RESEARCH ARTICLE

Invasive round goby shows higher sensitivity to salinization than native European perch

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Abstract

Salinity is an influential abiotic environmental factor in aquatic species, specifically in freshwater, where salinization causes ecosystem degradation. Secondary salinization, that is increases in salinity due to anthropogenic activities, can affect both osmoregulation and behaviour in freshwater fishes. It is generally believed that invasive species handle climatic change and environmental degradation better than native species, which is one reason for their invasion success. However, how invasive and native species cope with salinity changes remains little understood. Therefore, we investigated how low (500 μS/cm) and high salinity (2000 μS/cm) conditions affected oxygen consumption and behaviour in the invasive round goby (*Neogobius melanostomus*) and the native European perch (*Perca fluviatilis*). Our results showed that in round goby oxygen consumption increased and swimming and non-swimming movements changed in response to salinity increments, whereas European perch was not affected by salinity. Thus, it seems as if the invasive round goby is more sensitive to changes in salinity than the native European perch. Our results fit with the minority of studies indicating invasive species being less tolerant than some native species to environmental changes. This finding could be explained by the adaptation of round goby to low salinity due to its long establishment in River Rhine. Further, our results are also confirming that the effect of salinity is species-specific. In addition, European perch and round goby show diametrically different behavioural response to disturbance which could be an effect of holding different ecological niches as well as their anatomical differences.

Keywords

European perch, exploratory behaviour, general activity, metabolic rate, risk behaviour, round goby

Introduction

Salinization is one of the major causes of biological changes in river ecosystems (Vander Laan et al. 2013). Salinity is a very important abiotic environmental factor influencing aquatic species, to the extent that aquatic species are normally divided into groups based on living environment concerning salinity, such as freshwater, brackish water, or seawater species. Increases in salinity can occur via natural accumulation of salts. This is called primary salinization and the time-scale is typically very long (~100 000 years with some variances) (Herbert et al. 2015). Secondary salinization, on the other hand, is caused by anthropogenic activities such as vegetation clearance, intensive irrigation, river regulation, mining and extraction, and de-icing salts, and for secondary salinization the time-scale is much shorter than for primary salinization (decades or shorter) (Herbert et al. 2015).

Secondary salinization can have adverse effects on aquatic animals because changing salinities could affect the metabolic cost of the organism (Hart et al. 1991). For instance, many freshwater fish species have their optimal growth and typically lower metabolic rates when exposed to intermediate salinities, while increased salinities seem to reduce food intake and growth in fish (Bœuf and Payan 2001). However, fish condition has been observed to increase in spite of the reduced food consumption rate (Hintz et al. 2017). Increased riverine salinities as a result of road salt have been shown to be toxic for fathead minnows (*Pimephales promelas*) (Corsi et al. 2010) and anthropogenic increases of salinity in the Great Menderes Basin in Turkey have led to the extinction of the previously most abundant fish, namely carp (*Cyprinus carpio*) and also Wels catfish (*Silurus glanis*) (Koç 2008).

Various behavioural traits of freshwater fish are affected by salinity, although no pattern seems apparent. For instance, with increasing salinity Eastern mosquitofish (*Gambusia holbrooki*) decreased their aggressive behaviour and needed more time to capture prey (Alcaraz et al. 2008). By contrast, in an invasive cichlid aggression increased when exposed to brackish water compared to freshwater (Lorenz et al. 2016). Exposure to increased salinity increased swimming activity in pikeperch (*Sander lucioperca*) (Scott et al. 2008), and reduced anti-predator responses in fathead minnows (Hoover et al. 2013). However, there are studies showing no effects of salinity upon behaviour in other freshwater fish, such as in the Iberian barbel (*Luciobarbus bocagei*) (Leite et al. 2019).

In general, it is considered that invasive aquatic species can handle environmental change better than native species in freshwater ecosystems (Bates et al. 2013), with invasive species being more tolerant to temperature fluctuation. The pattern seems to be true for temperature tolerance in the River Rhine, because native fishes in the Rhine seem to be more negatively affected by the temperature changes during the last century (lower minimum and higher maximum) than invasive fishes (Leuven et al. 2011). Salinity sensitivity difference between invasive and native freshwater fishes has not been studied extensively. Invasive freshwater suckermouth armoured catfish (Loricariidae: *Pterygoplichthys*) has been shown to tolerate brackish water in south-

eastern Mexico (Capps et al. 2011), indicating that it could spread to a larger area. There are also some reports concerning invasive species being less tolerant to salinity than native species. For instance, the *Aphanius iberus* seems to handle salinity better than Eastern mosquitofish, but it is however also sensitive and grows worse in higher salinity environment (Sgarzi et al. 2020).

Based on this background, we wanted to investigate how an invasive fish and a native fish from River Rhine responded to different salinities. The species, the native European perch (*Perca fluviatilis*) and the invasive round goby (*Neogobius melanostomus*), were chosen based on their prevalence in the Rhine, where secondary salinization via mine water emission is extant (Wisotzky et al. 2018; Schulz and Cañedo-Argüelles 2019). Earlier studies have shown that both European perch (Overton et al. 2008; Christensen et al. 2019a) and round goby have a wide range of salinity tolerance (Karsiotis et al. 2012; Hempel and Thiel 2015). Further, European perch has a higher standard metabolic rate, that is minimum metabolic rate needed to sustain life for a specified temperature, (Ern et al. 2014; Christensen et al. 2019b), and round goby has a lower standard metabolic rate (Behrens et al. 2017) in brackish water compared to fresh water. Thus, we hypothesized that round goby would be less sensitive than European perch to an environmentally relevant increase of salinity. Specifically, we tested if oxygen consumption and/or exploratory behaviour, general activity and risk behaviour of both species were affected by salinity increments that could occur both naturally and following anthropogenic influences in the Rhine River.

Materials and methods

Experimental animals

The species chosen for this study, round goby and European perch, are not only invasive and native respectively. The round goby is typically a benthivore and the European perch a benthivore as juvenile and a piscivore as adult, thus having different ecological niches (Herlevi et al. 2018). European perch and round goby were caught by electrofishing in the Rhine River system (the tributary river Lahn at a conductivity [salinity] of 611 μS/cm and the actual river Rhine at a conductivity [salinity] of 493 μS/cm) in September 2020 near Koblenz (50°21'12.85"N, 7°34'43.79"E) in Germany, and brought in oxygenated containers to the holding facilities of University Koblenz-Landau (electrofishing was done by The German Federal Institute of Hydrology during fish stock assessments). During acclimation, the fishes were kept in two separate tanks (120 × 100 × 116 cm, filled to ~700 L) with de-ionized tap water amended for aquarium use (Borgmann 1996; Richter et al. 2018) with addition of common table salt (NaCl, Aquasale Grobes Meersalz naturbelassen, Südwestdeutsche Salzwerke AG, Heilbronn, Germany) to have a conductivity (salinity) of ~500 μS/cm, and from hereon we will use salinity interchangeable for conductivity for our experiment to provide an easier comparison to other studies. The tanks were kept at ambient temperature with enrichment (stones

and plastic tubes), a photoperiod set to 14 h light/10 h dark, and fishes were fed with dry fish feed granules (sera Vipagran Nature, sera GmbH, Heinsberg, Germany). The fishes were kept in the tanks for at least 1 week before the experiment.

The methodology of this study was conducted in accordance with the Guidelines of the European Union Council (86/609/EU). The experiments were approved by the Federal Investigation Office (Landesuntersuchungsamt, Koblenz, Germany; approval number: 23 177-07/G 20-20-062) according to § 8a of the German law for animal welfare.

Experimental set-up

Fish for the experiment were randomly selected, lightly anaesthetised using Tricaine methanesulfonate (MS-222; ~25 mg/L), measured, weighed, marked individually via fin clip, which is typically temporary and thus would not affect the fish after the release (Delcourt et al. 2018), and put into one of two aquaria on day 1 (body mass and length; European perch: 6.51 ± 1.71 g and 7.49 ± 0.63 cm [mean ± standard derivation (SD), N=24], round goby: 10.19 ± 4.91 g and 7.62 ± 1.04 cm [mean ± SD, N=24]). The aquaria (120 × 50 × 50 cm, filled to ~150 L [25 cm] with the same de-ionized tap water amended for aquarium use as in the tanks) were divided into three separate compartments, one compartment was for filtration and oxygenation and the other two compartments for the fishes (one for each species). The compartments for fishes were the same size (48 × 50 × 25 cm, ~60 L de-ionized tap water amended for aquarium use) with gravel as substrate and additional enrichment (round goby: plastic tubes based on Hempel and Thiel (2015); European perch: plastic plants based on Magnhagen (2012)). Each compartment held 6 fish of similar size from the same species (10 L water per fish). Fishes were fed 5% of their bodyweight with dry fish feed (sera Vipagran Nature, sera GmbH, Heinsberg, Germany), and the aquaria had a photoperiod set to 14 h light/10 h dark.

The two aquaria were separated into two different acclimations, namely low salinity condition (LS; 500 μS/cm) and high salinity condition (HS; 2000 μS/cm) based on the normal level and expected level after mine water emission, and on day 2 salinity change was initiated in the HS aquarium, whereas the LS aquarium was kept at the original salinity of 500 μS/cm. The salinity change was done by dissolving common table salt (NaCl, Aquasale Grobes Meersalz naturbelassen, Südwestdeutsche Salzwerke AG, Heilbronn, Germany) with water from the aquarium, and then pouring the solution into the compartment for filtration and oxygenation. A maximum change of 500 μS/cm per day was used to minimize acute stress for the fishes, and the final salinity of 2000 μS/cm for the high salinity condition was reached on day 4. Salinity, temperature and pH were measured regularly. The experimental set-up was run twice to acclimate 12 fish per group and species.

Oxygen consumption

After at least three days of habituation to the final salinity condition, oxygen consumption in fishes was measured using an automated intermittent flow respirometer (Q-Box

AQUA, Qubit Systems, Kingston, Canada). An individual fish was transferred to a respiration chamber (3.8 × 15.3 cm, 140 mL), which was submerged in an oxygenated acclimation water bath (LS or HS). The respiration chamber allowed the fish to move, but the fish were not able to swim freely. Oxygen consumption was then measured over eight 5-min periods when the chamber was closed (no circulation of water from the water bath) separated by eight 2.5-min periods when the chamber was opened (circulation of water from water bath leading to renewed oxygen), leading to a total time of 60 min. This means that, for practical reasons including trying to keep the fish holding as short as possible, we measure something between routine metabolic rate (RMR) and active metabolic rate (AMR) (White et al. 2016). After the oxygen consumption measurement, the fishes were put back into the aquaria for behavioural tests the following day. The oxygen consumption was measured on 4 fishes per day, and was done on day 3–5 under low salinity conditions and on day 6–8 under high salinity conditions between 08:00 and 15:00.

Behavioural tests

The day after the oxygen consumption measurement between 08:00 and 15:00, fishes were transferred individually to behavioural test arenas. The arenas (66 × 45 × 23 cm) were filled to ~ 25 L with treatment water from the aquarium and had an air stone in one corner. In the arenas several different behaviours were quantified in the following order:

1. Exploratory behaviour (EB)
   Fishes were filmed with a Raspberry Pi with a camera module for 10 min immediately after the introduction of the fish into the tank (Cerqueira et al. 2016).
2. General activity (GA)
   Fishes were filmed for 30 min after a 60 min habituation period after novel environment behaviour (70 min post introduction).
3. Risk behaviour (RB)
   Fishes were filmed for 30 min after the disturbance (start 100 min post introduction). The disturbance was applied by dropping a 50 ml Falcon tube filled with gravel into one side of the test arena (Millot et al. 2009).

From the films of the different tests, 10 minutes of each was analysed for behaviour. The following was quantified in all of the videos: 1) percentage of time swimming, 2) percentage of time resting, 3) percentage of time hiding by the air stone or the falcon tube (only in RB), 4) percentage of time spent in non-swimming movement (moving less than a body length), 5) time to initiated swimming (s; with a maximum of 600 s), and 6) time to hiding (s; with a maximum of 600 s). The general activity and risk behaviour were quantified from the 10 minutes directly before and after the disturbance respectively. Each fish was registered as performing one of the 4 behaviours (swimming, resting, hiding, or non-swimming movement) at every moment. As in the oxygen consumption test, 4 fishes were tested each day, on day 4–6 under low salinity

conditions and day 7–9 under high salinity conditions. In total, two rounds were made to reach an N of 12 for each species and treatment (a total of 48 fish). After the end of the experiment, the fish were returned to the Rhine River system.

Statistical analyses

Normality of the data and homogeneity of variances were tested with Shapiro-Wilk tests, and data were analysed using parametric tests (ANOVA) or non-parametric tests (Kruskal-Wallis test or Wilcoxon signed rank test). Oxygen consumption was compared between salinity conditions using a two-way repeated measure ANOVA (dependent: oxygen consumption, factors: salinity and time) in each species. Behavioural parameters were tested using Kruskal-Wallis test for the differences between salinity conditions within a species, and using Wilcoxon signed rank test to test the difference between before and after disturbance. Since the behavioural parameters are percentages, only two parameters were tested per behavioural test and that were one of the active and one of the inactive parameters. For exploratory behaviour and general activity swimming and resting were tested, and for risk behaviour non-swimming movement and hiding, based on the expected importance of the behavioural parameters depending on situation. Finally, the treatment effect upon the difference between before and after disturbance was tested using Kruskal-Wallis test on the difference of behaviour before disturbance with the behaviour after disturbance subtracted (as example: percentage of swimming during general activity - percentage of swimming during risk behaviour). For 2 fishes (one of each species) the video recording before the disturbance was shorter than the recording after the disturbance (~ 20 s) because of not turning on the recording at the right time. These data were used in the statistical analysis anyway by using percentage. The free software R for statistical computing (R Core Team 2020) using the integrated development environment RStudio (RStudio Team 2019) was used for all analyses. All data, if not stated otherwise, are presented as mean ± SD.

Results

Environmental data

During the experiment pH (LS: 7.52 ± 0.12; HS: 7.46 ± 0.04) and temperature (LS: 21.4 ± 1.0 °C; HS: 21.1 ± 0.4 °C) were similar between the salinity conditions, whereas salinity differed between the low salinity condition and high salinity condition (LS: 672 ± 30 µS/cm; HS: 2130 ± 0 µS/cm).

Oxygen consumption

Oxygen consumption decreased over time in both round goby (two-way repeated measure ANOVA; $F_{7,\,154}$ = 9.187, P < 0.0001; Fig. 1) and European perch (two-way repeated measure ANOVA; $F_{7,\,147}$ = 17.770, P < 0.0001; Fig. 2). In round goby there

was a significant effect of salinity condition (two-way repeated measure ANOVA; $F_{1, 22}$ = 6.445, P = 0.019; Fig. 1), with gobies under high salinity condition having a higher oxygen consumption than gobies under low salinity condition. However, in European perch there was no effect of salinity condition upon oxygen consumption (two-way repeated measure ANOVA; $F_{1, 21}$ = 0.774, P = 0.398; Fig. 2).
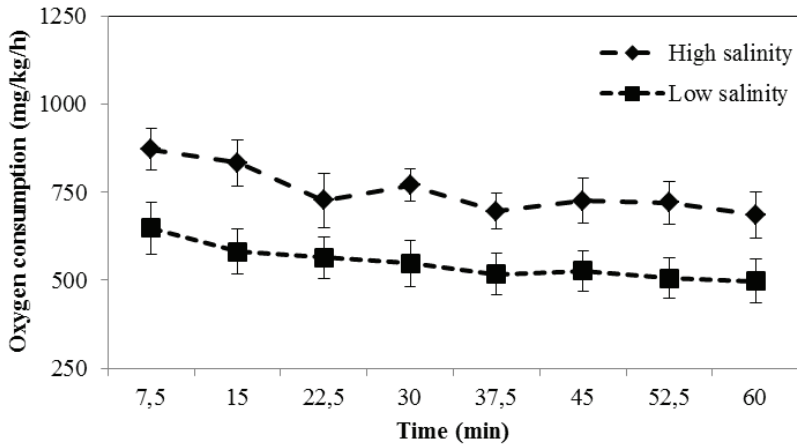

Figure 1. Oxygen consumption (mg/kg/h) in round goby from low salinity condition (square and densely dashed line) and high salinity condition (diamond and loosely dashed line) over time with a significant difference between conditions. Values are mean ± S.E.M.
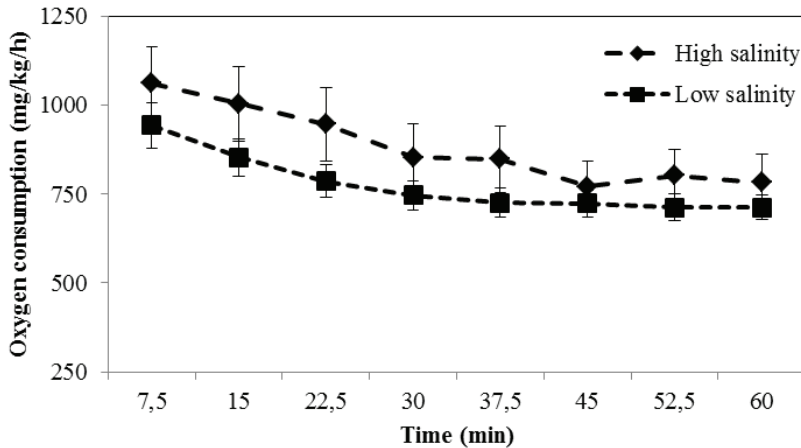

Figure 2. Oxygen consumption (mg/kg/h) in European perch from low salinity condition (square and densely dashed line) and high salinity condition (diamond and loosely dashed line) over time with no significant difference between conditions. Values are mean ± S.E.M.

Behaviour

Salinity condition affected exploratory behaviour immediately after transfer to the test arena in invasive round goby but not in native European perch. This difference in exploratory behaviour was seen in percentage of swimming with gobies from high salinity condition swimming more than those from low salinity condition (LS: 6 ± 12%, N = 12; HS: 12 ± 13%, N = 12; Kruskal-Wallis chi-squared = 4.4622, df = 1, P = 0.035; Table 1). However, there were no other differences in hiding, resting, non-swimming movement apparent, time to initiated swimming or time to hiding (Table 1). European perch from the different salinity conditions did not differ in any behavioural parameters directly after transfer to the test arena (Table 1).

While general activity, measured 70 min after the transfer to the test arena, was not affected by salinity conditions in either of the species (Table 1), the risk behaviour, measured directly after a disturbance (100 min after transfer to the test arena), was affected by salinity condition in round goby but not in European perch. Round gobies from the high salinity condition were doing more non-swimming movement, that is moving less than a body length, than gobies from the low salinity condition (LS: 0 ± 0%, N = 12; HS: 1 ± 2%, N = 12; Kruskal-Wallis chi-squared = 6.4022, df = 1, P = 0.011; Table 1). Beyond non-swimming movement in round goby, the rest of the behavioural parameters were similar in both species during risk behaviour, regardless of salinity condition (Table 1).

To test if the additional disturbance in a stressful situation affected the behaviour in the fishes and whether that depended upon the salinity condition, the same behaviours were compared between before and after the disturbance. While for both species the effects of the disturbance were evident, only for round goby the salinity condi-

Table 1. Behaviour across different situational contexts under two different salinity conditions in European perch and round goby.

Situation	Species	Condition	Swimming (%)	Resting (%)	Hiding (%)	Non-swimming movement (%)	Initiated swimming (s)	Time to hiding (s)	N
Exploratory behaviour	European perch	Low salinity	3 ± 4	45 ± 48	52 ± 49	0 ± 0	0 ± 0	211 ± 288	12
		High salinity	6 ± 10	62 ± 41	32 ± 38	0 ± 0	0 ± 0	260 ± 300	12
	Round goby	Low salinity	6 ± 12	45 ± 45	49 ± 44	0 ± 1	104 ± 198	265 ± 274	12
		High salinity	12 ± 13 *	29 ± 34	58 ± 40	1 ± 1	25 ± 37	159 ± 208	12
General activity	European perch	Low salinity	0 ± 0	42 ± 51	58 ± 51	0 ± 0	600 ± 0	250 ± 309	12
		High salinity	0 ± 0	58 ± 51	42 ± 51	0 ± 0	600 ± 0	350 ± 309	12
	Round goby	Low salinity	2 ± 5	23 ± 39	74 ± 41	1 ± 2	485 ± 207	131 ± 233	12
		High salinity	9 ± 13	40 ± 44	49 ± 46	2 ± 2	280 ± 262	239 ± 293	12
Risk behaviour	European perch	Low salinity	1 ± 2 +	25 ± 45	74 ± 45	0 ± 0	301 ± 312	152 ± 270	12
		High salinity	3 ± 4	49 ± 48	48 ± 49	0 ± 0	154 ± 269	206 ± 291	12
	Round goby	Low salinity	1 ± 1	16 ± 38 +	83 ± 39 +	0 ± 0	400 ± 295	51 ± 173	12
		High salinity	2 ± 3	6 ± 20	91 ± 24	1 ± 2 *	150 ± 271	38 ± 124	12

Values are mean ± S.D.

* Indicates that high salinity gobies behaviour is significantly higher compared to low salinity gobies (P < 0.05, Kruskal-Wallis test).

+ Indicates that there is a significant change in behaviour after disturbance (risk behaviour) for the low salinity and high salinity conditions combined compared to before disturbance (general activity) (P < 0.05, Wilcoxon signed rank test).

tion affected the behavioural responses. Round gobies from high salinity condition increased their resting after disturbance compared to gobies from low salinity condition (Kruskal-Wallis chi-squared = 4.4005, df = 1, P = 0.036). Following the disturbance, round gobies decreased resting (Wilcoxon signed rank test, V = 78, P = 0.003; Table 1) and increased hiding (Wilcoxon signed rank test, V = 62, P = 0.038; Table 1), while European perch increased swimming (Wilcoxon signed rank test, V = 0, P < 0.001; Table 1). No other behaviours were affected.

Discussion

Based on previous research, with lower standard metabolic rate in round goby (Behrens et al. 2017) and higher standard metabolic rate in European perch (Ern et al. 2014; Christensen et al. 2019b) in brackish water compared to fresh water, we had expected that the invasive round goby would be less sensitive to a higher salinity condition than the native European perch. However, round goby in high salinity condition showed an increase in oxygen consumption as well as changed behaviour compared to low salinity condition, and none of these differences were apparent in European perch. Thus, it seems as if the invasive round goby in the Rhine River system is more sensitive to increases in salinity than the native European perch.

Interestingly, it seems as if invasive species can differ in behaviour depending on time since colonization. For instance, in cane toad (*Rhinella marina*) anti-predatory responses differ between their native and invasive range with invasive toads being less likely to flee (Hudson et al. 2017). In addition, individuals from the invasive front were less likely to flee compared to toads from long-colonized areas and individuals even differed in morphology and locomotory traits between the invasive front and long-colonized areas (Hudson et al. 2017; Hudson et al. 2020). Further, native cane toad had higher stress responses than invasive cane toad at a similar climate (Kosmala et al. 2020a), and invasive cane toad had adapted to invasive areas abiotic challenges such as temperature (Kosmala et al. 2018), as well as moisture (Kosmala et al. 2020b). Round goby has also been reported to differ in several traits between the invasion front and long-colonized areas. Round goby at the invasion front were bolder, had a higher dispersal potential and higher resting metabolic rate (RMR) than gobies from long-colonized areas (present for approximately 10 years) (Myles-Gonzalez et al. 2015). Further, some studies also indicate that round goby have rapid adaptive traits in novel environments. For instance, it seems as if sperm velocity is adapting rapidly to novel salinity levels (Green et al. 2020). It has also been reported that genetic differentiation happened in fewer than ten generations of round goby in the southern Baltic Sea (Björklund and Almqvist 2010). Further, there is evidence that round goby differs in demography, morphology and feeding behaviour between an invasion front and a long colonised area (Brandner et al. 2013). The sampling site for round gobies of this study (Rhine-km ~590) can be considered a long-colonised area, because this species had been detected in the Dutch Rhine delta 2004 and from higher up in Rhine (Rhine-km

718) in 2008 (Borcherding et al. 2011). Consequently, the round goby used in this study might be more sensitive to salinity because they are long established and therefore could have adapted to the environment as previously shown in cane toad. There are some studies indicating that round goby could be sensitive to freshwater salinity levels. For instance, heat shock protein 70 (hsp70) expression was highest in round goby in freshwater (0‰) compared to 10 and 30‰ salinities (Puntila-Dodd et al. 2021). However, it is still perplexing that European perch from the same area seem to be more tolerant towards salinity, but other factors than sensitivity to abiotic factors can also be important for the establishment of invasive species, such as sensory biology (Abrahams et al. 2017).

Our results that round goby increased oxygen consumption following high salinity condition were unexpected. Earlier studies have shown that round goby has a wide spectrum of tolerance for salinity (0–20‰ without problems) (Karsiotis et al. 2012) and growth rate did not change within the range of 0.1 to 15‰ (Hempel and Thiel 2015). Additionally, in contrast to our results, Behrens et al. (2017) reported that standard metabolic rate (SMR) was lower at 10‰ compared to 0‰ salinity, but found no differences in maximal metabolic rate (MMR) and aerobic scope (AS). There are, however, several differences between this study and our study concerning methodology. For instance, we used salinities of 500 and 2000 µS/cm, which are ~0.25‰ and 1.00‰ respectively, which led to a lesser difference between salinity conditions. Further, for practical reasons, we did not measure SMR or MMR, rather something between routine metabolic rate (RMR) and active metabolic rate (AMR) (White et al. 2016). Additionally, in our experiment, the gobies were acclimated for a short time (3–5 days) whereas in Behrens et al. (2017) the gobies were acclimated for at least 20 days. Thus, some of the different results could be attributed to methodological differences. However, in the desert goby (*Chlamydogobius eremius*) oxygen consumption was higher for gobies in higher salinities (35‰ and 70‰) compared to gobies in 0‰ (Thompson and Withers 2002), which is similar to our results.

Further, our results show that high salinity condition does not seem to have any significant effect on oxygen consumption in European perch. This result was unexpected because earlier reports have shown higher oxygen consumption, either in standard metabolic rate (SMR) or maximal metabolic rate (MMR), in perch exposed to higher salinities. For instance, European perch had a higher SMR in brackish water (10‰) compared to fresh water (0‰) (Ern et al. 2014). Further, SMR seems to be increased in 15‰ compared to 0 and 10‰ at 10 °C and 20 °C, but MMR seems to be lower at 5 °C in 15‰ compared to 0 and 10‰ (Christensen et al. 2017), however this was in perch from brackish water. Salinity did not affect MMR in European perch from low respectively high saline background (Christensen et al. 2019b), but SMR was higher at 10‰ than in 0‰ in perch from high saline background (Christensen et al. 2019b). There are again several differences between these studies and our study. For instance, we used a lesser difference between salinity conditions compared to the other studies. As mentioned above, we measured something between routine metabolic rate (RMR) and active metabolic rate (AMR) (White et al. 2016). This could make comparisons

difficult. However, we suggest that our results with no difference between treatments could be attributed to the freshwater origin of the perch as well as not measuring SMR, and thus fitting in with perch from low saline background showing no differences in SMR and MMR between 0‰ and 10‰ salinity (Christensen et al. 2019b).

In our study, we showed that oxygen consumption decreased during the exposure time in both European perch and round goby. This indicates that the initiation of the procedure, netting the fish from the aquarium and putting it into the respirometer chamber, was stressful and that the fishes acclimated to the situation over time. Our set-up was similar to White et al. (2016), which also had preliminary data suggesting the first measurements were higher based on a stress response following the transfer to the respirometer chamber. That respirometry can induce a stress response has also been reported in rainbow trout (*Onchorhynchus mykiss*) (Murray et al. 2017). Further, the respirometry chamber could also be considered to induce stress of confinement (Ellis et al. 2012). Thus, we believe that we have measured an initial stress response following the short chasing as well as confinement, but that the fishes acclimated and therefore decreased their oxygen consumption to normal levels.

We had expected that both species would change their behaviour following salinity increment. However, while in our study elevated salinity seemed to have no effect on behaviour in European perch, in round goby there were several significant differences between the salinity treatments. In general, the activity was increased at elevated salinity in round goby. This could be an increase of activity to avoid the salinity by changing location. Further, round goby has been proposed to use risky strategies during starvation and winter conditions (Fortes Silva et al. 2019). Consequently, it seems possible that they would also be using riskier strategy when exposed to elevated salinity, and thus have an overall increased activity. There are several studies indicating behavioural effects of salinity in freshwater fishes. For instance, male desert goby exert less aggressive behaviour but do not change courtship behaviour at elevated salinity (Lehtonen et al. 2016). Further, fathead minnows exposed to 8000 ppm salinity spent less time moving compared to 4000 and 1000 ppm salinity (Hoover et al. 2013). Additionally, the fathead minnows exposed to 4000 and 8000 ppm salinity could not distinguish between low and high alarm cues shown through anti-predator behaviour, which minnows exposed to 1000 ppm salinity could (Hoover et al. 2013). Furthermore, sailfin molly (*Poecilia latipinna*) prefer to remain in freshwater with predator cue in comparison to salt water (Tietze and Gerald 2016). Elevated salinity in combination with elevated temperature negates the increased interspecific aggression following only elevated temperature in Eastern mosquitofish and Australian bass juveniles (*Macquaria novemaculeata*) (Lopez et al. 2018). However, some fish do not change behaviour when exposed to salinity such as Iberian barbel (Leite et al. 2019). Thus, our results are confirming that the effect of salinity in freshwater fishes do give disparate results, and that each species should be studied to be able to predict how salinity would affect them, and that the specific salinities should also be considered thoroughly.

There were also distinct differences between European perch and round goby in their behavioural response to disturbance i.e., comparing risk behaviour with general

activity. European perch increased their swimming and tended to rest less after disturbance. On the other hand, round goby hid more and tended to swim less after disturbance. Thus, it seems as if the responses to disturbance between the two species are diametrically different, and could be interpreted as European perch trying to flee the disturbance and round goby trying to hide from the disturbance. This could be an effect of their differences in ecology such as being a benthivore and a piscivore, as well as their anatomical differences.

Acknowledgements

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