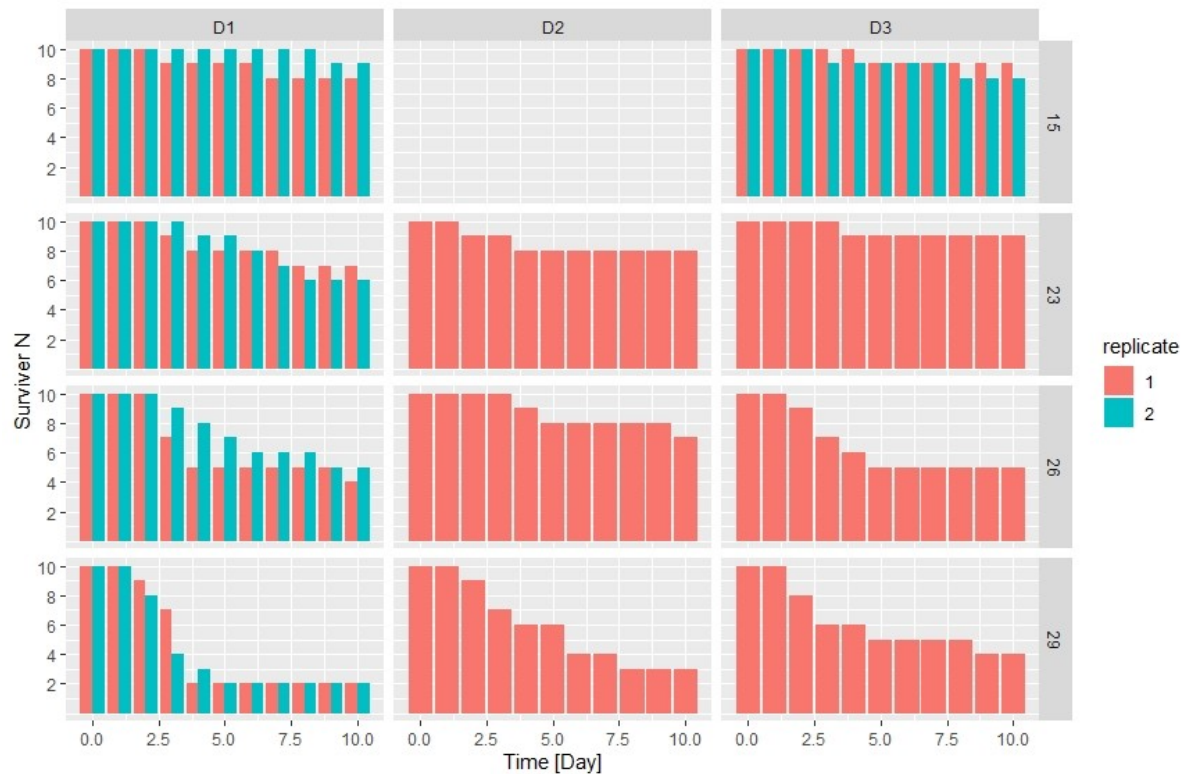


Fig. 3.8 larval mortality within 10 days after experiment start

Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

Tab. 3.3. Measured salinity during experiment mean value & sdv

	15-S1-1	15-S1-2	15-S3-1	15-S3-2	23-S1-1	23-S1-2	23-S2-1	23-S3-1
mean	15,05	15,01	15,09	15,02	22,98	22,99	22,99	22,97
sdv	0,08	0,06	0,06	0,11	0,11	0,09	0,11	0,13
	26-S1-1	26-S1-2	26-S2-1	26-S3-1	29-S1-1	29-S1-2	29-S2-1	29-S3-1
mean	25,95	25,98	26	26,02	28,97	28,96	28,97	28,95
sdv	0,10	0,10	0,07	0,32	0,13	0,11	0,173	0,14

## Discussion

### Method

The Method of mass rearing of larvae with repeated sampling to measure growth in a lab is an established procedure and was often used before, but an ad libitum food supply can constitute a problem. For this experimental design and tank-setup, there was an issue with the feeding of *Euritemora affinis* as a washing out from the rearing tank to the water reservoir of the water circulation system appeared. To prevent this, tanks were equipped with additional meshes at in- and outflow. Therefore food supply is assumed to be ad libitum, since in pre-tests the amount of food was not completely consumed during a day. To reliably measure the development of larvae a sufficient number of larvae needs to hatch at one day since regularly sampling and natural death reduce the numbers fast. In this experiment the amount of eggs per batch was limited, and only few larvae hatched on the same day. To compensate for this by increasing the number of breeding fish was not possible due to limited facility space. A specific trigger for stimulation of hatch like it was used e.g. for herring (Kiorboe *et al.*, 1985) is not known for round goby.

It takes typically ~ 10 days until all eggs from one batch are hatched (Chapter 2). With batch sizes reared in the laboratory of ~400 eggs (Chapter 2) of the number of hatching larvae at one day is mostly well below 100. We, therefore, had to restrict the samples to only 10 larvae per day and batch.

### Larval growth

Within the first days post hatch marine larval fish use their yolk sac as an energy resource. This phase can span from < 1 day in warm water species to > 30 in cold-water species (Peck *et al.*, 2012). The time for yolk sac depletion for round goby is not documented, but following the pictures taken for this experiment at 18°C it is likely within the first 10 days post-hatch. The data shown in figure 5 hint at a reduction in growth for some batch groups after 10 days. This may indicate a problem in changing from internal to external feeding after the yolk sac depletion. For the grass goby, *Zosterisessor Ophiocephalus*, the yolk sac is absorbed after 4 days post hatch which is associated with a larval mortality of 71%

(Privileggi *et al.*, 1997). These high mortality rates were not observed for round goby, nevertheless, one critical point in the larval stage is changing from internal to external feeding. After a period of starvation, larvae can reach a “point of no return” (PNR) that involves irreversible tissue damage leading to death even if prey is available.

Investigations on cod suggest that the first feeding success is related to the mouth gape which is a function of the larval size (Knutsen and Tilseth, 1985). Considering the average larval length at hatch (Tab.1) it becomes obvious that this could not be the only explanation for the limited growth in this experiment since for example at 10 PSU batch no. 2, which exhibited poor growth had a similar mean length like batch no. 1 (7.8437mm / 7.7289mm) that grew best. Nevertheless one would have expected that some larvae of a batch may have a problem with the first feeding, but why most larvae of certain batches have problems and stop growing remains unclear and the mechanism needs further investigations. Possibly genetic or epigenetic effects are involved, causing different salinity resistance, but there is no literature on the topic for round goby larvae. An effect of food availability is unlikely, since food was supplied in the same way for all batches, but can't be excluded finally.

Fig 3.5 and Fig. 3.6b illustrate a negative growth in length and a weight loss for some batches with increasing age. This negative growth in weight and length reflects probably the shrinking of larvae that can occur in starvation by exhausting lipid reserves and other body tissues like it was noticed for salmonids (Huusko *et al.*, 2011). It was further checked if the batch size, which is consequently linked to the number of larvae reared in the tank, had influenced growth differences. But no relation and therefore no effect was found. Another explanation for the smaller larvae at later stages of the experiment might be that the “bigger” larvae simply died first, and only the smaller ones survived due to lower metabolic rates.

Effects of salinity on growth rate are higher for stenohaline fishes than for euryhaline (Altinok and Grizzle, 2001). Round gobies are euryhaline, therefore effect is assumed to be smaller. Nevertheless, the experiment suggests that osmoregulation is a factor that influences weight gain (Fig.3.6). The weight at length was highest for fish reared at 10 PSU. Mechanisms involved in osmoregulation lead in most teleost to a blood osmolality of 280–360 mosM kg<sup>-1</sup>, corresponding to ~10-12 PSU (Varsamos *et al.*, 2005).

Moreover it was found that the highest aerobic scope, in adult round goby, appears at a salinity of 10 PSU with lower values above and below (Behrens *et al.*, 2016). Therefore the available energy for weight



and length gain in salinities differing from 10 PSU is reduced which may explain the reduced weight at length in higher and lower salinities.

The weight length relationship of larval fish was investigated in detail by (Pepin, 1995). The values for the slopes of the length-weight relationship of different species given in this paper are similar to the slope of the 10 PSU trial.

### AARS as growth proxy

#### *Method*

It was predicted to find a clear relationship between spAARs and growth, but in this case the method did not deliver the expected results. A statistical relation to growth was not determinable. This may be caused by the limited sample size in combination with the high growth variability. For the AARS measures, only 3 larvae were chosen from each batch since sufficient numbers of larvae for the dry weight determination was needed. Given the high variance in the AARS-Data it is obvious that the number of tested samples was too low. Further experiments with a higher number of tested larvae may gain different results, but with the present set up, this method did not work.

#### *General*

AARS activity measurements were used as an index for somatic growth in zooplankton (Yebra and Hernández-León, 2004). AARS is linked to protein synthesis which in turn has a positive relationship to growth (Houlihan et al., 1990; Sveier et al., 2000 as cited in Yebra and Hernández-León, 2004). Therefore we hypothesized that the AARS could work as a proxy for growth. The method of AARS activity measurements was not often used for fish before with the exception of a study on larval herring (Herrera et al., 2009) where somatic growth and AARS activities followed similar patterns.

## Survival Experiment

This experiment was conducted to simulate the transfer from 15 PSU into higher salinities without any ramping. This certainly stresses the larvae but was chosen to mimic a sudden release from the ballast water tank into a new environment. Larvae were hatched and reared at 15 PSU and it was assumed that 15psu has no relevant effect on survival.

The idea behind this experiment was to mimic the transport situation of the larvae: they either hatch in a ballast water tank, or in a harbour and get sucked into ballastwater tank and then develop with a suitable salinity for a time of about 20 days. After this period they are released in a new foreign environment with differing salinity.

The handling seems to have had a minor influence since in 15PSU only two larvae died in each beaker. Since mortality increases gradually with increasing salinity it appears that there is no clearly defined threshold. Some of the larvae even survive the highest tested salinity of 29 PSU.

Marbel goby larvae *Oxyeleotris marmoratus*, with an optimum salinity for survival at 10 PSU, showed increasing survival rates with increasing age in different salinities. (Senoo *et al.*, 2008). This might also apply for round goby. It is interesting to note the pronounced difference between these approximately 3-week old larvae and the freshly hatched larvae that are not able to cope with 25 PSU. Adult round goby are able to maintain blood osmolality stable up to 25psu (Behrens *et al.*, 2016). This indicates some kind of a physiological threshold that may also be crucial for post hatched larvae.

For juvenile round goby (TL 53-75mm) a survival of 100% is reported over 12 weeks at salinities of 30 PSU, adapted stepwise (Hempel and Thiel, 2015). Whether survival would still be 100% without stepwise adaption to salinity is unknown, nevertheless age is an important factor for salinity tolerance within the early life of round goby. Conspicuous is the reduced mortality in later hatched larvae, that is noticeable in all salinities. This is contrary to findings of marble goby, where early and late hatched larvae showed higher mortalities (Senoo *et al.*, 1994). Nevertheless the limited current data set suggests that later hatched larvae might be less sensitive to higher salinities. This may indicate again that some parts of the osmoregulatory metabolism still develop post-hatch, possibly osmotic active tissues like skin, gills and gut as described for flatfish (Schreiber, 2001). This should be addressed in further experiments.

## Conclusion

The question of the salinity bottleneck of distribution might be answered now. A successful egg hatching is observed at a maximum of 25 PSU. However freshly hatched larvae did not survive 25 PSU longer than a day. Whereas older larvae were also able to handle 29 PSU in a short term and juveniles also survive in high salinities (Hempel and Thiel, 2015) This leads to the conclusion that the early larval stages are most vulnerable in high salinities and form the crucial bottleneck for successful distribution into marine waters.

## APPENDIX

### APP. 3.1 Length salinity

Day1

Kruskal-Wallis rank sum test

Kruskal-Wallis chi-squared = 41.064, df = 4, p-value = 2.607e-08

Comparison	Z	P.unadj	P.adj
(sal. groups)			
1 05 - 10	-0.8273601	4.080330e-01	1.000000e+00
2 05 - 15	3.0876907	2.017183e-03	2.017183e-02
3 10 - 15	3.8771636	1.056813e-04	1.056813e-03
4 05 - 20	2.8534592	4.324609e-03	4.324609e-02
5 10 - 20	3.7931768	1.487322e-04	1.487322e-03

# Salinity a bottleneck?

Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

6	15 - 20	-0.8612374	3.891073e-01	1.000000e+00
7	05 - 25	4.7758204	1.789761e-06	1.789761e-05
8	10 - 25	5.4052116	6.473193e-08	6.473193e-07
9	15 - 25	2.2957275	2.169147e-02	2.169147e-01
10	20 - 25	3.2083945	1.334783e-03	1.334783e-02

Day 10

Kruskal-Wallis rank sum test

Kruskal-Wallis chi-squared = 11.332, df = 3, p-value = 0.01006

Comparison	Z	P.unadj	P.adj
(sal. groups)			
1 05 - 10	-1.1812768	0.237492776	1.000000000
2 05 - 15	1.7579147	0.078762016	0.472572097
3 10 - 15	3.2587745	0.001118946	0.006713673
4 05 - 20	0.7824921	0.433925449	1.000000000
5 10 - 20	2.3512899	0.018708453	0.112250719
6 15 - 20	-1.3009362	0.193280308	1.000000000

Day 20

Kruskal-Wallis rank sum test

Kruskal-Wallis chi-squared = 9.6068, df = 3, p-value = 0.02222

Salinity a bottleneck?  
Growth and fitness of post hatched round goby (*Neogobius melanostomus* (Pallas, 1814)) affected by salinity

---

Comparison	Z	P.unadj	P.adj
(sal. groups)			
1 05 - 10	-2.55549256	0.010603763	0.06362258
2 05 - 15	-1.35991818	0.173855817	1.00000000
3 10 - 15	1.26151810	0.207122244	1.00000000
4 05 - 20	0.01987837	0.984140400	1.00000000
5 10 - 20	2.75762008	0.005822382	0.03493429
6 15 - 20	1.49146651	0.135839057	0.81503434

### APP. 3.2 ANNOVA spAARS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
salinity	1	69	68.61	1.578	0.2116
day	1	202	202.08	4.648	0.0331 *
batch	1	0	0.01	0.000	0.9897
length	1	3	2.65	0.061	0.8055
weight	1	3	2.71	0.062	0.8032
Residuals	117	5087	43.48		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Salinity preference of round goby (*Neogobius melanostomus*) an experimental approach with fresh- and brackish water origin fish

### Abstract

*Since the first report of the round goby in the Baltic sea and in European rivers, many questions were addressed about the influence of local conditions on further dispersal and the potential threat to other areas that are not yet populated by round goby. Consequently linked with these questions is the query about preferences of round goby on the physical characteristics of the habitats. The challenge was to provide a suitable experiment setup fitting to this particular species with its given behavioural patterns. We tested a shuttle box and exposed round gobies from freshwater and a brackish water habitat to a choice of salinities. We found slight differences in salinity preference between the different origins (brackish and freshwater). Both preferred salinities below 20 PSU. Fish from freshwater showed a tendency for lower salinities (45.49 % below 10 PSU) in comparison to those from brackish water (37.18% below 10 PSU). Moreover, did 1/3 of brackishwater fishes stopped shuttling in high salinities whereas 2/3 of freshwater fishes stopped- this hints at a higher physiological impact for freshwater fish.*

### Introduction

Round goby is increasing its dispersal within the Baltic Sea towards the Skagerrak and marine waters. Besides this expansion round goby has established populations in multiple rivers and freshwater systems in northern Europe and northern America e.g.(Skora and Stolarski, 1993; Wiesner, 2005; Beek, 2006; Hayden and Miner, 2009a; Borcharding *et al.*, 2011; Hempel and Thiel, 2013; Azour *et al.*, 2015; Nurkse *et al.*, 2016; Šlapanský *et al.*, 2017). Round goby dispersed rapidly within the last decades and showed high plasticity in salinity tolerance from fresh water to salinities above 20 PSU. Investigations how salinity influences the osmoregulation capacity revealed that higher salinities affect the aerobic scope and the maximum metabolic rate of round goby. Nevertheless, it was noticeable that some individuals were less affected than others (Behrens *et al.*, 2016). Mortalities in their study were lowest around 10-15 with 89% surviving and highest at 25 and 30 PSU with 61% surviving for 3 months. Another study investigated the

weight gain of juvenile round goby in different salinities (Hempel and Thiel, 2015). The highest mean weight gain was observed at 7.5 PSU decreasing in salinities below and above. This experiment listed only 1 dead specimen in freshwater, all others survived also 30 PSU within the experiment time of 12 weeks. Both studies are based on round gobies with a brackish water origin. In an investigation on salinity tolerance with round gobies from freshwater lake Erie in the United State best growth rates were found between 5 and 10 PSU. This result is similar to those produced with fish with a brackish origin, but mortality rates were higher in freshwater. At 20 PSU, only 10% of the freshwater fish survived for only 2 months, while 5% survived for as long as 4 months. At salinity above 20 PSU most died within the first month. (Karsiotis *et al.*, 2012). These results show that differences exist with regard to salinity tolerance between populations originating from different habitats. However, it remains to be investigated if there is also a difference in salinity preference of round goby depending on their origin.

A setup in which fish can actively avoid unpleasant and select preferred conditions is a shuttle box (Horner *et al.*, 1961; Behrend and Bittermann, 1964; Woodard and Bitterman, 1971; Kovačević, 1978; Simón and Trincker, 1981; Schurman *et al.*, 1991; Bushnell *et al.*, 1994; Serrano *et al.*, 2010; Christensen and Grosell, 2018; Macnaughton *et al.*, 2018).

Although widely used, it comes along with various points to consider within the experiment setup, since every species has its peculiarities of behavior. In a shuttle box, the fish has the chance to actively avoid unpleasant conditions. Nevertheless, the individual must understand that there is another place with more comfortable conditions. These experiments aimed to test a method suitable for round goby. Round goby with 2 different origins, the Trave river mouth (brackish water) and the Rhine (fresh water) were tested.

The experiment should reveal the actual preferences and whether there are differences in salinity preferences depending on the origin, i.e. brackish or fresh water.

## Material and Methods

### Fish

Round gobies *Neogobius melanostomus* were caught first on 24.08.2016 in Travemünde (Germany) in the “Trave” river at Lübeck Herrenwyk by angling, and second at Rhein km 845 by angling on 8 - 9.11.2016. After catch fish were transported in an aerated tank to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Trave gobies were kept in aerated tanks (1m x 1m x 0.5m) connected to an recirculation system at 18 °C and 15 PSU. Rhein gobies were kept in freshwater tanks connected to arecirculation system at 18°C. Before the experiment started, the individuals were kept in the experiment set up for 24 hours to adapt to the shuttle box system. Acclimatization was filmed and recordings were inspected to control if fish performed spontaneous shuttling between the tanks. Salinities and temperature during the acclimatisation phase were similar to those in the maintenance tanks .

### System

A variety of different setups exists to test salinity preferences. A review of Jutfelt et al. (Jutfelt *et al.*, 2017) shows the most popular setups to test individual avoidance or preference of conditions. For the here described experiments, a shuttle box was assumed to be the best setup as other systems do not fit to the round goby behaviour, details are discussed below.

The shuttle box system was set up as shown in Figures 4.1 and 4.2. The tanks had a diameter of 23.5cm and a height of 30cm. The drain was installed at 13.5cm which limited the water level. The connection between both tanks was 1.5cm long and had a diameter of 5cm. To reduce the water exchange between the tanks it was equipped with a “curtain” produced out of a view foil of ~0.1mm thickness. The foil was gashed as shown in Fig. 4.3. The camera used was a raspberry pi camera with a resolution of 1024 x 800 pixels, a frame rate of 32, ISO 200, and a shutter speed of 60000. Valves used to control the waterflow (position see Fig. 4.2) were controlled by air pressure. The pumps were adjusted to a volume flow of 2 liters per minute. For salinity measurements, a WTW ProfiLine Cond 3310 was connected to the controlling computer. The tanks were equipped with a transparent ground. Under the tanks, an indirect



infrared led light source was mounted to achieve a reflection-free uniform illumination at day and night time.

The shuttle box was programmed to change salinities automatically dependend of the fish position and the retention time at this position within the system. The ambient salinity changed depending on the behavior of the fish. The position of the fish was tracked by a camera to control the aea in which the fish was staying. For this three areas were defined, tank 1, tank 2, and the pipe in the middle. The salinity delta between the tanks was always at 5 PSU with a tolerance of 0.5 PSU. If a fish did not move, the salinity changed every 30 minutes by 2 PSU in both tanks simultaneously. The lower and the upper limit were a salinities of 1 and 33 PSU. One tank was defined to be allways more saline, the other to be less saline. If the fish stayed in the less saline tank the salinity decreased, if it stayed in the higher saline tank, the salinity increased. If the fish was sitting in the connection pipe, both tanks kept its salinity, no changes took place. If the fish stayed in the less salinie tank at the threshold of 1 PSU longer than 30 minutes, the program changed the salinity difference of the tanks, such that the less saline one became the one with higher salinity and other way round. If the fish stayed at the high saline limit the system did not react and maintained the salinity at the threshold level of 33 PSU.

One fish (Rhine 1, first in the experiment) was accidentally exposed to another program; In deviation from standard program the order of the high and less saline tank changed also if a the fish stayed at upper salinity threshold of 33 PSU for 30 minutes.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

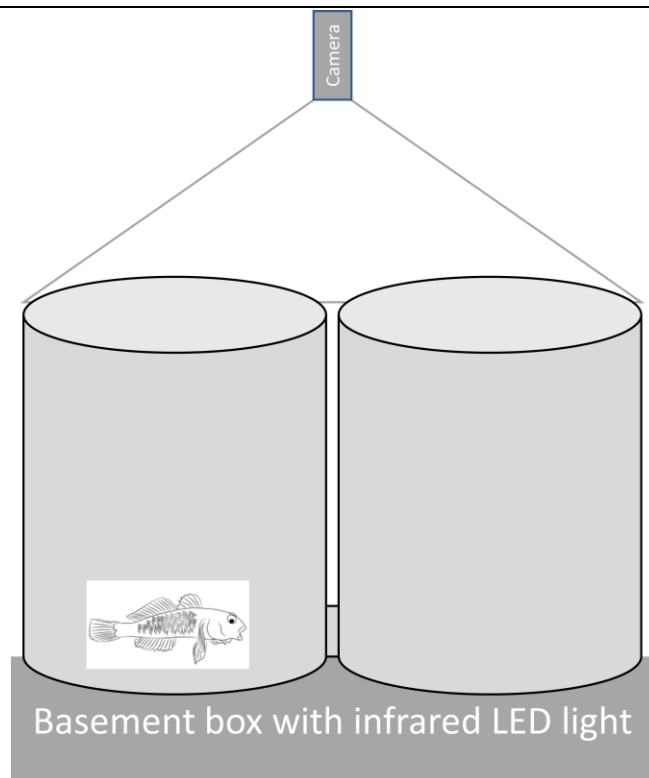


Fig. 4.1: Shuttle-Box overview

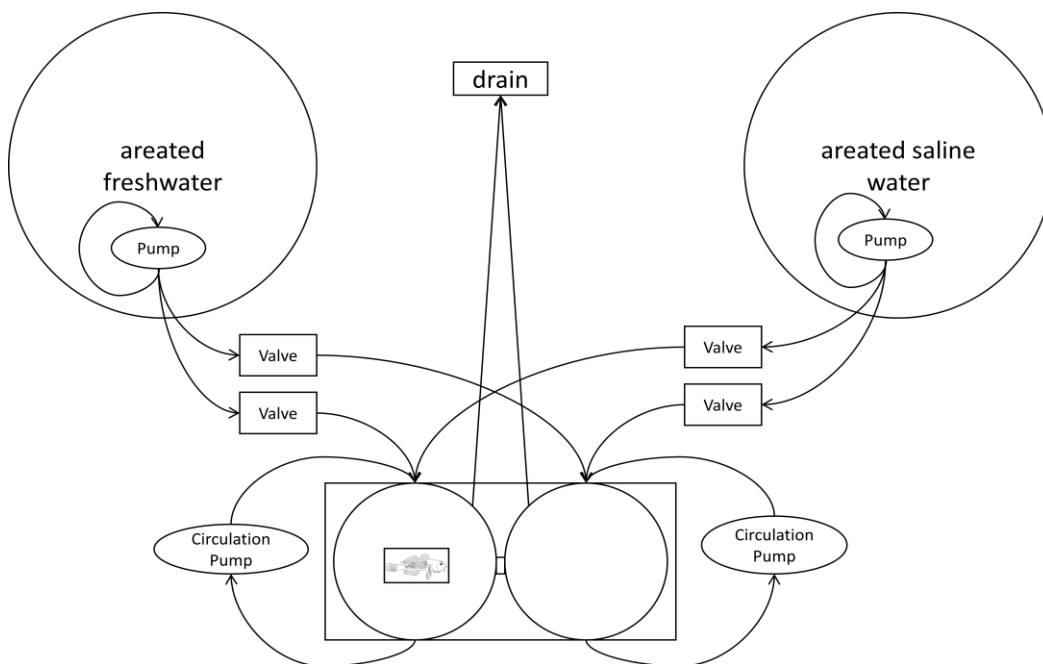


Fig. 4.2: schema of the system setup.

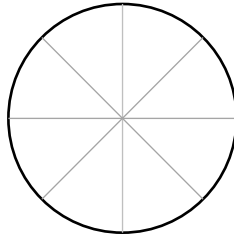


Fig. 4.3: barrier in the connection pipe, lines within the circle indicate how the foil was gashed to guarantee a pass through for the fishes

## Results

6 fishes from the Trave and 9 fishes from the Rhine were tested for their salinity preference. All fishes during this experiment shuttled between the tanks. All fishes survived the experiment, but especially those that were exposed longer to high salinities were weak and needed some time to recover.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Trave River

Trave - Fish 1

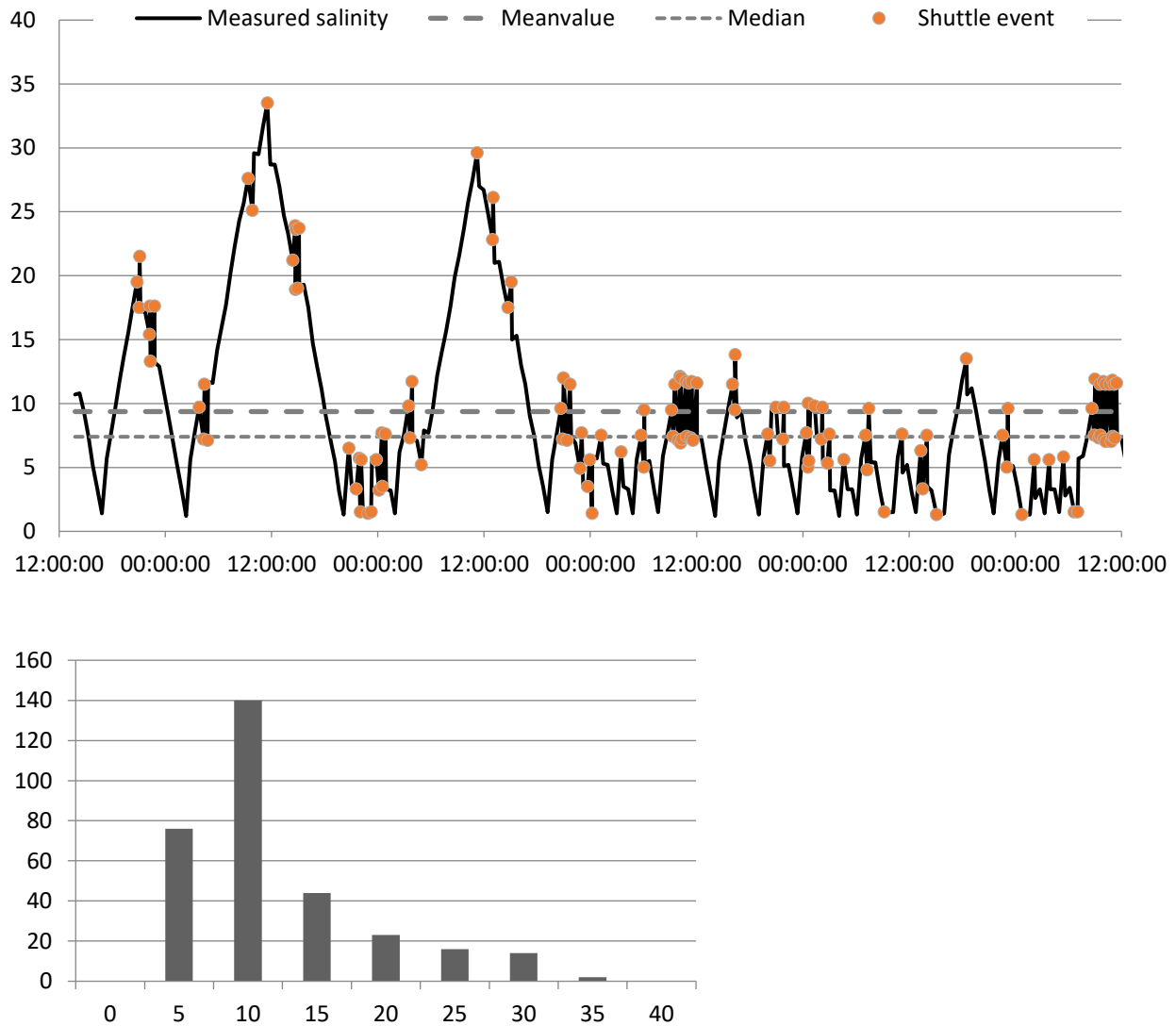


Fig. 4.4 Results of Trave fish no. 1 - upper graph chosen salinity vs. time, points indicate shutteling events, lower graph frequency of chosen salinity. (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The first Trave fish (Fig.4.4) shuttled multiple times and managed to control the salinity effectively. Mean salinity was at 9.348 PSU and median at 7.4 PSU. Besides two times at noon on the 2<sup>nd</sup> and 3<sup>rd</sup> day, there

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

were no peaks in salinities above or near 30 PSU. Also, salinities higher than 20 PSU did only occur in one peak. The fish stayed most of the time in a salinity range between 5 and 10 PSU.

*Trave- Fish 2*

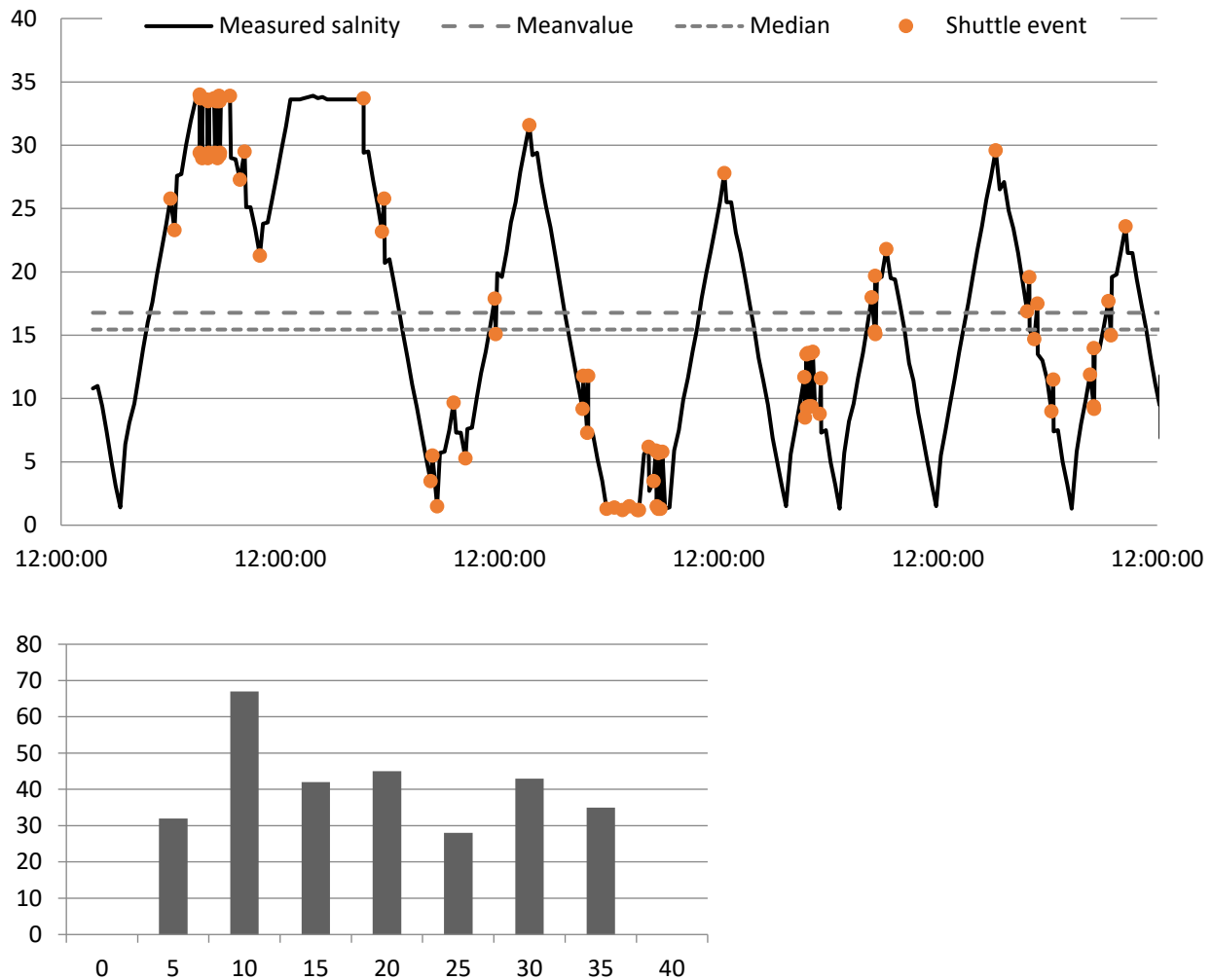


Fig.4.5 Results of Trave fish no. 2 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The second fish with a Trave origin showed a different behavior than the first one. Mean salinity was at 16.77 PSU and median at 15.45 PSU. The salinity was often in a range above 20 PSU and very frequent also in a range above 30 PSU. This fish nevertheless was most frequently exposed to 10 PSU

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Trave Fish 3

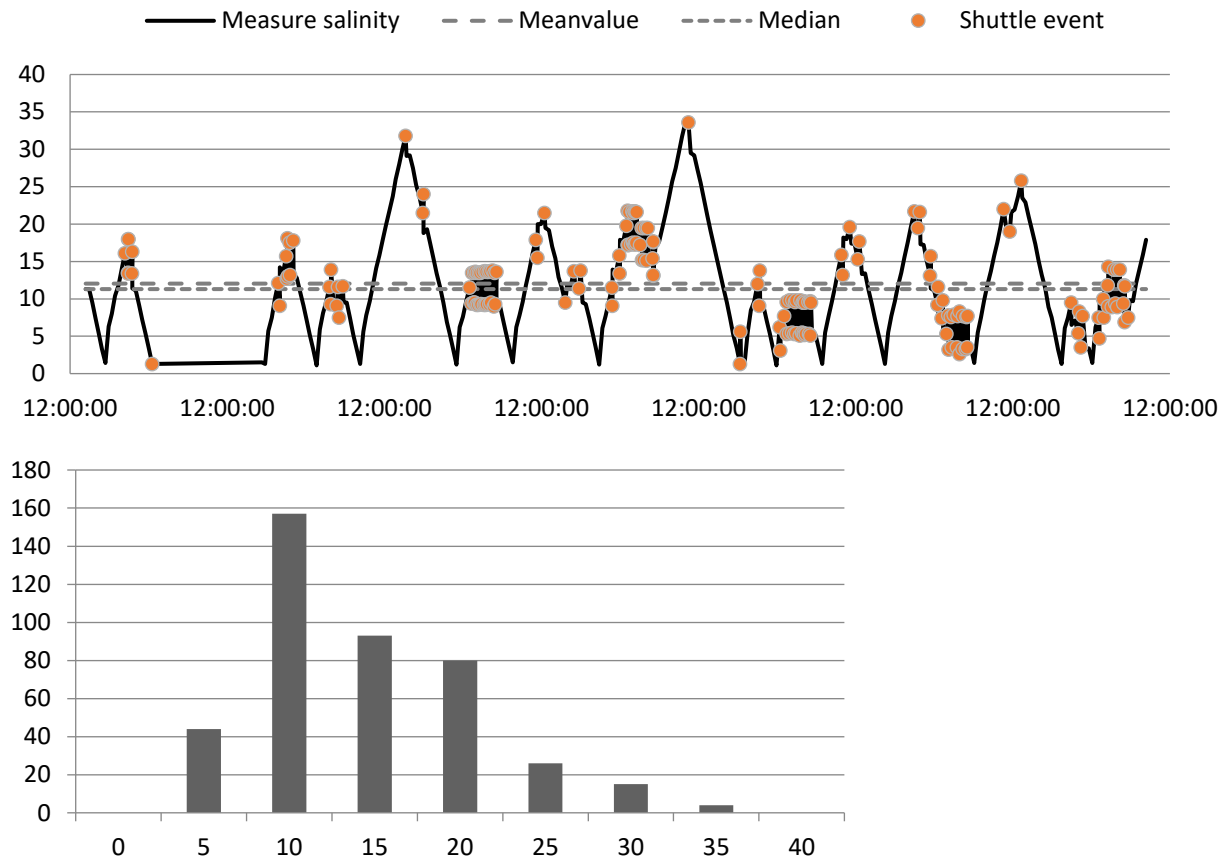


Fig. 4.6 Results of Trave fish no. 3 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The 3<sup>rd</sup> fish managed to maintain the salinity most of the time below 20 PSU and showed like the first one only two peaks in the salinity range of 30 PSU. Mean salinity was at 12.04 PSU and median at 11 PSU. The highest number of salinity measurements was again observed in a range of 10 PSU followed by 15 and 20 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Trave – Fish 4

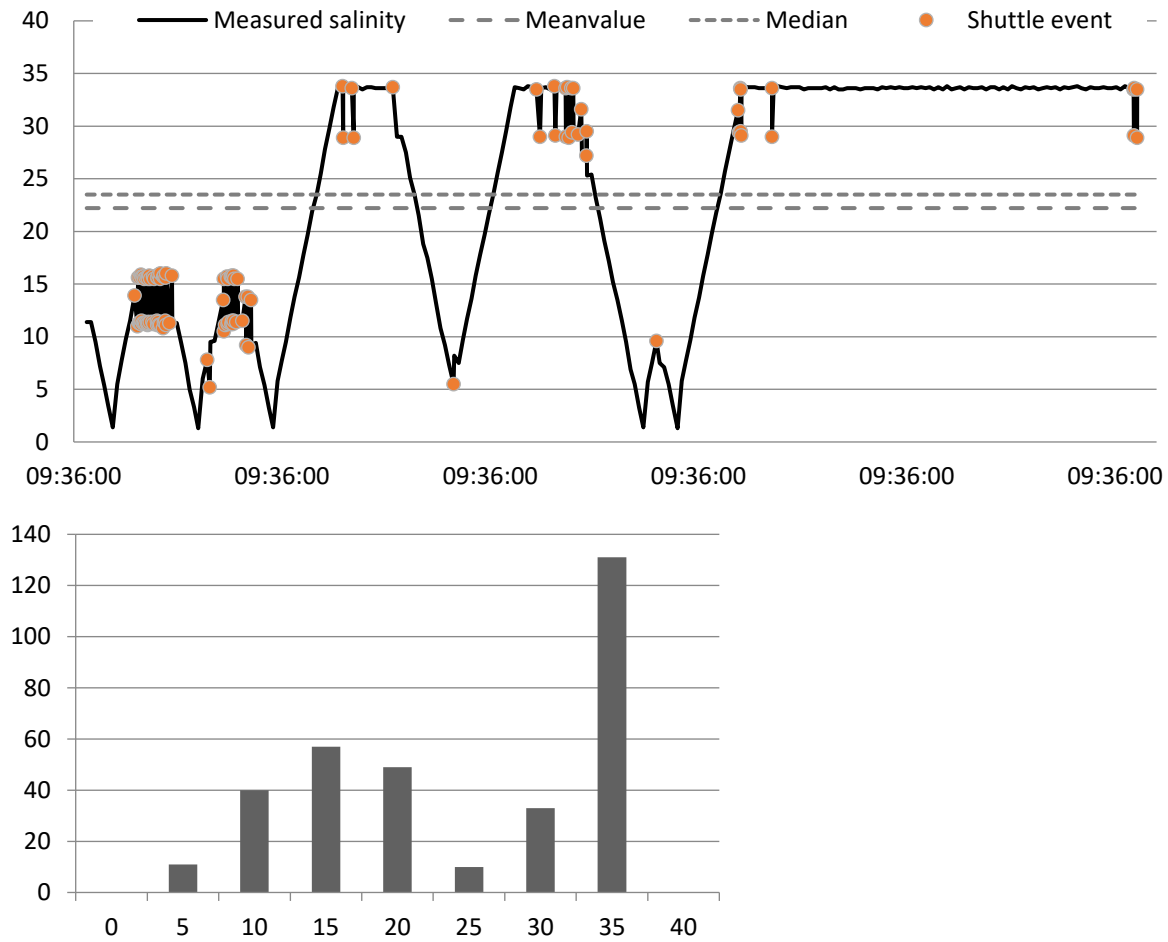


Fig. 4.7 Results of Trave fish no. 4 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The fourth Trave fish managed to maintain the salinity below or in the proximity of 15 PSU within the first 24 hours, with a high amount of changes between the chambers. After this period, the amount of changes decreased and the salinity was most of the time, besides three peaks, above or around 30 PSU. Mean salinity was at 22.22 PSU and median at 23.5 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Trave Fish 5

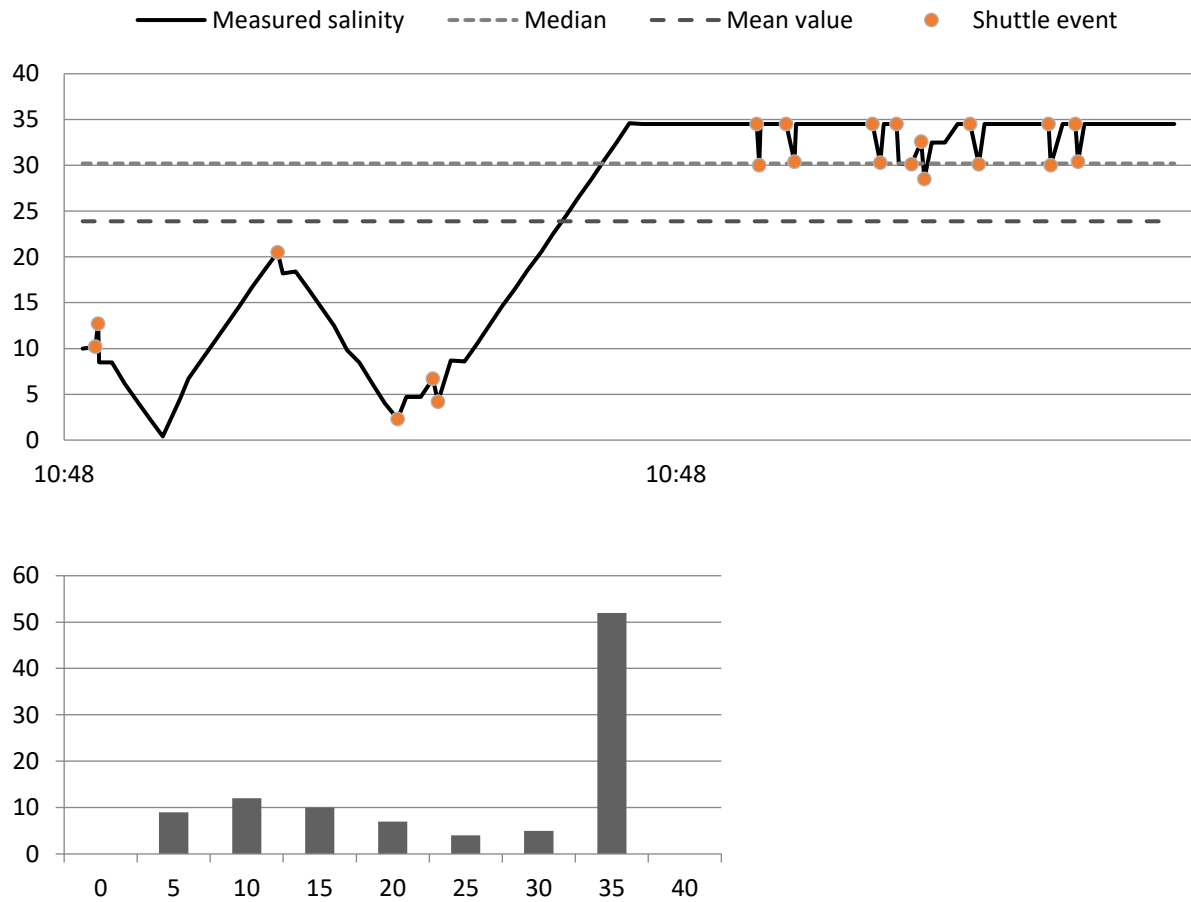


Fig. 4.8 Results of Trave fish no. 5

upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The fish no. 5 with a Trave origin shuttled sometimes, maintaining a salinity below 20 PSU in the -first hours. After a journey to high salinities above 30 PSU, the fish shuttled multiple times but stayed not long enough in a low salinity tank to regulate the salinity back down. Mean salinity was at 23.9 PSU and median at 30.2 PSU.



Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Trave Fish 6

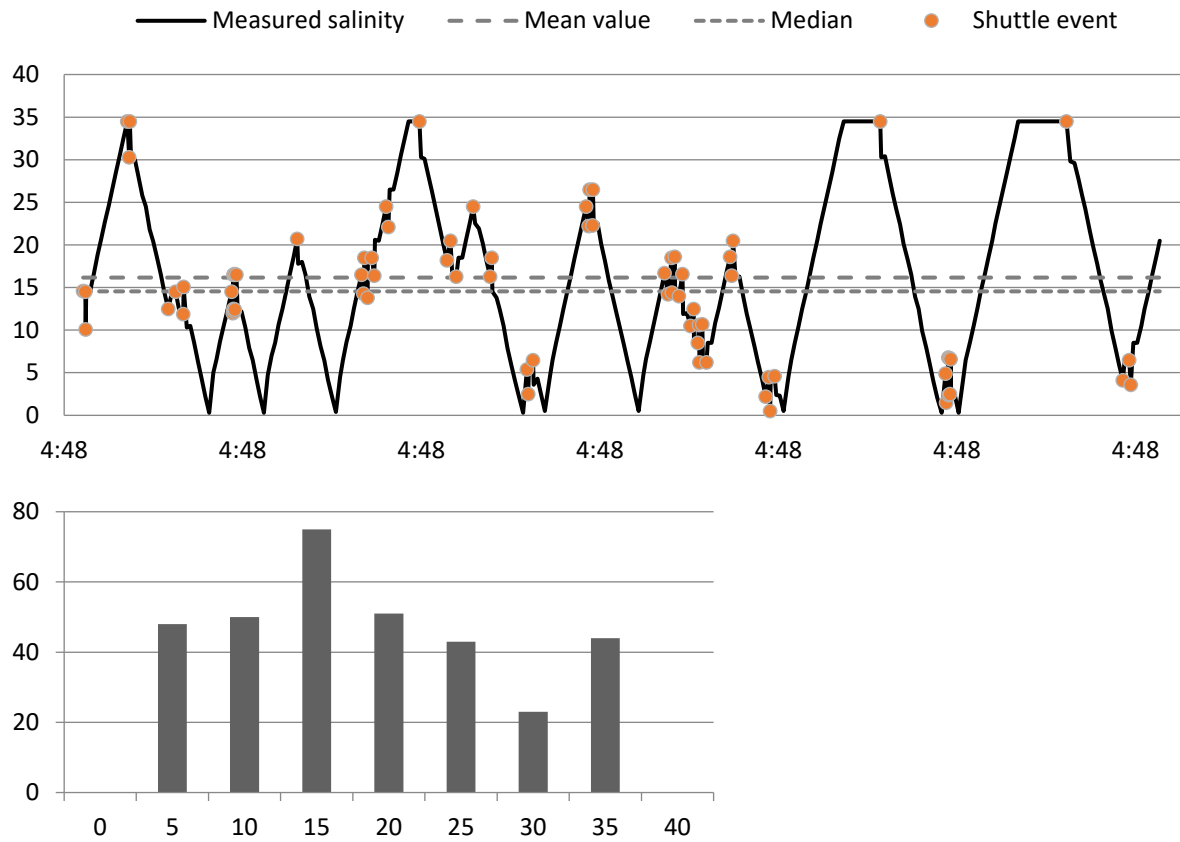


Fig. 4.9 Results of Trave fish no. 6 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The Trave fish no.6 was often exposed to the salinity change from 1 to 35 PSU but controlled now and then in-between the salinity, mostly below 20 PSU. The all-over most frequent measured salinity was 15 PSU followed by 10 and 20 PSU. Mean salinity was at 16.17 PSU and median 14.55 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

## Rhine River

9 fish with a Rhine origin were tested. All fish shuttled between the tanks, but show different success in controlling the salinity.

### *Rhine Fish 1*

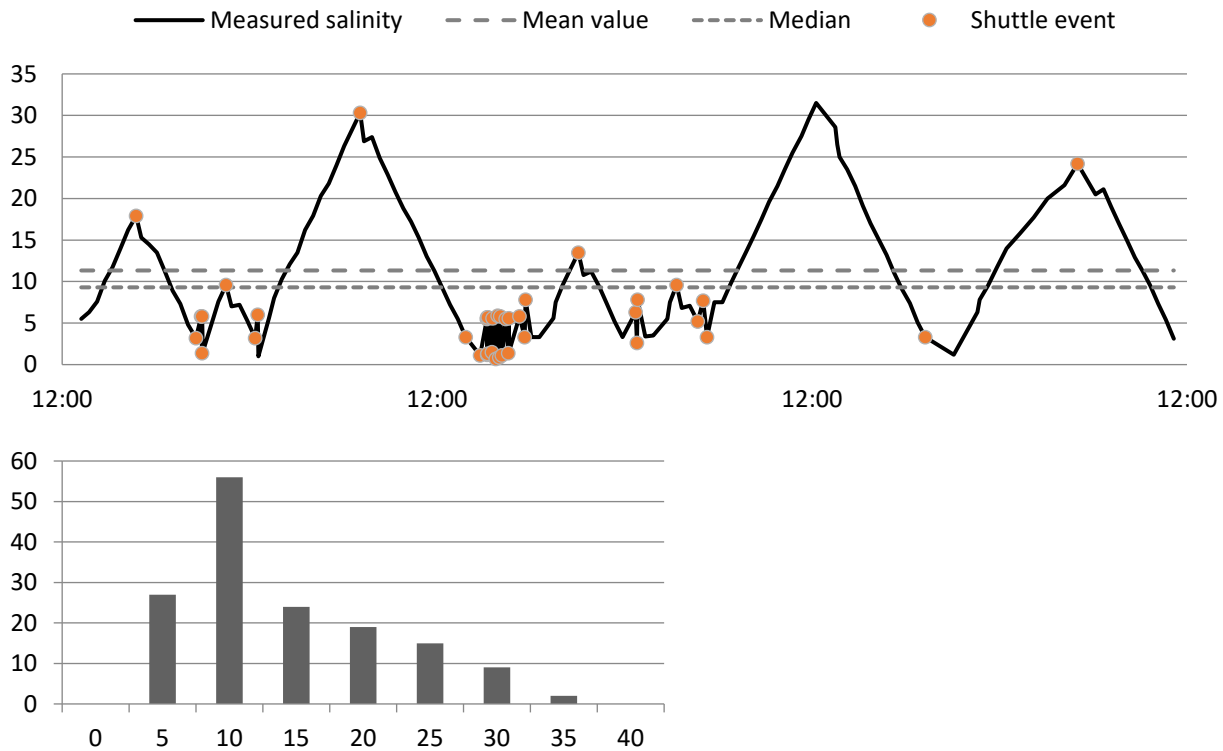


Fig. 4.10 Results of Rhine fish no.1 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The first tested Rhine fish managed to keep the salinity most of the time below 20 PSU, only 3 peaks were higher. The frequency of measured ambient salinity shows that the fish prefer salinities around 10 PSU. From 152 ambient salinity measurements only 26 times the salinity was higher than 20 PSU. Mean salinity was at 11.33 PSU and median 9.3 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine Fish 2

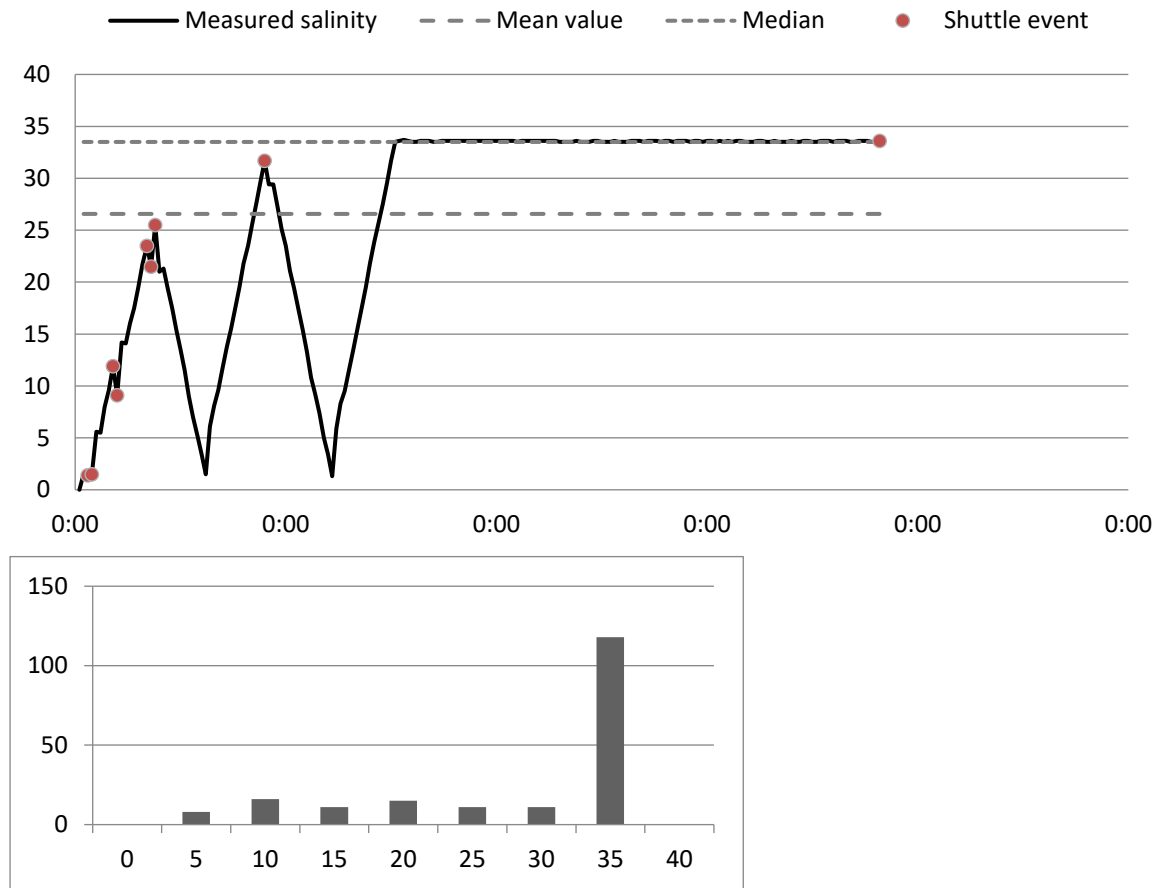


Fig. 4.11 Results of Rhine fish no.2 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The second round goby from the Rhine did not show a behavior controlling the salinity although some shuttles between the tanks take place. This fish traveled through the salinities several times and ended up at 35PSU. Mean salinity was at 26.57 PSU and median 33.5 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine fish 3

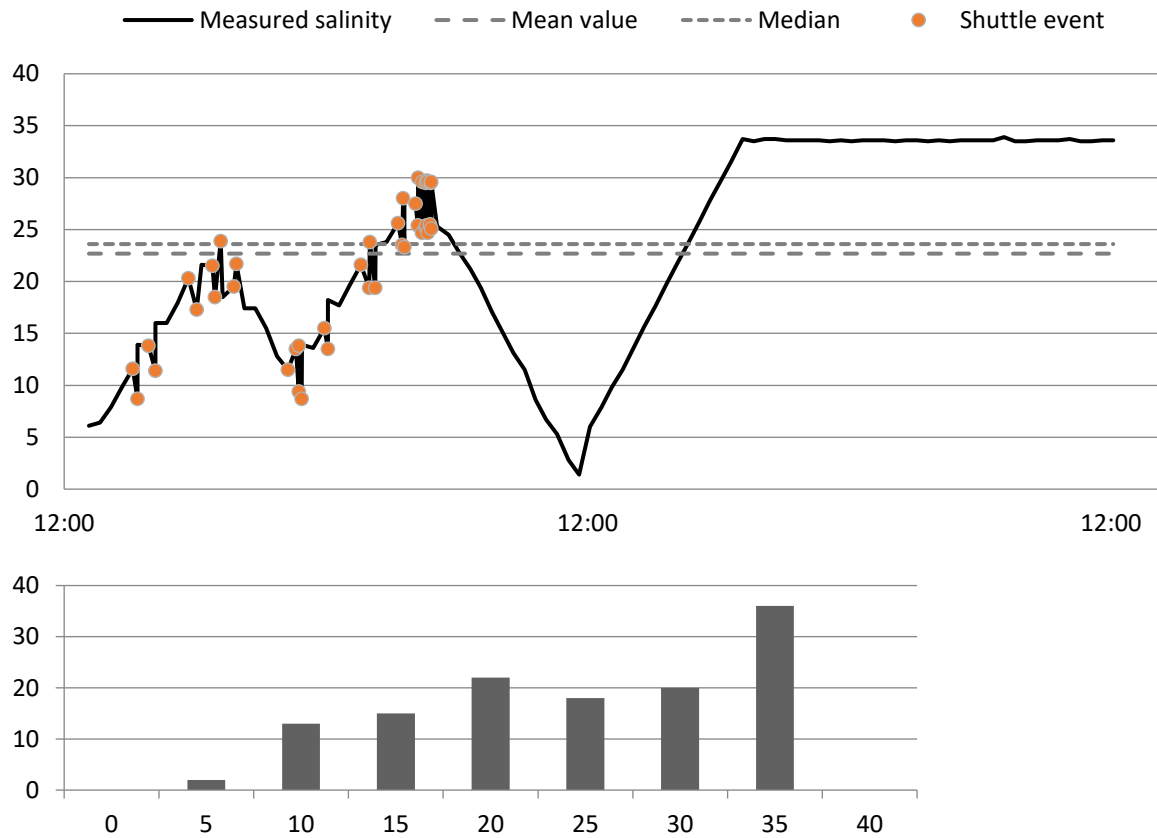


Fig. 4.12 Results of Rhine fish no.3 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

This fish shuttled often within the first hours of the experiment, but with reduced success in controlling the salinity. The most frequent salinity measurements, besides 35 PSU were observed at 20 PSU. Mean salinity was at 22.68 PSU and median 23.6 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 4

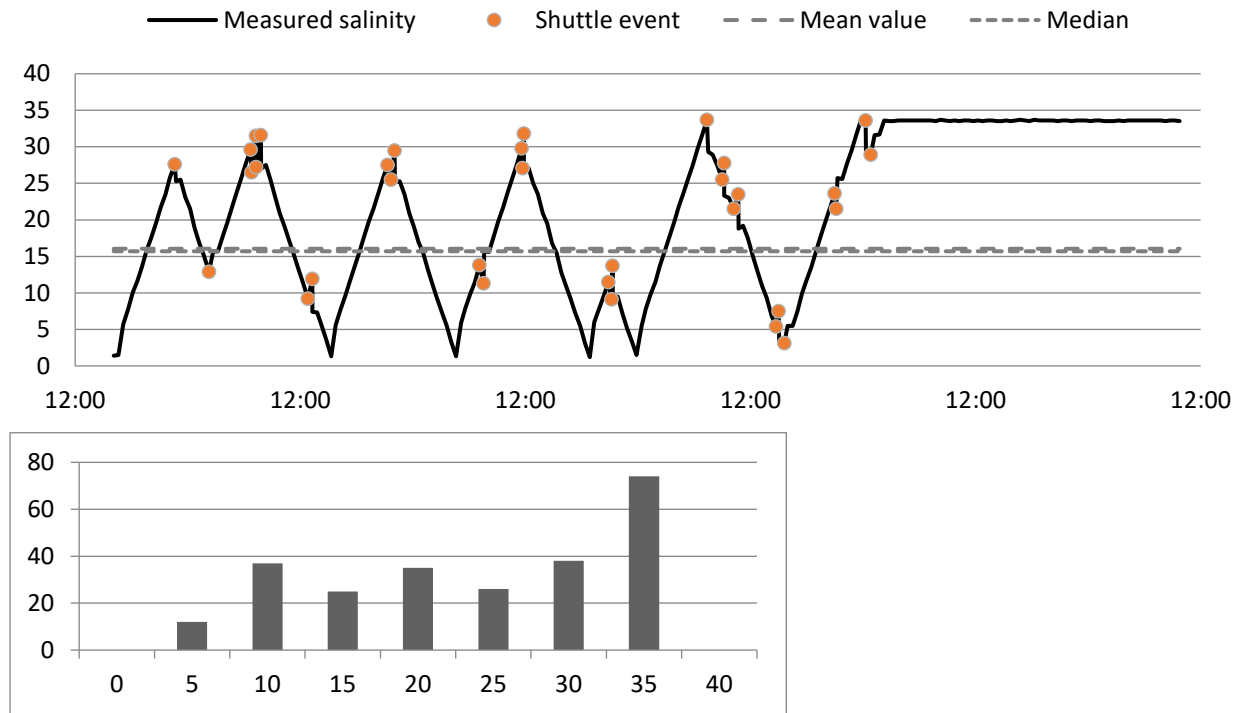


Fig. 4.13 Results of Rhine fish no.4 - upper graph is chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The fourth fish from the Rhine took nearly 5 journeys from fresh to saltwater by shuttling between the tanks. In the end, it stayed in the higher salinity tank. Nevertheless due to the two peaks between 10 and 15 PSU, besides 30 and 35 PSU, the most frequent ambient PSU was the 10 PSU group. Mean salinity was at 16.04 PSU and median 15.7 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 5

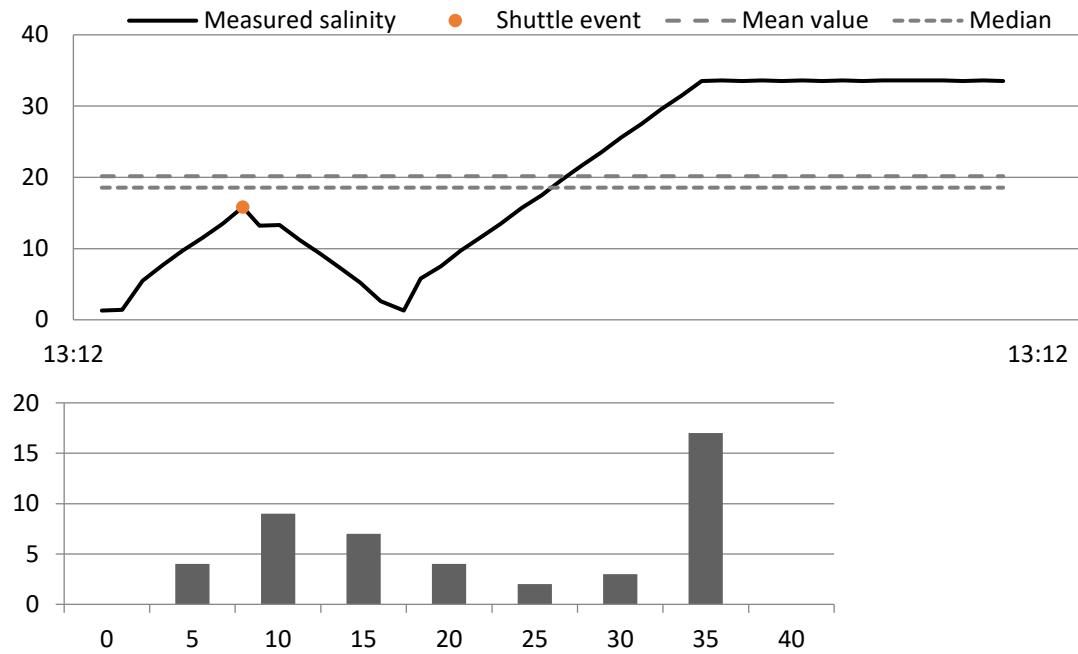


Fig. 4.14 Results of Rhine fish no.5 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The fifth fish from the Rhine showed a very limited shuttle behavior. After 5 shuttles, it ended up in 35 PSU water. Mean salinity was at 20.15 PSU and median 18.55 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 6

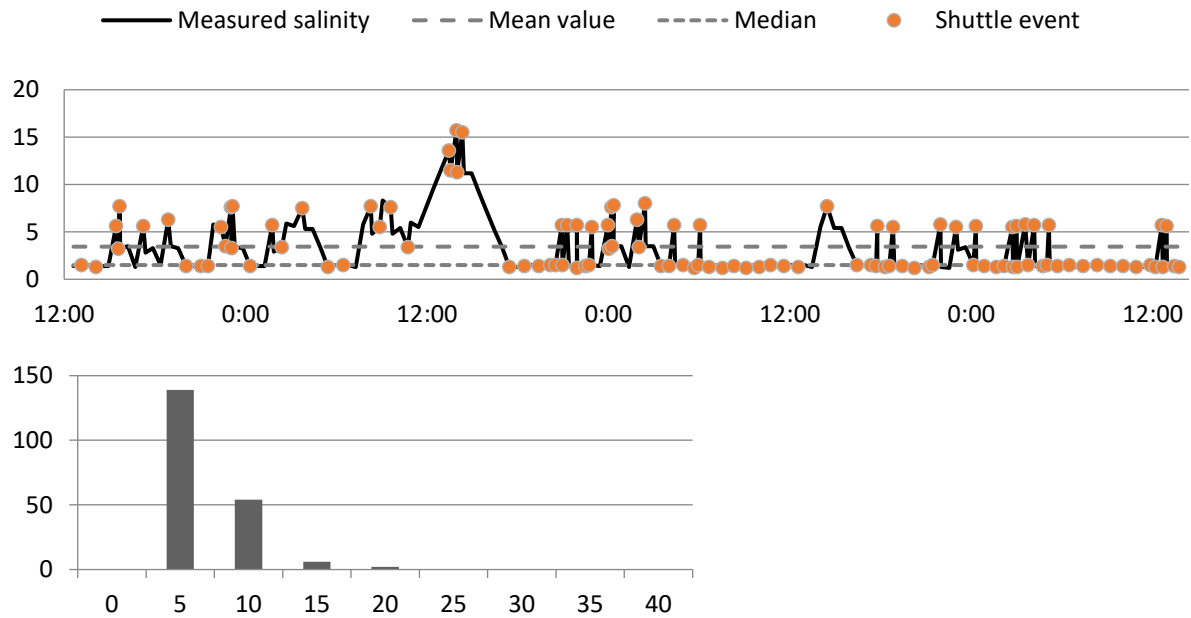


Fig. 4.15 Results of Rhine fish no.6 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

This fish controlled the shuttle box very efficiently. It shuttles frequently between the two tanks and managed with it to keep the salinity most of the time between 0 and 5 PSU only followed by the 10 PSU group from 5 to 10 PSU. Only two times the fish was in salinity above 15PSU. Mean salinity was at 3.44 PSU and median 1.5 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 7

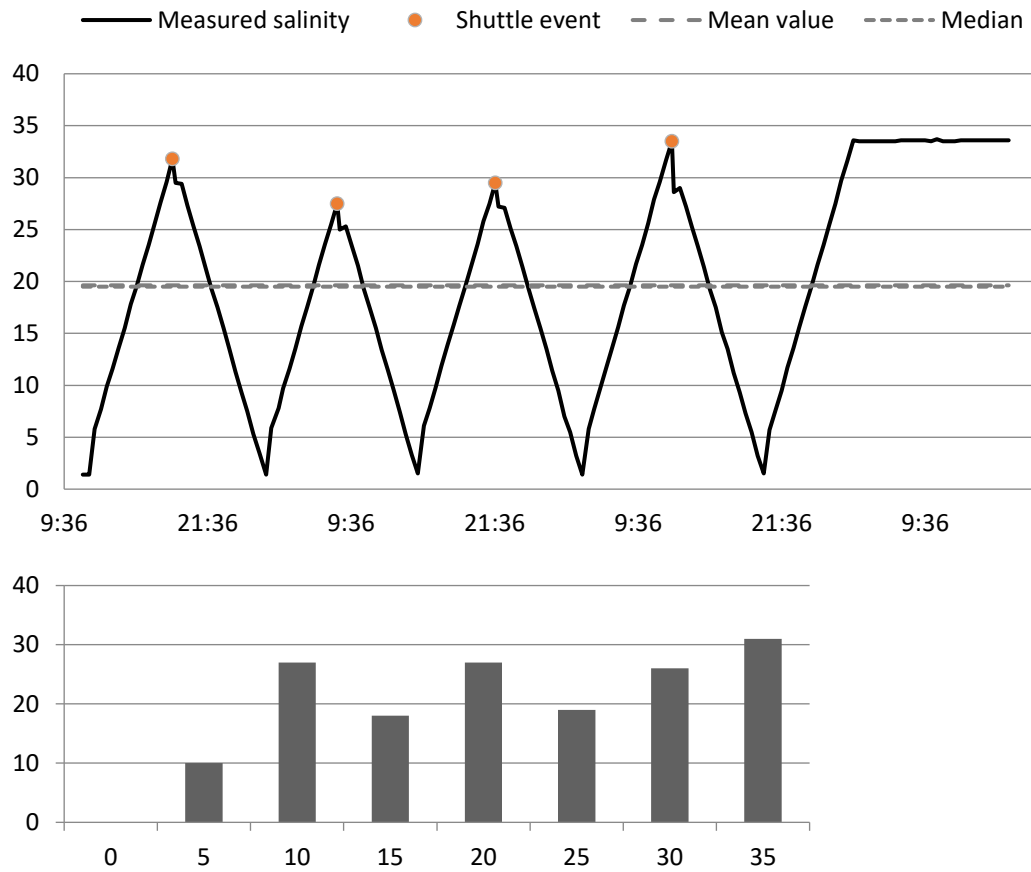


Fig. 4.16 Results of Rhine fish no. 7 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The seventh Rhine fish only shuttled only in extreme situations at 0 and 35 PSU, at the end it stopped at 35 PSU. Mean salinity was at 19.63 PSU and median 19.5 PSU.



Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 8

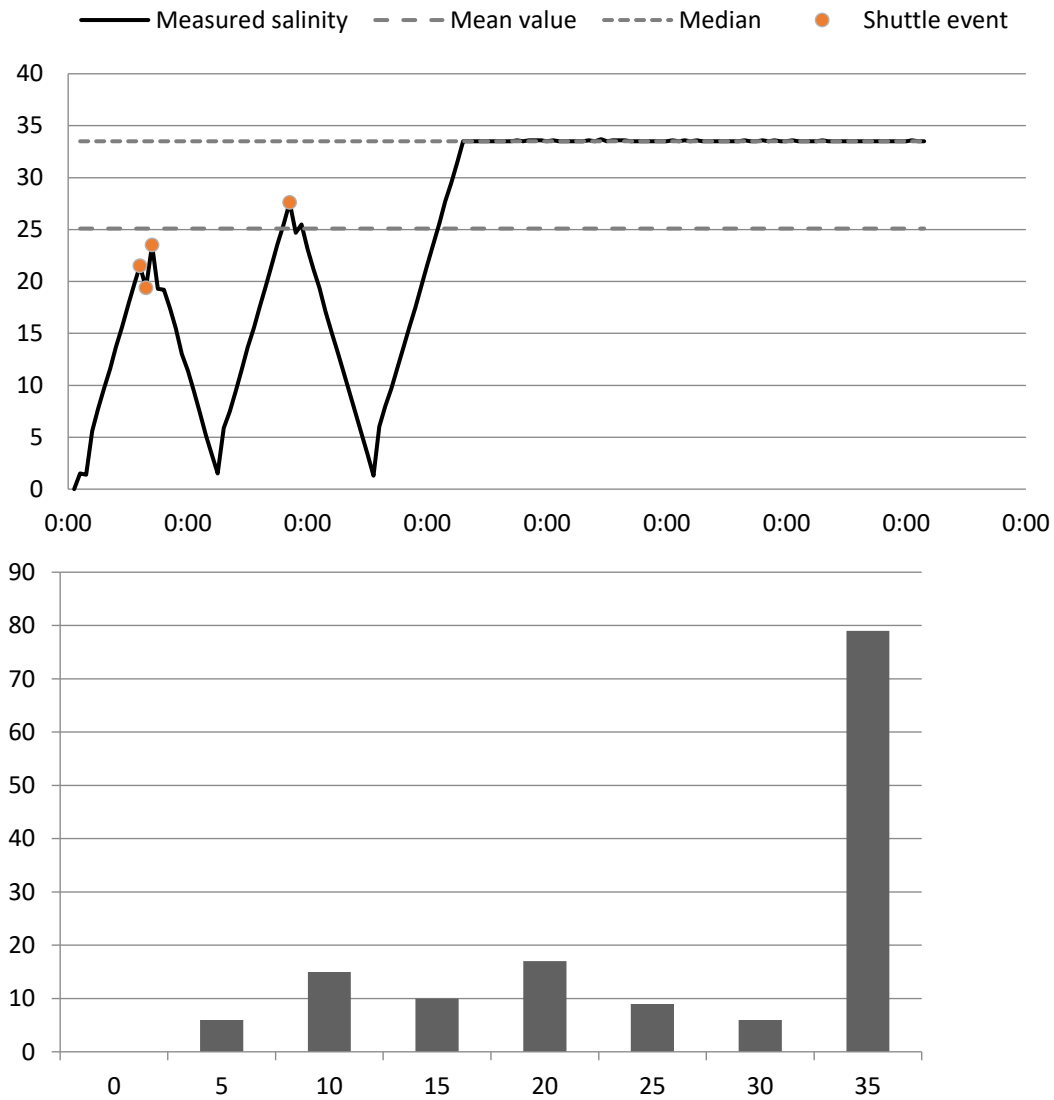


Fig. 4.17 Results of Rhine fish no.8 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The 8<sup>th</sup> fish also shuttled limited times and ended up in 35 PSU without further shuttle attempts. Mean salinity was at 25.09 PSU and median 33.5 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Rhine 9

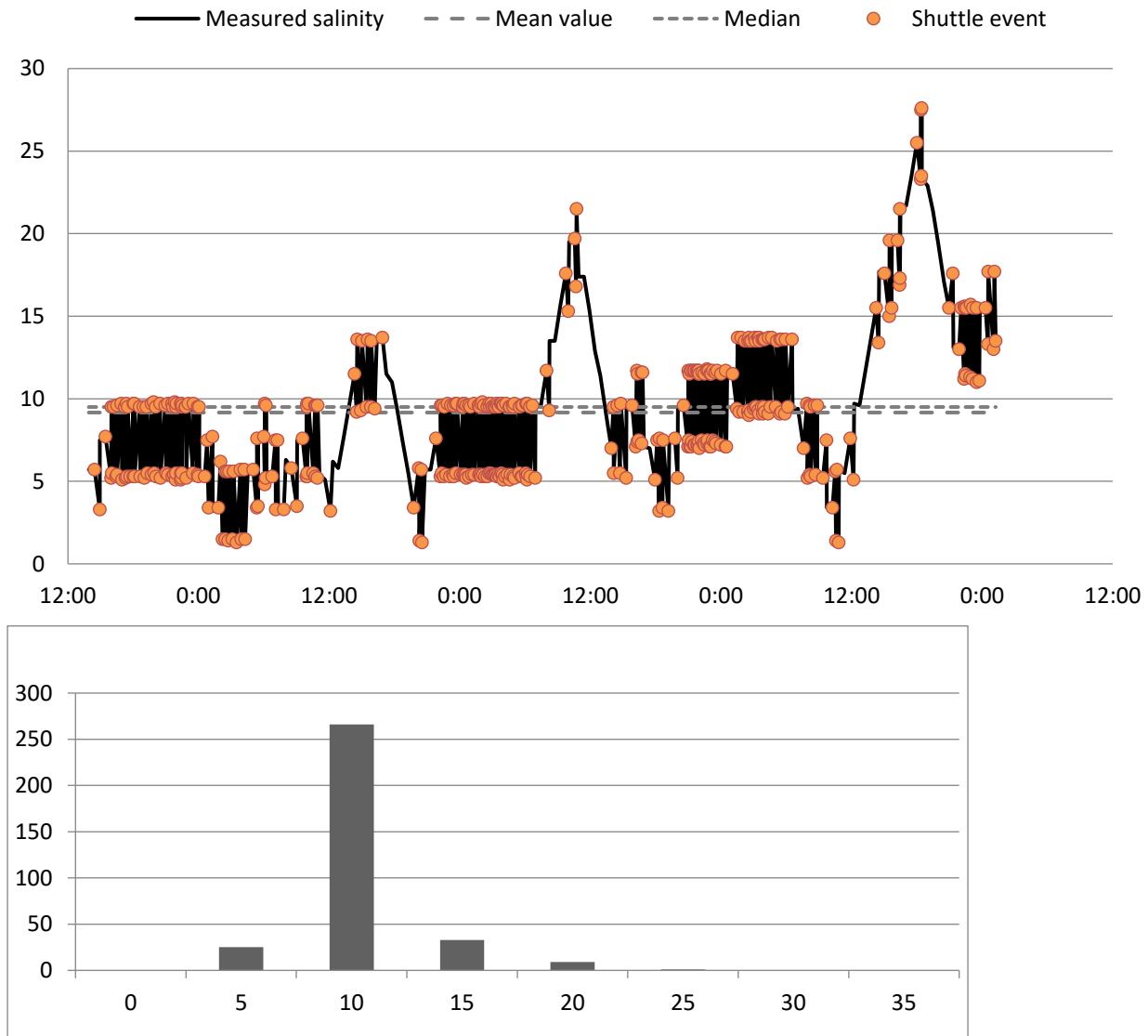


Fig. 4.18 Results of Rhine fish no.9 - upper graph chosen salinity vs. time, points indicate shutteling events, lowergraph frequency of chosen salinity, frequency~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...)

The last Rhine fish showed a successful shuttle behavior. It managed to keep the salinity very most of the time between 5 and 10 PSU. Followed by staying in the range between 10 and 15 PSU and 0 to 5 PSU. A very limited time it stayed in ambient salinities above 15 PSU. Mean salinity was at 9.16 PSU and median 9.5 PSU.

Salinity preference of round goby (*Neogobius melanostomus*)  
an experimental approach with fresh- and brackish water origin fish

Combined results

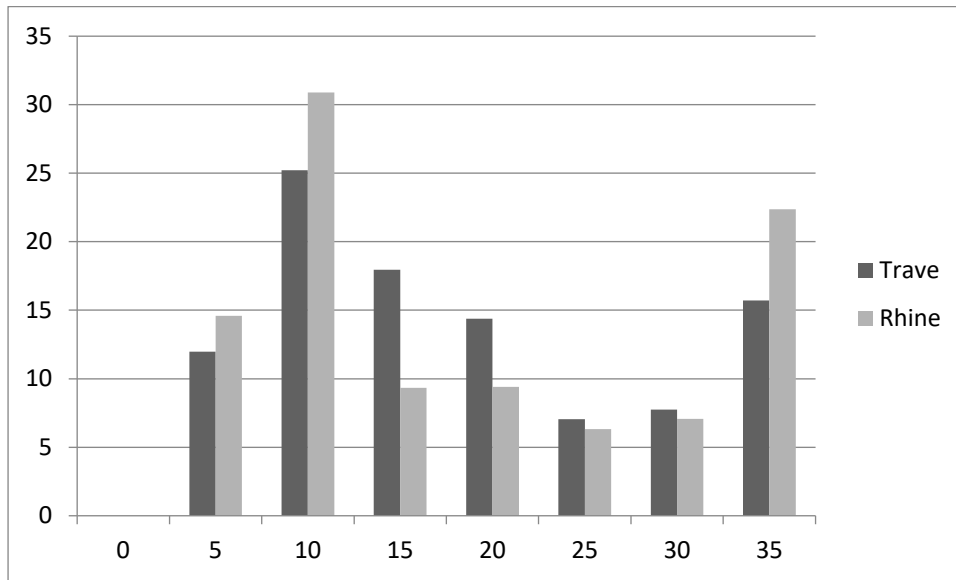


Fig.4.19 Combined results, frequency [%]~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...) overall tested fishes, grouped by Trave and Rhine.

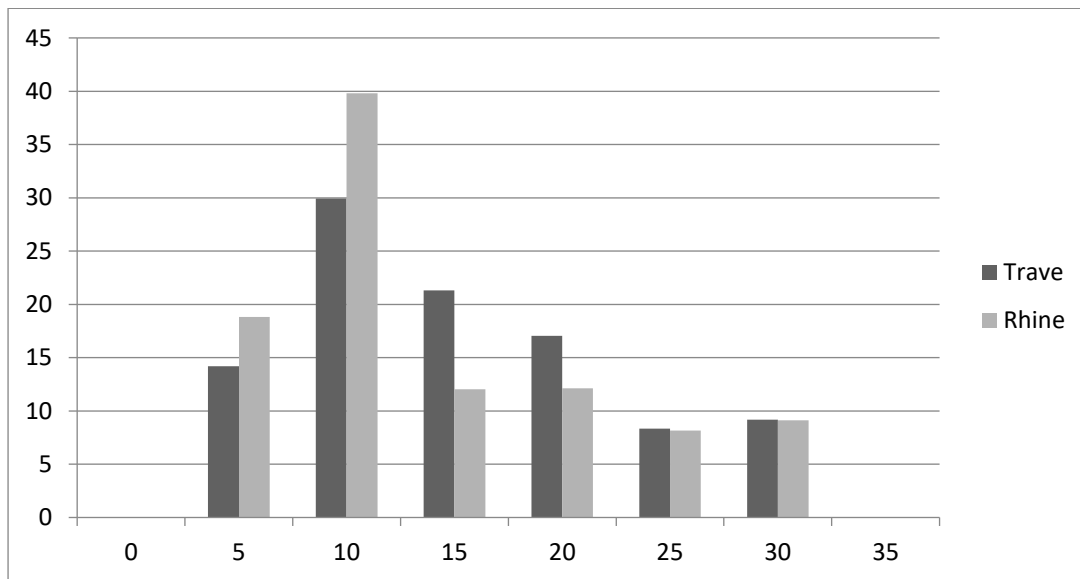


Fig.4.20 Combined results without 35 PSU, frequency [%]~ salinity (Frequency in groups. 0=0 | 5=0.1-4.9 | 10=5-9.9 | 15= 10-14.9 | ...) overall tested fishes, grouped by Trave and Rhine.

The combined data revealed a similar pattern in salinity preference for the two origins. Both showed the highest frequencies for the 10 PSU salinity group (5-10 PSU). In the group of Trave fishes, this is followed by 15 PSU, in Rhine fishes by 35 PSU, which is for Trave fish the 3<sup>rd</sup> most frequently measured salinity group, followed by 20, 5, 30 and 35 PSU. The Rhine fish choose as 3<sup>rd</sup> most frequent salinities between 0 and 5 PSU, followed by the 15 and 20 PSU groups. The salinity groups 30 and 25 PSU were as for the Trave fish rarely encountered.

If the 35 PSU group is excluded from the results, since most of the measurements were generated due to fish that stopped shutteling (Fig. 4.20), the picture becomes clearer. Nearly 40% of measurements of the Rhine fish were in the 10 PSU group. For Trave it is also the most frequent group with 30% of the events in this salinity class.

## Discussion of Method

The results show that not all fish were able to control the system. This might be caused by the setup of the experiment. The fish need to actively explore the tanks and to “understand” that the connection between the tanks is the link between different salinities. Nevertheless was this setup considered to be adequate, since there are only few alternative methods to test the preference of salinity in a laboratory environment. Some of the methods are based on currents with different physical properties, which are used multiple times for different experiments to avoid unpleasant conditions e.g. (Shelford and Allee, 1913; Chidester, 1921; Cherry and Cairns, 1982; Gerlach and Lysiak, 2006). In this approach, the fish must constantly swim against the current, which consists of several parallel currents next to each other, each with different conditions. The fish can therefore decide between these by changing position. These current based systems would most likely cause problems in experiments with round goby. Round goby can maintain a swimming speed over longer periods of 35.5cm/s and burst >75cm/s (up to 30sec) (Burger, 2019) which might be sufficient for such an experiment. However, Gobidae can also hold on substrates, some Gobidae even can climb up waterfalls with their fused pelvic fins, that function as a sucker (Schoenfuss and Blob, 2003; Blob *et al.*, 2006). This pelvic fin adaption exists across Gobidae and will possibly have an effect on the behavior in a current-based system. The fish can save energy in a metabolic critical environment like high salinities by attaching to the ground. This would influence results in a hardly controllable way.

Other systems use the different density properties of saltwater, whereby layers of different saline water are generated that do not mix due to density differences. These systems are probably also not ideal since round goby is a bottom-living species that seldom swims into the pelagic. Therefore the fish will stay most of the time in the bottom area and will not experience the differences in salinity between the different water layers and consequently not learn about the opportunity to avoid unpleasant conditions.

The here used shuttlebox system was equipped with a curtain, that was used in the setup to reduce water exchange between the two chambers. The curtain could possibly influence the willingness of the fish to shuttle. However in our pre-tests multiple round goby swam through the curtain multiple times without hesitation without any other disturbance or salinity changes and therefore no external reason to shuttle. Therefore and because all goby in the experiments shuttled it is assumed that the curtain did not have any or only very minor effects on the result. Hence the system was considered to be the most promising one.

As mentioned before one of the Rhine fish was exposed to another program controlling the shuttlebox. This was erroneously started. Nevertheless, this fish did not behave differently than the other fish, since the fish only once stayed long enough in the threshold salinities to start the automatical change of the salinity differences between the boxes. In pre-tests, this program led to a passive behavior of the round goby. The fishes stayed in one tank and suffered through the unpleasant conditions. Since the Rhine round goby did not show this passive behavior the data were considered to be comparable with the others and were included.

The time frame of 30 min for the change of salinity was chosen for two reasons. Longer periods increase the water consumption, which is a logistical and also financial issue. A shorter period would stress the metabolism of the fish more and the metabolism of the fish might be unable to cope with the rapid changes of salinities. This would possibly lead to the fish becoming unable to control the system as was also observed in a number of individuals even in the 30 min interval setting. Hence since the 30 min worked in pretests this period was applied in the experiments.

## Discussion of Results

The results show that some fishes were able to control the salinity within the tanks by shuttling and with it maintain the salinity in an area that is assumed to be physiologically optimal. The high numbers of 35 PSU salinity measurements are assumed to be an artificial effect, that can not be assessed as salinity preference, but was instead caused by a stop of shuttling. Without the fishes that stopped shuttling the frequency of high saline measurements would be much lower. With the 35 PSU salinity class included 44.87 % Trave and 45.18 % Rhine measurements were above 20 PSU. Without 35% PSU class 34.59% of the Trave and 29.38% of the Rhine fish measurements were above 20 PSU. It is most likely that the stop of shuttling in high saline water is caused by metabolic stress. Investigation on grey snapper with a preferred salinity of 9-23 PSU revealed reduced activity in extreme salinity >30 PSU (Serrano *et al.*, 2010), which might also be the case for round goby. Findings of salinity-dependent survival of round goby (Karsiotis *et al.*, 2012; Hempel and Thiel, 2015) support this interpretation. Hence the stress due to the quick salinity changes seems to affect the fishes with a freshwater origin to a higher degree than those with a brackish water background. The cause of these differences might be related to osmoregulation. Behrens *et al.*, (2016) showed that osmolality, in brackish round goby, is relatively constant from 0 till 20 PSU with a slight but insignificant increase at 25 PSU. However, above 25 PSU the osmolality increased significantly. Therefore, the amount of energy for maintaining the osmolality increases, leading to a decrease of the aerobic scope and with it the available energy to shuttle. The fish with a brackish origin chose in 69,5% a salinity of 20PSU or below. This is also true for the Rhine fishes, but those choose 4.94% less often salinities between 15 and 20 PSU, hence show a higher tendency to salinity below 15 PSU. Unfortunately osmolality data for freshwater round goby are not available. Nevertheless, these results point to differences in osmoregulatory capacity. Similar results were achieved in investigations of perch from different salinity habitats. Those with a brackish water origin tolerated salinities up to 17,5 PSU whereas in freshwater fish salinity tolerance was substantially lower with 10 PSU (Christensen *et al.*, 2019). Investigations of yellow perch *Perca flavescens* with fish acclimates to two different salinities, one group acclimated to freshwater and one to 10 PSU, revealed slight differences in salinity preference. Freshwater individuals preferred a mean salinity of about 9.7 PSU and brackish ones a salinity of 11.1 PSU (Christensen and Grosell, 2018).

One-third ( 2 out of 6) tested Trave fish stopped shuttling at 35 PSU whereas two-third (6 out of 9) of the Rhine fishes did. This difference could be caused, besides the physiological reasons, by a learned

behavior of the fishes. In the Trave estuarine area, the salinity is changing and round goby can learn to avoid these by changing position, which leads to a trained evasion behavior. In contrast, the Rhine area fish were caught from a homogeneous fresh water system. Hence, the possibility of avoiding of unpleasant salinity is not known to these fishes. This may explain the less efficient avoidance behavior in the shuttle system.

Even if the lack of experience with a salinity avoidance behavior is possibly affecting the results it became quite obvious that round goby from freshwater origin do not prefer freshwater habitats. This is congruent to the results of Behrens *et al.* (2016) who identified an optimal metabolic range at 10 PSU for round goby. Further is this supported by different maximum lengths of round gobies depending on their habitat. Freshwater goby are reported to be smaller in length with a maximum length of 117mm (Thompson and Simon, 2015) whereas in brackishwater lengths up to 235mm are reported (Sokołowska and Fey, 2011). This supports the assumption, that freshwater is not optimal and that osmoregulation is expensive with the consequence of reduced energy for growth.

## Comparison with other shuttle box experiments

The application of shuttle boxes for the determination of salinity preference is limited. Unlike temperature preference setups the water needs to be dumped after usage, only a very complex tank system with tanks for each salinity might allow reuse. If such a system is not realized a lot of saline water needs to be available. This is feasible for small species with small tank sizes, but nearly impossible with bigger species and large tanks. The salinity preference test on yellow perch *Perca flavescens* was performed with a setup similar to ours (Christensen and Grosell, 2018). The main difference is a longer junction pipe between the two tanks in the perch setup. If a fish stays in this pipe the salinity remains stable and does not change. The perch spent up to 33% the time in this junction zone and were able to maintain the salinity in a preferred range without the necessity to shuttle.

Research on grey snapper (Serrano *et al.*, 2010) was conducted with a similar shuttle box setup including a long junction zone. The species in this experiment seemed to control the box in a more explicit way, the extreme measurements were less often found. This could be either species-dependent or such a longer junction zone could lead to more distinct results since a preferred salinity can be “locked” by the fish. If such a longer junction pipe were also used in the system for round goby, it could help to identify more precisely the preferred salinity range. However, since round goby is a bottom-living species that

inhabit caves, a certain risk exists that round goby would possibly just “sit” in the junction zone and ignore the experimental set-up.

Another investigation on sailfin molly *Poecilia latipinna* uses a shuttle box system to investigate salinity preference in combination with antipredator behavior (Tietze and Gerald, 2016). In this experiment, the fish has only the choice of fresh or saltwater with a fixed gradient and additional to chemical cues. The result show that the fish favour the predator avoidance over osmoregulatory demands. A setup that uses only 2 different salinities would be useful as a consecutive experiment to determine more detailed results by enforcing a choice between two distinct salinities, for example, salinity that was most frequent in this experiment.

## Conclusion

The results hint at a slight difference in salinity preference depending on origin. The data also showed that fish with a freshwater origin were limited in controlling in comparison to fish with brackish water origin. Hence the fishes are adapted to the habitats somehow. The chosen system worked with constraints for round goby. For a better data quality, a longer junction zone could be helpful. Subsequently consecutive experiments should use a more limited salinity range to identify smaller differences in salinity preference between populations of the different habitats.



---

## General Discussion

This thesis was started under the umbrella of the research project BIO-C3. The three c's represent; changes, causes and consequences in the Baltic Sea. One of the changes within the shorter history of the Baltic Sea was the introduction of the invasive round goby *Neogobius melanostomus*. Since its first appearance, its distribution has increased. As illustrated in figure 1.2 from Kotta *et al.*, (2016) the number of reports of new gobies increased considerably, especially from 2010 onwards. Multiple questions were addressed within this BIO\_C3, also concerning the effects of salinity and its influence on the spatial distribution of *Neogobius melanostomus*. Since only limited knowledge about salinity influence on round goby was gathered from field observations, the here presented experiments were conducted to better understand the influence of salinity on the hatching success and the ability to grow in brackish environments.

### Salinity affects the reproduction of round goby

The influence of salinity on the reproduction of round goby begins with the path of sperm to the egg and the fertilization process. Many organisms use internal fertilization for high fertilization success. Round goby, similar to many other aquatic organisms, employ external fertilization. The sperm of round goby is influenced in velocity and motility by salinity. Moreover, does the effect of salinity vary across locations with higher and lower salinity (Green *et al.*, 2019). Thus, salinity influences reproduction even before the actual fertilization process has begun. This part was not investigated in this thesis. However, it must not be disregarded, since this can already make fertilization of the eggs impossible.

Furthermore, the reproduction behavior is affected by salinity. Round goby males show two different fertilization behaviors (Corkum *et al.*, 1998). Normal males (type 1) are large and invest more in growth than in reproduction. They defend their territory, court females, have increased sexual hormone concentrations, and show secondary sexual characteristics. Whereas the type 2 male is smaller, invests more in reproduction than in growth and shows no secondary sexual characteristics. Instead of courting females, they add their ejaculate to eggs in burrows that are watched by other males, slipping in quickly while the guarding male is careless, or imitating the appearance of females to gain entry (Oliveira *et al.*, 2008). This "sneaking" behavior could possibly be less efficient if, due to increasing salinity, the

swimming performance of the round goby decreases. This could further have an influence on the fertilization of eggs. Assuming that 100% fertilization of eggs is more likely by insemination of two males, a reduced success of sneaker males could hypothetically have an impact of the overall reproduction.

Recent investigations found that round goby sperm from freshwater origin behave differently than those from a brackish environment. In freshwater, a decrease in sperm velocity and viability was found. Freshwater males were bigger at same age with higher energy reserves, and had a lower gonosomatic index (GSI), than those in a brackish environment. Hence the freshwater males individuals invest more into growth until a size that enables to be a good nestholder (Green *et al.*, 2020). This emphasizes the role of salinity in reproduction of round goby and highlights that a suitable environment is crucial for the survival of species in newly colonized areas via influences in the very early stages of reproduction. Hence to guarantee a successful reproduction salinity needs to be in a suitable range at spawning, egg maturation, -hatching as well as growth in the early life stage.

With a view on the salinity map of the northwestern Baltic Sea (Fig. 4.1), in combination with the results of the previous chapters, some points become obvious. In chapter two it was shown that the highest hatching success can be expected in salinities of 15 PSU and below. In 20 PSU the hatching success was less than 50% with one outlier. Hence the coastlines of the western Baltic Sea differ in their suitability for successful hatching. Along the German coast the area north of Kiel is characterized by a salinity range where only a strongly reduced hatching success is likely. This is supported by a study on competition between round and black goby in 2014 (Matern *et al.*, 2021), where no round goby were caught further north than Kiel. Hence a spreading further north with successfully established populations is unlikely or will progress only slowly. One way for a successful invasion of these areas would be settle first in river mouths with freshwater influence.

The northeastern coastline of the Swedish mainland and Danish islands Sjaelland and Lolland has a more suitable salinity range. The map in Fig. 4.1 also includes round goby catches from this region (Azour *et al.*, 2015) indicating established populations. Round gobies are also established further north in Gothenburg harbor (Sweden) where salinity is influenced by the river Göta älv (Green *et al.*, 2019).

According to our results is a distribution further north on the German and Danish mainland coast - except from estuarine areas - unlikely. Along the Swedish Kattegat-coastline, considering only the salinity conditions and no other factors like suitable breeding grounds, food availability, predation pressure, the invasion possibly goes further in the northern direction. However, we have observed outliers in our experiments, which were able to cope with higher salinity levels better than others. Should this

phenotype prevail, a spread, albeit slow, towards the North Sea can occur also on the German –Danish coast. Since we observed that no larvae survived 25 PSU for longer periods, the larval stage might be the most critical stage preventing an rapid extension into northern and more saline areas. .

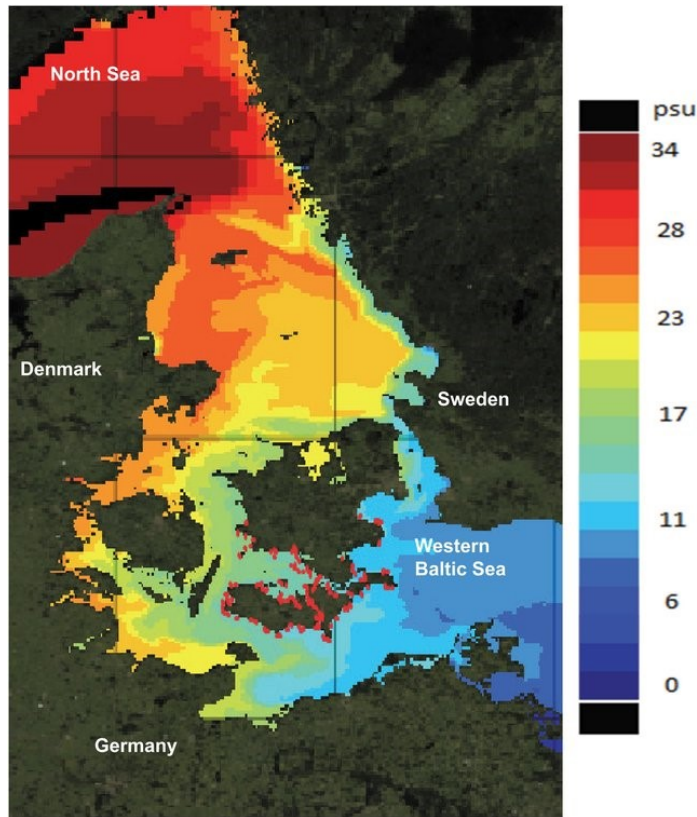


Fig.4.1 Occurrence of round goby (red dots, from Azour et al. as cited in (Behrens et al., 2016)) in the Baltic Sea-North Sea transition zone in relation to salinity at 0-5m depth. Salinity data (yearly mean) are provided by Myocean and are output from the Baltic Sea 3D physical model code HBM (HIROMB-BOOS) with a spatial resolution of 2 km and temporal resolution of 1 hour **copied** from(Behrens et al., 2016)

## Genetic role

The results of the shuttle box chapter showing - although only minor - different salinity preferences of fresh and brakish water round goby suggested that the same species can have a varying preference for habitat conditions. In addition our experiments revealed the existence of “outliers”, e.g. individuals that were better able to cope with extreme salinities.

---

Phenotypic plasticity is the capability of organisms to create different physical or physiological properties from the same genotype to adjust to different environments. This means that the expression of genotypes is different in response to environmental influences. Phenotypic plasticity buffers organisms against environmental change and likely supports initial establishment of non-native species in new environments and range expansion after establishment. For plants, a study indicates that invasive species have greater phenotypic plasticity than co-occurring non-invasive ones (Davidson *et al.*, 2011). Hence it can be assumed that successful invaders, like the round goby, have higher plasticity for adaptation than less invasive species. A comparison between the successful invader round goby and less successful tube nose goby confirmed this. It was found that round goby has a greater response in transcriptional changes on thermal impacts than the tubenose goby (Wellband and Heath, 2017).

The degree of variability depends largely on whether the invasion results from single or multiple introductions (Bock *et al.*, 2015). Investigations on the American round goby population found a very high genetic diversity: almost all alleles of the southern Dnieper River at Khersin, Ukraine do occur in North American samples (Brown and Stepien, 2009) hence indicating a very large number of introduced individuals. This explains at least partially the success of round goby within the Great Lakes. High variation within the start population forms an excellent base for a successful spreading by providing high numbers of fishes with individual characteristics. Hence the probability that some individuals have a variance that fits the local conditions best has been very high.

Investigation on round goby suggest that maternal RNA incorporates environmental information which could be a non-genetic pathway of inheritance (Adrian-kalchhauser *et al.*, 2018). This would suggest that rapid adaptation of invaders is common and not limited by genetic variation (Bock *et al.*, 2015). Hence the flexibility in transcription and with it the ability to “adapt” to the environment conditions is in combination with the information “transported” via the maternal RNA a big advantage for a successful invader.

---

## Mechanisms of spreading

Over short distances active swimming is the most likely way of distribution. Investigations at Danish coastlines in the Baltic Sea revealed a spreading by swimming of approximately 30km per year (Azour *et al.*, 2015). The authors could also show that newly established populations have a better fitness than established high-density populations. This is in line with observations of fisherman in the Trave river who reported that round goby were well fed in the early period after the initial immigration and a strong decrease of condition to very thin fishes at the time peak densities. Another investigation within two non-navigable rivers in the Czech Republic and Slovakia revealed a dispersal rate of only 1.2 to 3.2km per year (Šlapanský *et al.*, 2017). It is unclear if the difference in spreading distance in comparison to the investigation of Azour *et al.* (2015) is caused by salinity differences or other influences like predator pressure or food availability. Salinity could probably contribute to this difference because its effect on aerobic scope, which is higher in brackish water (Behrens *et al.*, 2016) compared to freshwater. Hence salinity can possibly also influence invasion speed, if the invasion takes place by swimming.

Besides actively swimming there are other means of distribution. For another goby species, the bighead goby *Ponticola kessleri*, genetic analysis of Swiss and German samples revealed patterns that are inconsistent with genetic isolation by distance. (Adrian-Kalchhauser *et al.*, 2016). If the individuals are separated by a longer distance they are expected to differ genetically. If these differences are not as expected, an exchange over the long distance is likely. Hence some sort of long distance travel of the gobies takes place. As gobies are not swimming long distances like catadromous fishes an artificial transport is most likely, e.g. via ships.

Genetic analysis of north American round goby revealed, that the invasion originated from the mouth of the southern Dnieper River at Kherushon Ukraine (Brown and Stepien, 2009). Thus the question of transport over long distances of thousands of km arises. There are two main theories on how this transport can take place. One is the transport of larval individuals via ballast water tanks. Investigations within Lake Erie (USA) showed that early juvenile round gobies prey on plankton at night in pelagic water (Hayden and Miner, 2009b). Hence a realistic chance exists that larvae are sucked into ballast water tanks and get transported over long distances. The results of chapter three show how well larvae can cope with salinity. The survival experiment revealed that even very young larvae (of brackish origin) are at least for short term able to survive sudden exposure to higher salinities. However freshly hatched

---

(first day post-hatch) larvae did not survive 25 PSU. However, if ballast water is taken in a habitat where round goby larvae do already occur, the salinity of the ballast water will be suitable during the transport. If the ballast water is exchanged in a new habitat with a higher salinity at a time then the larvae are already older and more resistant. Juvenile round goby (mean total length 63mm) survived after acclimatization a 84 day long experiment at 30 PSU (Hempel and Thiel, 2015). This suggests that the age of the introduced larvae is playing a role in survival in new places. A measure that would reduce the survival of at least young larvae in ballast water tanks would therefore be to replace the brackish water with fully marine water at high seas.

Another potential transport mechanism is the attachment of eggs on ship hulls. Especially hollows and notches within the hull with cave-like structures can be potentially be used for spawning eggs by round goby. A common measure against organisms growing on hulls is an antifouling layer which is mainly used to maintain the hydrodynamic of the ship. As this needs to be renewed from time to time, it can also become less effective or even ineffective, especially in commercial cargo shipping. Regular cleaning of ship hulls is not only an environmental issue but also an economic advantage due to reduced fuel costs (Adland *et al.*, 2018). The composition of attached organisms is depending on the used anti fouling materials (Swain *et al.*, 2007). If and how round goby eggs are influenced by anti-fouling is not known. Hence there is a theoretical chance that round goby females can spawn eggs on a ship hull. But so far there is no direct evidence that round goby do attach their eggs to ship hulls (Adrian-kalchhauser *et al.*, 2017). Therefore an investigation into this process and the influence of antifouling on round goby eggs is advisable.

Measures against invading species are of political interest, having lead to a regulation on the prevention and management of the introduction and spread of invasive alien species (European Union, 2014). In it is stated in article 21: *"A large proportion of invasive alien species are introduced unintentionally into the Union. It is therefore crucial to manage the pathways of unintentional introduction more effectively. Action in this area should be gradual, given the relatively limited experience in this field. Action should include voluntary measures, such as the actions proposed by the International Maritime Organisation's Guidelines for the Control and Management of Ships' Biofouling, and mandatory measures. Action should build on the experience gained in the Union and in Member States in managing certain pathways, including measures established through the International Convention for the Control and Management of*

---

*Ships Ballast Water and Sediments adopted in 2004. Accordingly, the Commission should take all appropriate steps to encourage Member States to ratify that Convention.”*

In case of round goby a mandatory exchange of ballast water in fully marine conditions for ships with departure in areas inhabited by round goby could be a first measure. This could potentially minimize the amount of larval survival within the tanks without any harm to the environment since no chemicals are used. Furthermore, the risk of establishing an unwanted population of round goby through such a procedure is very low. Some older individuals may survive this, if they enter the ballast water tanks at all, but successful reproduction is not possible, as shown above.

The results of the hatching success experiment showed that due to the reduced development with decreasing temperature long journeys as an egg become possible. Due to the slower development, it seems theoretically possible that a high salt content, as is the case during an Atlantic crossing, can also be partially compensated for by the eggs. In the port of destination with better salinity conditions, the larvae can then hatch, but a lower success rate is to be expected. Detailed investigations on the influence of changing salinities during the maturation of eggs need to be done as a continuation of the here presented results. If this turns out to be a potential distribution vector, the only way to stop round goby spreading by attached eggs on ship hulls would be through mechanical or chemical solutions.

---

## Implications of invasion

Alien species can have major impacts on existing ecosystems, their populations and biodiversity (Wilcove *et al.*, 1998; Grosholz, 2002; Olden *et al.*, 2004; Bruno *et al.*, 2005; Bando, 2006). It is suggested that invaders affect the “surrounding” communities differently, depending on their trophic level. Negative effects within a trophic level are often caused by competition, whereas also positive effects can occur e.g. in higher trophic levels by serving as a new food source (Thomsen *et al.*, 2014). This is also the case for round goby, as in the Baltic Sea new links to higher trophic levels are established. The invasive round goby appears as prey of cod (Funk *et al.*, 2020) and in cormorants the amount of round goby in the diet changed from 1% in 2010 up to 47% in 2015 (Oesterwind *et al.*, 2017). A similar picture can be found in the Great Lakes, where Burbot *Lota lota* changed diet from native species to high amounts of round goby in Lake Huron 77% and Lake Michigan 53% (Hensler *et al.*, 2008). However, also other effects are noticeable in the Baltic: juvenile turbot changed its dominant food item from mysids towards crangon after the appearance of round goby (Ustups *et al.*, 2016). Not all potential negative effects materialize, e.g. it was assumed that eggs of Western Baltic Spring Spawning Atlantic herring might be a preferred prey for round goby, but investigations on the impact of round goby on herring eggs in the Western Baltic Sea found a non-existing temporal spatial overlap between both (Wiegand *et al.*, 2018). Moreover coexistence is possible despite competition as shown for logperch and round goby in Duluth-Superior Harbour, where round goby negatively affected the logperch on rocky substrate. Nevertheless the logperch population remains close to pre-invasion levels since the greater area of soft bottom, unsuitable for round goby, is still inhabited by logperch (Leino and Mensinger, 2017).

Hence gaining the best possible knowledge of existing invasions can help to understand the potential risks and to improve the assessment of future invasions.



---

## References

- Able, K. W., and Palmer, R. E. 1988. Salinity Effects on Fertilization Success and Larval Mortality of *Fundulus heteroclitus*. *Copeia*: 345–350.
- Adland, R., Cariou, P., Jia, H., and Wolff, F. C. 2018. The energy efficiency effects of periodic ship hull cleaning. *Journal of Cleaner Production*, 178: 1–13. Elsevier Ltd. <https://doi.org/10.1016/j.jclepro.2017.12.247>.
- Adrian-kalchhauser, I., Guyen, A. N., Hirsch, P. E., and Burkhardt-holm, P. 2017. The invasive round goby may attach its eggs to ships or boats – but there is no evidence. *Aquatic Invasions*, 12: 263–267.
- Adrian-kalchhauser, I., Burkhardt-holm, P., Adrian-kalchhauser, I., Walser, J., Schwaiger, M., and Burkhardt-holm, P. 2018. RNA sequencing of early round goby embryos reveals that maternal experiences can shape the maternal RNA contribution in a wild vertebrate. *BMC Evolutionary Biology*. *BMC Evolutionary Biology*. <https://bmcevolbiol.biomedcentral.com/articles/10.1186/s12862-018-1132-2>.
- Adrian-Kalchhauser, I., Hirsch, P. E., Behrmann-Godel, J., N'Guyen, A., Watzlawczyk, S., Gerten, S., Borchering, J., *et al.* 2016. The invasive bighead goby *Ponticola kessleri* displays large-scale genetic similarities and small-scale genetic differentiation in relation to shipping patterns. *Molecular Ecology*, 25: 1925–1943.
- Adrian-Kalchhauser, I., N'Guyen, A., Hirsch, P. E., and Burkhardt-Holm, P. 2017. The invasive round goby may attach its eggs to ships or boats – but there is no evidence. *Aquatic Invasions*, 12: 263–267.
- Alderdice, D. 1988. Osmotic and ionic regulation in teleost eggs and larvae. *In* *Fish physiology*, pp. 163–251. Ed. by W. S. Hoar and D. J. Randall.
- Almqvist, G., Strandmark, A. K., and Appelberg, M. 2010. Has the invasive round goby caused new links in Baltic food webs? *Environmental Biology of Fishes*, 89: 79–93. <http://link.springer.com/10.1007/s10641-010-9692-z> (Accessed 28 July 2014).
- Altinok, I., and Grizzle, J. M. 2001. Effects of brackish water on growth, feed conversion and energy absorption efficiency by juvenile euryhaline and freshwater stenohaline fishes. *Journal of Fish Biology*, 59: 1142–1152.
- Aykanat, T., Thrower, F. P., and Heath, D. D. 2011. Rapid evolution of osmoregulatory function

- 
- by modification of gene transcription in steelhead trout. *Genetica*, 139: 233–242.
- Azour, F., Deurs, M. Van, Behrens, J., Carl, H., Hüssy, K., Greisen, K., Ebert, R., *et al.* 2015. Invasion rate and population characteristics of the round goby *Neogobius melanostomus* : effects of density and invasion history, 24: 41–52.
- Bailey, K. M., and Houde, E. D. 1989. Predation on Eggs and Larvae of Marine Fishes and the Recruitment Problem. *Advances in Marine Biology*, 25: 1–83.
- Bando, K. J. 2006. The roles of competition and disturbance in a marine invasion. *Biological Invasions*, 8: 755–763.
- Barton, D. R., Johnson, R. A., Campbell, L., Petruniak, J., and Patterson, M. 2005. Effects of Round Gobies (*Neogobius melanostomus*) on Dreissenid Mussels and Other Invertebrates in Eastern Lake Erie, 2002–2004. *Journal of Great Lakes Research*, 31: 252–261. <http://linkinghub.elsevier.com/retrieve/pii/S038013300570318X>.
- Beek, G. C. W. Van. 2006. The round goby *Neogobius melanostomus* first recorded in the Netherlands: 42–43.
- Behrend, E. R., and Bittermann, M. E. 1964. AVOIDANCE-CONDITIONING IN THE FISH: FURTHER STUDIES OF THE CS-US INTERVAL. *The American Journal of Psychology*, 77: 15–28.
- Behrens, J. W., van Deurs, M., and Christensen, E. A. . 2016. Evaluating Dispersal potential of an invasive fish by the use of aerobic scope and osmoregulation capacity. (review) *Plos One*: 1–19. <http://dx.doi.org/10.1371/journal.pone.0176038>.
- Bergstrom, M. a., and Mensinger, A. F. 2009. Interspecific Resource Competition between the Invasive Round Goby and Three Native Species: Logperch, Slimy Sculpin, and Spoonhead Sculpin. *Transactions of the American Fisheries Society*, 138: 1009–1017. <http://www.tandfonline.com/doi/abs/10.1577/T08-095.1> (Accessed 22 July 2014).
- Bird, A. 2007. Perceptions of epigenetics. *Nature*, 447: 396–398.
- Björklund, M., and Almqvist, G. 2009. Rapid spatial genetic differentiation in an invasive species, the round goby *Neogobius melanostomus* in the Baltic Sea. *Biological Invasions*, 12: 2609–2618. <http://link.springer.com/10.1007/s10530-009-9669-z> (Accessed 21 July 2014).
- Blob, R. W., Rai, R., Julius, M. L., and Schoenfuss, H. L. 2006. Functional diversity in extreme environments: Effects of locomotor style and substrate texture on the waterfall-climbing

- 
- performance of Hawaiian gobiid fishes. *Journal of Zoology*, 268: 315–324.
- Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., Turner, K. G., *et al.* 2015. What we still don't know about invasion genetics. *Molecular Ecology*, 24: 2277–2297.
- Bolla, S., and Ottesen, O. H. 1998. The influence of salinity on the morphological development of yolk sac larvae of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Aquaculture Research*: 203–209.
- Borcherding, J., Staas, S., Krüger, S., Ondračková, M., Šlapanský, L., and Jurajda, P. 2011. Non-native Gobiid species in the lower River Rhine (Germany): Recent range extensions and densities. *Journal of Applied Ichthyology*, 27: 153–155.
- Brandner, J., Pander, J., Mueller, M., Cerwenka, a F., and Geist, J. 2013. Effects of sampling techniques on population assessment of invasive round goby *Neogobius melanostomus*. *Journal of fish biology*, 82: 2063–79. <http://www.ncbi.nlm.nih.gov/pubmed/23731152> (Accessed 14 July 2014).
- Brothers, E. B., Mathews, C. P., and Lasker, R. 1976. Daily growth increments in otoliths from larval and adult fishes. *Fishery Bulletin*, 74: 1–8.
- Brown, J. E., and Stepien, C. A. 2009. Invasion genetics of the Eurasian round goby in North America: Tracing sources and spread patterns. *Molecular Ecology*, 18: 64–79.
- Brownscombe, J. W., and Fox, M. G. 2012. Range expansion dynamics of the invasive round goby (*Neogobius melanostomus*) in a river system. *Aquatic Ecology*, 46: 175–189.
- Brownscombe, J. W., Masson, L., Beresford, D. V., and Fox, M. G. 2012. Modeling round goby *Neogobius melanostomus* range expansion in a Canadian river system. *Aquatic Invasions*, 7: 537–545.
- Bruno, J. F. J., Fridley, J., Bromberg, K., and Bertness, M. 2005. Insights into biotic interactions from studies of species invasions. *Species invasions: insights into ecology, evolution, and biogeography*: 13–40. <http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Insights+into+biotic+interactions+from+studies+of+species+invasions#0>.
- Buckley, L. J. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology*, 80: 291–298.
- Burger, C. 2019. A Review of Round Goby Swimming Capabilities , Behaviors , and Habitat

## References

- 
- Preferences to inform Colonization Deterrence Applications in Waterways. Vancouver, WA. 1–32 pp. <http://foxlocks.saturn.stellarbluetechnologies.com/wp-content/uploads/2019/04/Task-1-Round-Goby-Lit-Review.pdf>.
- Bushnell, P. G., Steffensen, J. F., Schurmann, H., and Jones, D. R. 1994. Exercise metabolism in two species of cod in arctic waters. *Polar Biology*, 14: 43–48.
- Carlton, J. T., and Geller, J. B. 1993. Ecological Roulette: The Global Transport of Nonindigenous Marine Organisms. *Science*, 261: 78–82.
- Charlebois, P. M., Corkum, L. D., Jude, D. J., and Knight, C. 2001. The Round Goby (*Neogobius melanostomus*) Invasion: Current Research and Future Needs. *Internat. Assoc. Great Lakes Res.*
- Cherry, D. S., and Cairns, J. 1982. Biological Monitoring Part V - Preference and Avoidance Studies. *Water Research*, 16: 263–301.
- Chidester, F. E. 1921. A simple apparatus for studying the factors influencing fish migration. *Proceedings of the Society for Experimental Biology and Medicine*, 18: 175–177.
- Christensen, E. A. F., and Grosell, M. 2018. Behavioural salinity preference of juvenile yellow perch *Perca flavescens*. *Journal of Fish Biology*: 1–7.
- Christensen, E. A. F., Grosell, M., and Steffensen, J. F. 2019. Maximum salinity tolerance and osmoregulatory capabilities of European perch *Perca fluviatilis* populations originating from different salinity habitats. *Conservation Physiology*, 7: 1–5.
- Clapp, D. F., Schneeberger, P. J., Jude, D. J., Madison, G., and Pistis, C. 2001. Monitoring Round Goby (*Neogobius melanostomus*) Population Expansion in Eastern and Northern Lake Michigan. *Journal of Great Lakes Research*, 27: 335–341. <http://linkinghub.elsevier.com/retrieve/pii/S0380133001706491>.
- Clemmesen, C., and Doan, T. 1996. Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. *Marine Ecology Progress Series*, 138: 33–39.
- Corkum, L., MacInnis, A., and Wickett, R. 1998. Reproductive habits of round gobies. *Great Lakes Res.* 3: 13–20. <http://web2.uwindsor.ca/courses/biology/corkum/PDFs/Corkum,MacInnis - 1998.pdf> (Accessed 15 December 2014).
- Corkum, L. D., Sapota, M. R., and Skora, K. E. 2004. The Round Goby, *Neogobius*

- 
- melanostomus, a Fish Invader on both sides of the Atlantic Ocean. *Biological Invasions*, 6: 173–181. <http://link.springer.com/10.1023/B:BINV.0000022136.43502.db>.
- Cowan, J. H., Houde, E. D., and Rose, K. a. 1996. Size-dependent vulnerability of marine fish larvae to predation: an individual-based numerical experiment. *ICES Journal of Marine Science*, 53: 23–37. <http://icesjms.oxfordjournals.org/cgi/doi/10.1006/jmsc.1996.0003>.
- Crain, C. M., Halpern, B. S., Beck, M. W., and Kappel, C. V. 2009. Understanding and managing human threats to the coastal marine environment. *Annals of the New York Academy of Sciences*, 1162: 39–62.
- Cushing, D. 1990. Plankton production and year-class strength in fish populations - an update of the match mismatch hypothesis. *Advances in Marine Biology*, 26: 249–293.
- Cushing, D. H. 1975. *Marine Ecology and Fisheries*. Cambridge University Press.
- Davidson, A. M., Jennions, M., and Nicotra, A. B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, 14: 419–431.
- Davidson, I. C., Ashton, G. V., Ruiz, G. M., Scianni, C., Brown, C. W., Pagenkopp-Lohan, K., and Fleischer, R. 2013. Richness, extent, condition, reproductive status, and parasitism of fouling communities on commercial vessels. Report to the California State Lands Commission, Marine Invasive Species Programme. 51 pp.
- Dietrich, M. 2017. Investigations on the salinity effect on sperm motility and viability in round goby. Universität Hamburg. 32 pp.
- Dobrindt, A. B. für V. und digitale I., and Hendrick, B. Bundesministerin für Umwelt, Naturschutz, B. und R. 2014. Dritte Verordnung zur Änderung umweltrechtlicher Vorschriften in der Seeschifffahrt 1. Bundesgesetzblatt, 1: 1371–1382.
- Ducrotoy, J.-P., and Elliott, M. 2008. The science and management of the North Sea and the Baltic Sea: Natural history, present threats and future challenges. *Marine Pollution Bulletin*, 57: 8–21. Pergamon. <https://www.sciencedirect.com/science/article/pii/S0025326X08002282> (Accessed 8 February 2018).
- Ellis, S., and Macisaac, H. J. 2009. Salinity tolerance of Great Lakes invaders. *Freshwater Biology*, 54: 77–89.
- European Union. 2014. Regulation (EU) No 1143/2014 of the European Parliament and the

- 
- Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32014R1143>.
- Ferron, A., and Legget, W. C. 1994. An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology* 1, 30: 217–303.
- Florin, A.-B., Hüsey, K., Blass, M., Oesterwind, D., Puntila, R., Ustups, D., Albrecht, C., *et al.* 2017. How old are you-Evaluation of age reading methods for the invasive round goby (*Neogobius melanostomus*, Pallas 1814). *Journal of Applied Ichthyology*: 1–6. <http://doi.wiley.com/10.1111/jai.13596>.
- Fonds, M., and Van Buurt, G. 1974. The influence of temperature and salinity on development and survival of goby eggs (Pisces, Gobiidae). *Hydrobiological Bulletin*, 8: 110–116.
- Funk, S., Frelat, R., Möllmann, C., Temming, A., and Krumme, U. 2020. The forgotten feeding ground: patterns in seasonal and depth-specific food intake of adult cod *Gadus morhua* in the western Baltic Sea. *Journal of Fish Biology*.
- Galil, B. S., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., Narščius, A., Ojaveer, H., and Olenin, S. 2014. International arrivals: widespread bioinvasions in European Seas. *Ethology Ecology & Evolution*, 26: 152–171.
- Gerlach, G., and Lysiak, N. 2006. Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour*, 71: 1371–1377.
- Gong, X., Huang, X., and Wen, W. 2018. Influence of salinity on the early development and biochemical dynamics of a marine fish, *Inimicus japonicus*. *Journal of Oceanology and Limnology*, 36: 427–437.
- Gozlan, R. E., St-Hilaire, S., Feist, S., Martin, P., and Kent, M. L. 2005. Disease threat to European fish. *The Nature*, 435: 1046.
- Green, L., Havenhand, J. N., and Kvarnemo, C. 2019. Evidence of rapid adaptive trait change to local salinity in the sperm of an invasive fish. *Evolutionary Applications*: 533–544.
- Green, L., Niemax, J., Herrmann, J. P., Temming, A., and Kvarnemo, C. 2020. Alternative reproductive tactics are associated with sperm performance in invasive round goby from two different salinity environments. *Ecology and Evolution*, 10: 9981–9999.
- Green, L. (University of G. 2017. Sperm adaptations in euryhaline gobies. University of Gothenburg. 72 pp.

## References

- 
- Groen, M., Sopinka, N. M., Marentette, J. R., Reddon, A. R., Brownscombe, J. W., Fox, M. G., Marsh-Rollo, S. E., *et al.* 2012. Is there a role for aggression in round goby invasion fronts? *Behaviour*, 149: 685–703.
- Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology & Evolution*, 17: 22–27.
- Gutowsky, L. F. G., and Fox, M. G. 2011. Occupation, body size and sex ratio of round goby (*Neogobius melanostomus*) in established and newly invaded areas of an Ontario river. *Hydrobiologia*, 671: 27–37.
- Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J., *et al.* 2008. A Global Map of Human Impact on Marine Ecosystems. *Science*, 319: 948–953.
- Hayden, T. a., and Miner, J. G. 2009a. Rapid dispersal and establishment of a benthic Ponto-Caspian goby in Lake Erie: Diel vertical migration of early juvenile round goby. *Biological Invasions*, 11: 1767–1776.
- Hayden, T. A., and Miner, J. G. 2009b. Rapid dispersal and establishment of a benthic Ponto-Caspian goby in Lake Erie: Diel vertical migration of early juvenile round goby. *Biological Invasions*, 11: 1767–1776.
- Hempel, M., and Thiel, R. 2013. First records of the round goby *Neogobius melanostomus* (Pallas, 1814) in the Elbe River, Germany. *BioInvasions Records*, 2: 291–295.
- Hempel, M., and Thiel, R. 2015. Effects of salinity on survival, daily food intake and growth of juvenile round goby *Neogobius melanostomus* (Pallas, 1814) from a brackish water system. *Journal of Applied Ichthyology*, 31: 370–374.
- Hempel, M., Neukamm, R., and Thiel, R. 2016. Effects of introduced round goby ( *Neogobius melanostomus* ) on diet composition and growth of zander ( *Sander lucioperca* ), a main predator in European brackish waters, 11.
- Henne, J. P., and Watanabe, W. O. 2003. Effects of light intensity and salinity on growth, survival and whole-body osmolality of larval southern Flounder *paralichthys lethostigma*. *Journal of the World Aquaculture Society*, 34: 450–465.
- Hensler, S. R., and Jude, D. J. 2007. Diel Vertical Migration of Round Goby Larvae in the Great Lakes. *Journal of Great Lakes Research*, 33: 295–302. Elsevier.
- Hensler, S. R., Jude, D. J., and He, J. 2008. Burbot growth and diets in lakes Michigan and Huron: An ongoing shift from native species to round gobies. *Burbot: Ecology*,

- 
- management, and culture., 59: 91–107.
- Herrera, I., Borchardt, S., Santana del Pino, A., Peck, M. A., Yebra, L., and Hernández-León, S. 2009. Aminoacyl-tRNA synthetases (AARS) activity as an index of Atlantic herring (*Clupea harengus*) larvae growth. 50–73 pp.
- Herrera, I., Yebra, L., and Hernández-León, S. 2012. Effect of temperature and food concentration on the relationship between growth and AARS activity in *Paracartia grani nauplii*. *Journal of Experimental Marine Biology and Ecology*, 416–416: 101–109.
- Hjort, J. 1914. Fluctuations in the great fisheries of northern europe. *Rapp.P.-V.Reun.Cons.int. Explo*, 20: 1–228.
- Hjort, J. 1926. Fluctuations in the year classes of important food fishes. *J. Cons. int. Explor. Mer*, 1: 5–38.
- Horner, J. L., Longo, N., Bittermann, M. E., and Mawr, B. 1961. A Shuttle Box for Fish and a Control Circuit of General Applicability Author ( s ): J . L . Horner , Nicholas Longo , M . E . Bitterman Reviewed work ( s ): Source : The American Journal of Psychology , Vol . 74 , No . 1 ( Mar . , 1961 ), pp . 114-120 Publ. The American Journal of Psychology, 74: 114–120.
- Houde, E. D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symp.*, 2: 17–29.
- Houde, E. D. 2008. Emerging from Hjort’s shadow. *Journal of Northwest Atlantic Fishery Science*, 41: 53–70.
- Hüssy, K., Hinrichsen, H. H., and Huwer, B. 2012. Hydrographic influence on the spawning habitat suitability of western Baltic cod (*Gadus morhua*). *ICES Journal of Marine Science*, 69: 1736–1743.
- Huusko, A., Mäki-Petäys, A., Stickler, M., and Mykrä, H. 2011. Fish can shrink under harsh living conditions. *Functional Ecology*, 25: 628–633.
- Janssen, J., and Jude, D. J. 2001. Recruitment Failure of Mottled Sculpin *Cottus bairdi* in Calumet Harbor, Southern Lake Michigan, Induced by the Newly Introduced Round Goby *Neogobius melanostomus*. *Journal of Great Lakes Research*, 27: 319–328. <http://linkinghub.elsevier.com/retrieve/pii/S0380133001706478>.
- Javidpour, J., Sommer, U., and Shiganova, T. 2006. First record of *Mnemiopsis leidyi* A. Agassiz 1865 in the Baltic sea. *Aquatic Invasions*, 1: 299–302.



- 
- Johnson, J. H., Ross, R. M., McCullough, R. D., and Mathers, A. 2010. Diet shift of double-crested cormorants in eastern Lake Ontario associated with the expansion of the invasive round goby. *Journal of Great Lakes Research*, 36: 242–247.
- Johnson, T. B., Bunnell, D. B., and Knight, C. T. 2005. A Potential New Energy Pathway in Central Lake Erie: the Round Goby Connection. *Journal of Great Lakes Research*, 31: 238–251. Elsevier. <http://linkinghub.elsevier.com/retrieve/pii/S0380133005703178>.
- Jude, D. J., Reider, R. H., and Smith, G. R. 1992. Establishment of Gobiidae in the Great Lakes Basin. *Canadian Journal of Fisheries and Aquatic Sciences*, 49: 416–421. NRC Research Press. <https://doi.org/10.1139/f92-047>.
- Jutfelt, F., Sundin, J., Raby, G. D., Krång, A. S., and Clark, T. D. 2017. Two-current choice flumes for testing avoidance and preference in aquatic animals. *Methods in Ecology and Evolution*, 8: 379–390.
- Kalchhauser, I., Mutzner, P., Hirsch, P. E., and Burkhardt-holm, P. 2013. Arrival of round goby *Neogobius melanostomus* (Pallas , 1814) and bighead goby *Ponticola kessleri* (Günther , 1861) in the High Rhine (Switzerland). *BioInvasion Records*, 2: 79–83.
- Kappel, C. V. 2005. Losing pieces of the puzzle: threats to marine, estuarine, and diadromous species. *Frontiers in Ecology and the Environment*, 3: 275–282.
- Karlson, A. M. L., Almquist, G., Skóra, K. E., and Appelberg, M. 2007. Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *ICES Journal of Marine Science*, 64: 479–486.
- Karsiotis, S. I., Pierce, L. R., Brown, J. E., and Stepien, C. a. 2012. Salinity tolerance of the invasive round goby: Experimental implications for seawater ballast exchange and spread to North American estuaries. *Journal of Great Lakes Research*, 38: 121–128. Elsevier B.V. <http://linkinghub.elsevier.com/retrieve/pii/S0380133011002632> (Accessed 18 July 2014).
- Kiorboe, T., Munk, P., and Stottrup, J. G. 1985. First feeding by larval herring. *Dana*, 5: 95–107.
- Kjørsvik, E., Stene, A., and Lønning, S. 1984. MORPHOLOGICAL, PHYSIOLOGICAL AND GENETICAL STUDIES OF EGG QUALITY IN COD. *Flødevigen rapportser*, 1: 67–86.
- Kjørsvik, E., Pittman, K., and Pavlov, D. 2004. From Fertilisation to the end of metamorphosis - functional development. *In* *Culture of cold-water marine fish*, pp. 204–279. Ed. by E. Moksness, E. Kjørsvik, and Y. Olsen. Blackwell Publishing.
- Knutsen, G. M., and Tilseth, S. 1985. Growth, Development, and Feeding Success of Atlantic

- 
- Cod Larvae *Gadus morhua* Related to Egg Size. American Fisheries Society, 114: 507–511.
- Köster, F. W., Vinther, M., MacKenzie, B. R., Eero, M., and Plikshs, M. 2008. Environmental effects on recruitment and implications for biological reference points of eastern baltic cod (*Gadus morhua*). *Journal of Northwest Atlantic Fishery Science*, 41: 205–220.
- Kotta, J., Nurkse, K., Puntila, R., and Ojaveer, H. 2016. Shipping and natural environmental conditions determine the distribution of the invasive non-indigenous round goby *Neogobius melanostomus* in a regional sea. *Estuarine, Coastal and Shelf Science*, 169: 15–24. <http://linkinghub.elsevier.com/retrieve/pii/S0272771415301517>.
- Kovačević, N. S. 1978. Fish avoidance conditioning with tactile reinforcement. *Bolletino di zoologia*, 45: 41–44.
- Kovtun, I. F. 1978. On the fecundity of the round goby, *Gobius melanostomus*, from the Sea of Azov. *Journal of Ichthyologie*, 17: 566–573 (English translation of Vopr. Ikhtiol. 17: 566–573).
- Lederer, A. M., Janssen, J., Reed, T., and Wolf, A. 2008. Impacts of the Introduced Round Goby (*Apollonia melanostoma*) on Dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) and on Macroinvertebrate Community between 2003 and 2006 in the Littoral Zone of Green Bay, Lake Michigan. *Journal of Great Lakes Research*, 34: 690–697. Elsevier. <http://linkinghub.elsevier.com/retrieve/pii/S0380133008716113>.
- Leggett, W. C., and Deblois, E. 1994. Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? *Netherlands Journal of Sea Research*, 32: 119–134.
- Leino, J. R., and Mensinger, A. F. 2017. Interspecific competition between the round goby, *Neogobius melanostomus*, and the logperch, *Percina caprodes*, in the Duluth-Superior Harbour. *Ecology of Freshwater Fish*, 26: 34–41.
- Leppäkoski, E., Olenin, S., and Gollasch, S. 2002a. The Baltic Sea — A Field Laboratory for Invasion Biology. *In* *Invasive Aquatic Species of Europe. Distribution, Impacts and Management.*, pp. 253–259.
- Leppäkoski, E., Gollasch, S., Gruszka, P., Ojaveer, H., Olenin, S., and Panov, V. 2002b. The Baltic - —a sea of invaders. *Canadian Journal of Fisheries and Aquatic Sciences*, 59: 1175–1188.
- Leslie, J. K., and Timmins, C. A. 2004. Description of age-0 Round Goby, *Neogobius melanostomus* Pallas (Gobiidae), and ecotone utilisation in St. Clair Lowland Waters,

- Ontario. Canadian Field-Naturalist, 118: 318–325.
- Litvak, M. K., and Leggett, W. C. 1992. Age and size-selectiv predation on larval fishes: the bigger-is-better-hypothesis revisited. Marine Ecology Progress Series, 81: 13–24.
- Lohrer, A. M., Whitlatch, R. B., and Ecology, S. 2008. Interactions among Aliens : Apparent Replacement of One Exotic Species by Another Published by: Ecological Society of America INTERACTIONS AMONG ALIENS : APPARENT REPLACEMENT OF ONE EXOTIC SPECIES BY ANOTHER, 83: 719–732.
- Macinnis, A. J., and Corkum, L. D. 2000a. Fecundity and Reproductive Season of the Round Goby *Neogobius melanostomus* in the Upper Detroit River. American Fisheries Society: 136–144.
- Macinnis, A. J., and Corkum, L. D. 2000b. Fecundity and Reproductive Season of the Round Goby *Neogobius melanostomus* in the Upper Detroit River. American Fisheries Society, 129: 136–144.
- Macnaughton, C. J., Kovachik, C., Charles, C., and Enders, E. C. 2018. Using the shuttlebox experimental design to determine temperature preference for juvenile Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*). Conservation Physiology, 6: 1–10.
- Malik, A., Abbas, G., Ghaffar, A., Ferrando, S., Gallus, L., and Shah, S. S. A. 2018. Effect of different salinity level on breeding, fertilization, hatching and survival of nile tilapia, *oreochromis niloticus* (Linnaeus, 1758) in Captivity. Pakistan Journal of Zoology, 50: 539–547.
- Manné, S., Poulet, N., and Dembski, S. 2013. Colonisation of the Rhine basin by non-native gobiids: an update of the situation in France. Knowledge and Management of Aquatic Ecosystems: 02. <http://www.kmae-journal.org/10.1051/kmae/2013069>.
- Marsden, J., Charlebois, P., Wolfe, R., Jude, D., and Rudnika, S. 1996. The Round Goby, *Neogobius melanostomus* (Pallas), a Review of European and North American Literature. Zion, Illinois.
- Matern, S., Herrmann, J., and Temming, A. 2021. Differences in diet compositions and feeding strategies of invasive round goby *Neogobius melanostomus* and native black goby *Gobius niger* in the Western Baltic Sea, 16: 1–15.
- McCormick, S. D., Farrell, A. P., and Brauner, C. J. 2013. Euryhaline Fishes. Academic Press. 559 pp.

- Meekan, M. G., Vigliola, L., Hansen, A., Doherty, P. J., Halford, A., and Carleton, J. H. 2006. Bigger is better: Size-selective mortality throughout the life history of a fast-growing clupeid, *Spratelloides gracilis*. *Marine Ecology Progress Series*, 317: 237–244.
- Meunier, B., Yavno, S., Ahmed, S., and Corkum, L. D. 2009. First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus*). *Journal of Great Lakes Research*, 35: 608–612. <http://linkinghub.elsevier.com/retrieve/pii/S0380133009001695> (Accessed 5 August 2014).
- Michalek, M., Puntila, R., and Werner, M. 2012. ABUNDANCE AND DISTRIBUTION OF ROUND GOBY. HELCOM Baltic Sea Environment Fact Sheets. <http://helcom.fi/baltic-sea-trends/environment-fact-sheets/biodiversity/abundance-and-distribution-of-round-goby>.
- Mills, E. L., Leach, J. H., Carlton, J. T., and Secor, C. L. 1993. Exotic Species in the Great Lakes: A History of Biotic Crises and Anthropogenic Introductions. *Journal of Great Lakes Research*, 19: 1–54.
- Minchin, D., and Gollasch, S. 2002. Vectors - How exotics get around. *In* *Invasive aquatic species of Europe: distribution, impacts and management*, pp. 183–192. Springer, Dordrecht.
- Nikolai Vasil'evich, A., and Plotnikov, I. 2012. Sustainable society for the saving unique biodiversity and very rich biological resources of the Caspian Sea: 17–20.
- Nissling, A., Müller, A., and Hinrichsen, H. H. 2003. Specific gravity and vertical distribution of sprat eggs in the Baltic Sea. *Journal of Fish Biology*, 63: 280–299.
- Nissling, A., Johansson, U., and Jacobsson, M. 2006. Effects of salinity and temperature conditions on the reproductive success of turbot (*Scophthalmus maximus*) in the Baltic Sea. *Fisheries Research*, 80: 230–238.
- Nurkse, K., Kotta, J., Orav-Kotta, H., and Ojaveer, H. 2016. A successful non-native predator, round goby, in the Baltic Sea: generalist feeding strategy, diverse diet and high prey consumption. *Hydrobiologia*, 777: 271–281. Springer International Publishing.
- Oosterwind, D., Bock, C., Förster, A., Gabel, M., Henseler, C., Kotterba, P., Menge, M., *et al.* 2017. Predator and prey: the role of the round goby *Neogobius melanostomus* in the western Baltic. *Marine Biology Research*, 13: 188–197. <https://www.tandfonline.com/doi/full/10.1080/17451000.2016.1241412>.
- Ojaveer, H., Gollasch, S., Jaanus, A., Kotta, J., Laine, A. O., Minde, A., Normant, M., *et al.* 2007. Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea - A supply-side invader?

- 
- Biological Invasions, 9: 409–418.
- Okunsebor, S. A., Ofojekwu, P. C., Kakwi, D. G., and Audu, B. S. 2015. Effect of Temperature on Fertilization , Hatching and Survival Rates of *Heterobranchius bidorsalis* Eggs and Hatchlings. *British Journal of Applied Science & Technology*, 7: 372–376.
- Olden, J. D., Poff, N. L. R., Douglas, M. R., Douglas, M. E., and Fausch, K. D. 2004. Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution*, 19: 18–24.
- Oleksiak, M. F., Churchill, G. A., and Crawford, D. L. 2002. Variation in gene expression within and among natural populations. *Nature Genetics*, 32: 261–266.
- Oliveira, R. ., Taborsky, M., and Brockman, H. J. 2008. *Alternative Reproductive Tactics: An Integrative Approach*. Cambridge University Press, New York.
- Opstad, I. 2003. Growth and survival of haddock (*Melanogrammus aeglefinus*) larvae at different salinities. *Proceedings of the 26th Annual Larval Fish Conference*: 63–69.
- Peck, M. A., Huebert, K. B., and Llopiz, J. K. 2012. Intrinsic and Extrinsic Factors Driving Match-Mismatch Dynamics During the Early Life History of Marine Fishes. 177–302 pp.
- Pepin, P. 1995. An analysis of the length-weight relationship of larval fish: Limitations of the general allometric model. *Fishery Bulletin*, 93: 419–426.
- Petereit, C., Haslob, H., Kraus, G., and Clemmesen, C. 2008. The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae. *Marine Biology*, 154: 295–306.
- Privileggi, N., Ota, D., and Ferrero, E. A. 1997. Embryonic and larval development of the grass goby *Zosterisessor ophiocephalus* (Teleostei, Gobiidae). *Italian Journal of Zoology*, 64: 201–207.
- Puntila, R., Florin, A.-B., Naddafi, R., Kotta, J., Oesterwind, D., Putnis, I., Smolinsky, S., *et al.* 2018. Abundance and distribution of Round goby (*Neogobius melanostomus*). *In* HELCOM Baltic Sea Environment Fact Sheets. Online., p. <http://www.helcom.fi/baltic-sea-trends/environment>. <http://www.helcom.fi/baltic-sea-trends/environment>.
- Ray, W. J., and Corkum, L. D. 1997. Predation of zebra mussels by round gobies, *Neogobius melanostomus*. *Environmental Biology of Fishes*, 50: 267–273.
- Ricciardi, A., and Macisaac, H. J. 2000. Recent mass invasion of the North American Great Lakes by Ponto – Caspian species, 15: 62–65.

- Ruiz, G. M., Carlton, J. T., Grosholz, E. D., and Hines, A. H. 2012. Global Invasions of Marine and Estuarine Habitats by Non-Indigenous Species: Mechanisms , Extent , and Consequences Author ( s ): Gregory M . Ruiz , James T . Carlton , Edwin D . Grosholz , Anson H . Hines Reviewed work ( s ): Published by : Oxford Unive. Oxford University Press, 37: 621–632.
- Sampaio, L. A., Freitas, L. S., Okamoto, M. H., Louzada, L. R., Rodrigues, R. V., and Robaldo, R. B. 2007. Effects of salinity on Brazilian flounder *Paralichthys orbignyanus* from fertilization to juvenile settlement. *Aquaculture*, 262: 340–346.
- Sapkale, P. H., Singh, R. K., and Desai, A. S. 2011. Optimal water temperature and ph for development of eggs and growth of spawn of common carp (*Cyprinus carpio*). *Journal of Applied Animal Research*, 39: 339–345.
- Sapota, M. R., and Skóra, K. E. 2005. Spread of alien (non-indigenous) fish species *Neogobius melanostomus* in the Gulf of Gdansk (south Baltic). *Biological Invasions*, 7: 157–164.
- Schoenfuss, H. L., and Blob, R. W. 2003. Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): Vertical propulsion at the aquatic-terrestrial interface. *Journal of Zoology*, 261: 191–205.
- Schrandt, M. N., Stone, L. C., Klimek, B., Mäkelin, S., Heck, K. L., Mattila, J., and Herlevi, H. 2016. A laboratory study of potential effects of the invasive round goby on nearshore fauna of the Baltic Sea. *Aquatic Invasions*, 11: 327–335.
- Schreiber, A. M. 2001. Metamorphosis and early larval development of the flatfishes (Pleuronectiformes): An osmoregulatory perspective. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, 129: 587–595.
- Schurman, H., Steffensen, J. F., and Lomholt, J. P. 1991. The influence of hypoxia on the preferred temperature of rainbow trout. *Journal of Experimental Biology*, 157: 75–86.
- Senoo, S., Kaneko, M., Cheah, S. H., and Ang, J. 1994. Egg Development , Hatching , and Larval Development of Marble Oxyeleotris marmoratus under Artificial Rearing Conditions \*’ Goby of Fisheries at the expensive table fish in Malaysia . ` ) Fish farmers wish to. *Fisheries Science*, 60: 1–8.
- Senoo, S., Sow, H. S., and Mukai, Y. 2008. Effects of Different Salinity Levels on Growth and Sur vival of Marble Goby , *Oxyeleotris marmoratus* Juvenile.
- Serrano, X., Grosell, M., and Serafy, J. E. 2010. Salinity selection and preference of the grey

- 
- snapper *Lutjanus griseus*: Field and laboratory observations. *Journal of Fish Biology*, 76: 1592–1608.
- Shelford, V. E., and Allee, W. C. 1913. The reactions of fishes to gradients of dissolved atmospheric gases. *Journal of Experimental Zoology*, 14: 207–266.
- Shigesada, N., Kawasaki, K., and Takeda, Y. 1995. Modeling Stratified Diffusion in Biological Invasions. *The American Naturalist*, 146: 229–251.
- Simón, V., and Trincker, D. E. W. 1981. A new apparatus for the study of avoidance conditioning in fishes. *Behavior Research Methods & Instrumentation*, 13: 70–72.
- Skabeikis, A., and Lesutienė, J. 2015. Feeding activity and diet composition of round goby (*Neogobius melanostomus*, Pallas 1814) in the coastal waters of SE Baltic Sea. *Oceanological and Hydrobiological Studies*, 44. <http://www.degruyter.com/view/j/ohs.2015.44.issue-4/ohs-2015-0048/ohs-2015-0048.xml>.
- Skora, K., Olenin, S., and Gollasch, S. 1999. *Neogobius melanostomus* (Pallas, 1811). In: Gollasch, S., Minchin, D., Rosenthal, H., Voigt, M. (Eds.), *Case Histories on Introduced Species: Their General Biology, Distribution, Range Expansion and Impact*. Logos-Verlag, Berlin. *Case Histories on Introduced Species: Their General Biology, Distribution, Range Expansion and Impact*. Logos-Verlag, Berlin: 69–73. <http://www.gollaschconsulting.de/download/Neogobius.pdf>.
- Skora, K. E., and Stolarski, J. 1993. New fish species in the Gulf of Gdansk, *Neogobius* sp. (cf. *Neogobius melanostomus* (Pallas 1811)). *Bulletin of the Sea Fisheries Institute*, 1: 83–84.
- Šlapanský, L., Janáč, M., Kevin, R., Libor, M., and Pavel, J. 2017. Expansion of round gobies in a non-navigable river system. *Limnologica*, 67: 27–36.
- Sokołowska, E., and Fey, D. P. 2011. Age and growth of the round goby *Neogobius melanostomus* in the Gulf of Gdańsk several years after invasion. Is the Baltic Sea a new Promised Land? *Journal of fish biology*, 78: 1993–2009. <http://www.ncbi.nlm.nih.gov/pubmed/21651546> (Accessed 23 July 2014).
- Steinhart, G. B., Marschall, E. A., and Stein, R. A. 2004. Round Goby Predation on Smallmouth Bass Offspring in Nests during Simulated Catch-and-Release Angling. *Transactions of the American Fisheries Society*, 133: 121–131. <http://www.tandfonline.com/doi/abs/10.1577/T03-020>.
- Swain, G. W., Kovach, B., Touzot, A., Casse, F., and Kavanagh, C. J. 2007. Measuring the

- 
- performance of today's antifouling coatings. *Journal of Ship Production*, 23: 164–170.
- Tandler, A., Anav, F. A., and Choshniak, I. 1995. The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, *Sparus aurata*, larvae. *Aquaculture*, 135: 343–353.
- Thompson, H. A., and Simon, T. P. 2015. Age and growth of round goby *Neogobius melanostomus* associated with depth and habitat in the western basin of Lake Erie. *Journal of Fish Biology*, 86: 558–574.
- Thomsen, M. S., Byers, J. E., Schiel, D. R., Bruno, J. F., Olden, J. D., Wernberg, T., and Silliman, B. R. 2014. Impacts of marine invaders on biodiversity depend on trophic position and functional similarity. *Marine Ecology Progress Series*, 495: 39–47.
- Thorlacius, M., Ellström, G. H., and Rodin, T. B. 2015. Behavioral dependent dispersal in the invasive round goby *Neogobius melanostomus* depends on population age. *Current Zoology*, 61: 529–542.
- Tietze, S. M., and Gerald, G. W. 2016. Trade-offs between salinity preference and antipredator behaviour in the euryhaline sailfin molly *Poecilia latipinna*. *J Fish Biol*, 88: 1918–1931.
- Turbelin, A. J., Malamud, B. D., and Francis, R. A. 2017. Mapping the global state of invasive alien species: patterns of invasion and policy responses. *Global Ecology and Biogeography*, 26: 78–92.
- United States Coast Guard. 1993. Ballast water management for vessels entering the Great Lakes.
- Ustups, D., Bergström, U., Florin, A. B., Kruze, E., Zilniece, D., Elferts, D., Knospina, E., *et al.* 2016. Diet overlap between juvenile flatfish and the invasive round goby in the central Baltic Sea. *Journal of Sea Research*, 107: 121–129. Elsevier B.V. <http://dx.doi.org/10.1016/j.seares.2015.06.021>.
- Varsamos, S., Nebel, C., and Charmantier, G. 2005. Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 141: 401–429.
- VENTER, O., BRODEUR, N. N., NEMIROFF, L., BELLAND, B., DOLINSEK, I. J., and GRANT, J. W. A. 2006. Threats to Endangered Species in Canada. *BioScience*, 56: 903. <https://academic.oup.com/bioscience/article/56/11/903-910/272301>.
- Ware, D. M. 1975. Relation between egg size, growth, and natural mortality of larval fish. *Journal of the Fisheries Board of Canada*, 32: 2503–2512.



- Wellband, K. W., and Heath, D. D. 2017. Plasticity in gene transcription explains the differential performance of two invasive fish species. *Evolutionary Applications*, 10: 563–576.
- Whitehead, A., and Crawford, D. L. 2006. Neutral and adaptive variation in gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 5425–5430.
- Wickett, R., and Corkum, L. 1998. You have to get wet: A case study of the nonindigenous great lakes fish, round goby. *Fisheries*, 23: 26–27.
- Wiegleb, J., Kotterba, P., Hammer, C., and Oesterwind, D. 2018. Predation of the round goby (*Neogobius melanostomus* Pallas, 1814) on Atlantic herring eggs in the Western Baltic Sea. *Marine Biology Research*, 14: 989–1003. Taylor & Francis. <https://doi.org/10.1080/17451000.2019.1577977>.
- Wiesner, C. 2005. New records of non-indigenous gobies (*Neogobius* spp.) in the Austrian Danube. *Journal of Applied Ichthyology*, 21: 324–327.
- Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E. 1998. Quantifying Threats to Imperiled Species in the United States. *BioScience*, 48: 607–615. <https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1313420>.
- Williams, R. J., Griffiths, F. B., Van der Wal, E. J., and Kelly, J. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine, Coastal and Shelf Science*, 26: 409–420.
- Woodard, W. T., and Bitterman, M. E. 1971. Classical conditioning of goldfish in the shuttlebox. *Behavior Research Methods & Instrumentation*, 3: 193–194.
- Yebra, L., and Hernández-León, S. 2004. Aminoacyl-tRNA synthetases activity as a growth index in zooplankton. *Journal of Plankton Research*, 26: 351–356.
- Yebra, L., Hirst, A. G., and Hernández-León, S. 2006. Assessment of *Calanus finmarchicus* growth and dormancy using the aminoacyl-tRNA synthetases method. *Journal of Plankton Research*, 28: 1191–1198.
- Yebra, L., Berdalet, E., Almeda, R., Pérez, V., Calbet, A., and Saiz, E. 2011. Protein and nucleic acid metabolism as proxies for growth and fitness of *Oithona davisae* (Copepoda, Cyclopoida) early developmental stages. *Journal of Experimental Marine Biology and Ecology*, 406: 87–94. Elsevier B.V. <http://dx.doi.org/10.1016/j.jembe.2011.06.019>.
- Young, J. a. M., Marentette, J. R., Gross, C., McDonald, J. I., Verma, A., Marsh-Rollo, S. E.,

## References

- 
- Macdonald, P. D. M., *et al.* 2010. Demography and substrate affinity of the round goby (*Neogobius melanostomus*) in Hamilton Harbour. *Journal of Great Lakes Research*, 36: 115–122. <http://linkinghub.elsevier.com/retrieve/pii/S0380133009002032> (Accessed 18 August 2014).
- Zarev, V., Apostolou, A., Velkov, B., and Vassilev, M. 2013. Bulgarian black sea gobies as important object in the commercial fishing. *Bulgarian Journal of Agricultural Science*, 19: 233–236.

## Picture source

Goby drawing in system drafts copied from <https://www.drawingtutorials101.com/how-to-draw-a-goby>.

---

## Acknowledgements

First of all, I would like to thank my doctoral advisor Prof. Dr. Axel Temming. Thank you for the scientific advice, varied excursions also into other areas than research. Thank you for the patience to accompany this project over such a long time, for the encouraging words and the conveyance of the feeling that it is actually not all that bad, but also for the acceptance of my love for rather short and concise formulations instead of longer text sections. Finally, however, also for throwing me into the cold water of science to be able to find my own way and to be allowed to gather my own experiences in the project.

I thank you Prof. Dr. Christian Möllmann for your work as a reviewer, further thank you to all persons who are available as reviewers for the disputation.

Right after, my biggest thanks go to my family, my wife Anneke, my children and also my parents. I would like to thank you, Anneke, for making it possible for me to find so much time for this project especially in the final phase with the children demanding a lot of time. Thanks to my children Thies and Ronja for putting up with me sitting at my desk so often.

Thanks to my parents Ingrid and Ralf, who always motivated me to continue and reminded me, especially in the last years with a full time job outside of science and the family at home, that it would be stupid to ruin all the invested time by quitting.

Further, I would like to thank everyone at the Institute of Marine Ecosystem and Fisheries Sciences who was involved in my project. Jens-Peter Herrmann for many advices and the technical support, the support on land and water, in the lab and on the boat. Unfortunately, you left us far too early and were not able to see the good end of this work. I will always remember you!

Further to all technicians; Sven Stäcker and Stefanie Schnell who always supported me in all aspects of animal husbandry. Thanks André Eckhardt and Jan Helle who always supported me with the experimental setups and technical questions. Thanks Carmen Czerwinski for going through all the eggs pictures to determine their status. Also a thank you to Jochen Lütke for the support in all laboratory belonging and not to be forgotten to Patricia Gorre for all the administrativ work. A big thank you to Richard Klinger for supporting me during the experiments with a helping hand and also for going out fishing with me. A big thank you for support goes to Richard Klinger, Steffen Funk, Merten Saathof, Kevin Haase, Rene Plonus, Sven Matern and Max Dietrich for their support in fishing. Although this list might not be complete, I would like to thank Tim Dudeck, Björn Illing, Stefan Reiser,

## Declaration on oath

---

Alexander Keth, Frankziska Bills, Marta Moyano, Magarethe Nowicki, Claudia Günther, Saskia Otto, Rini Brachvogel, Rebecca Lauerburg, Kristin Hänselmann, Julia Frieze, Jens Flöter, Dominik Gloe, Sven Urban, Sabine Eberle, Dorothee Moll, Leon Green Eklin, Jane Behrens, Katja Heubel, Emil Aqutisaq Flindt, John Fleng Steffensen, Myron Peck, Rikka Puntilla, Paul Kotterba, Daniel Oesterwind, Jan Dierking and finally all that I have missed.

## Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, December 2021

Jan Niemax