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ORIGINAL PAPER

Indications of reproductive disturbance by the invasive round goby on a native salmonid

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Abstract Invasive species have a wide array of effects on species in their introduced ranges, including sub-lethal effects such as disruption of courtship or parental care, with potential negative influence on fitness of the native species. The invasive round goby (*Neogobius melanostomus*) is a door-knocker species to freshwater rivers and streams connected to the Baltic Sea. In the case of establishment of round goby in freshwater it may overlap spatially with native salmonids like Baltic Sea Atlantic salmon and sea trout, and we therefore experimentally studied interactions between round goby and salmon during salmon spawning. We found that salmon spawning behaviour was unaffected by the presence of round goby. However, salmon displayed aggression towards the round goby and the salmon male and female resided closer to each other in the presence of round goby at higher round goby densities, which we interpret to be a protective behaviour. In addition, salmon spawning was delayed in the presence of round goby. Altogether, our results imply that the reproductive success of vulnerable Baltic salmonids may be impaired under a scenario where round goby migrates upstream and establishes in Baltic rivers and streams. Consequently, we see the need for management actions to hinder spread and subsequent establishment of round goby in freshwaters along the Baltic coastline.

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Introduction

Invasive species have a wide array of effects on species in their introduced ranges (reviewed by Pyšek et al. 2020). They can negatively influence both individual survival and long-term population development of native species through e.g. predation (reviewed by Pyšek et al. 2020), but sub-lethal effects are also important (Gribben and Wright 2006). Disruption of courtship or parental care of native

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species by invasive species are examples of such sub-lethal effects, with potentially negative effects on fitness (e.g. Howe et al. 1997; Rincón et al. 2002; Steinhart et al. 2005; Brown et al. 2018). As species introductions have increased over time (Seebens et al. 2017), so has the need to investigate invasive species effects on the reproduction of native species (Steinhart et al. 2005).

The invasive round goby (*Neogobius melanostomus* Pallas, 1814), originating from the Ponto-Caspian region (reviewed by Kornis et al. 2012), was first found in the Baltic Sea in 1990, likely arriving with ballast waters from commercial ships (Skóra and Stolarski 1993). It is established in most coastal areas in the southern and central Baltic Sea (ICES 2022) and a range expansion is currently underway, where round goby is dispersing from the coast into adjacent freshwaters. So far, the round goby numbers in freshwater are low and there are currently no clear indications of self-sustaining round goby populations in the Baltic Sea drainage area (Verliin et al. 2017; Puntila et al. 2018; Rakauskas et al. 2018; Carl et al. 2019; Florin et al. 2021). However, the round goby can adapt to freshwater conditions, as demonstrated by experiments on sperm performance (Green et al. 2021) as well as aerobic scope and osmoregulatory capacity (Behrens et al. 2017). Furthermore, round goby forms thriving populations in the freshwater system of the invaded North American Great Lakes, as well as in rivers in central Europe (reviewed by Kornis et al. 2012 and Cerwenka et al. 2023) and can hence be considered a door-knocker species to freshwater ecosystems around the Baltic Sea. It is possible that we are experiencing a time lag before round goby starts to reproduce, and abundances increase, in freshwaters connected to the Baltic Sea, with potentially severe consequences for native species.

Round goby impacts on the reproductive success of native species have mainly been studied with focus on round goby predation on early life stages (e.g. Steinhart et al. 2004, 2005; Wiegleb et al. 2019; Lutz et al. 2020). However, there is also evidence from the Great Lakes of round goby behavioural interference with native fishes during spawning or parental care. Presence of round goby leads to both frequent attacks by the round goby on nest-guarding smallmouth bass males (*Micropterus dolomieu* Lacepède, 1802) and frequent chases of round goby by the smallmouth bass. This results in both greater energy expenditure in smallmouth bass, which could negatively impact future reproduction, and more time spent away from the guarded nest, which increases the risk of egg predation (Steinhart et al. 2005). Under experimental conditions, round goby successfully chases away nest-guarding mottled sculpin (*Cottus bairdii* Girard, 1850) males (Dubs and Corkum 1996; Janssen and Jude 2001) and feeds on the unprotected eggs (Janssen and Jude 2001). This behaviour might be one reason for the abrupt decline of mottled sculpin after round goby establishment in the Great Lakes (Janssen and Jude 2001). Round goby interactions with logperch (*Percina caprodes* Rafinesque, 1818) shows a similar pattern, with round goby being significantly more aggressive and superior at securing shelter, which indicates potential displacement of logperch and reproductive disturbance in the wild (Balshine et al. 2005). In general, round goby displacement of native species during reproduction could be a contributing factor behind population declines of several coastal fish species in the Great Lakes (Reid and Mandrak 2008). Undoubtedly, the importance of reproductive disturbance on native species by round goby has been extensively investigated in the Great Lakes, while such studies are lacking from the Baltic Sea where the environmental conditions, species composition and -interactions are fundamentally different.

The anadromous Baltic Sea salmonids, Atlantic salmon (*Salmo salar* Linnaeus, 1758) and sea trout (*Salmo trutta* Linnaeus, 1758), are top predators connecting freshwater stream habitats with the open brackish Baltic Sea through long-distance migrations (Thorstad et al. 2011, 2016). They migrate to rivers prior to spawning during autumn (Fleming and Einum 2011; Thorstad et al. 2016). When a suitable redd has been located, the salmonids display intricate spawning behaviours. The eggs are deposited in gravel, where they develop and hatch in early spring (ibid.). The salmon population trends in the Baltic Sea are diverse, with some populations doing well, primarily in the northern rivers, while others are developing poorly, primarily in the southern Baltic rivers (ICES 2023). For sea trout, the situation is similar with many populations doing poorly (HELCOM 2022). Migration barriers, overfishing and, for salmon, mortality related to the M74 syndrome are considered the major reasons for the historic population decline, while climate change is an emerging negative factor for population resilience (LaMere et al. 2020). In addition to these threats, invasive species can add extra pressure on the salmonid populations in and around the Baltic Sea. Specifically, they may be negatively affected by introduced pathogens and through genetic mixing with invasive salmonids (Josefsson and Andersson 2001; Gren et al. 2009), while knowledge about other types of potential impacts by invasive species, for example reproductive disturbance, is lacking.

Various interactions between invasive round goby and salmonids have been reported from the Great Lakes, such as round goby predation on eggs and larvae and salmonid predation on the round goby (reviewed by Hirsch et al. 2016). Further, the evidence from the Great Lakes of reproductive disturbance caused by round goby on native species (Dubs and Corkum 1996; Janssen and Jude 2001; Balshine et al. 2005; Steinhart et al. 2005) indicate potential risks also for fishes spawning in freshwaters connected to the Baltic Sea. If the round goby continues its expansion and establishes in salmonid rivers around the Baltic Sea, the risk of interference between round goby and native salmonids may increase with time.

We applied an experimental approach to investigate if round goby negatively influenced salmonid spawning. We did this by studying if salmon i) spawning behaviour, ii) movement patterns, iii) aggression and iv) probability and time until spawning was affected by the presence of round goby.

Materials and methods

The experiment was carried out at the Swedish University of Agricultural Sciences' Fisheries Research Station in Älvkarleby, Sweden. The station acts as a hatchery for a stocking program of Baltic salmon into the adjacent river Dalälven, and hence collects a yearly brood stock of ascending spawners. A limited number of these spawners could be used for the round goby interaction experiment annually, which lead to the experiment being conducted during two years, 2019 and 2021 (2020 was aborted due to COVID-19), to achieve sufficient sample size.

Fish collection and husbandry

Round goby was caught using fyke nets in September and October 2019 at Muskö (58.9741, 18.0907) and 2021 at Gävlebukten (60.701472, 17.271237) by commercial fishers. In 2019, the fish (n=486) was kept in tanks on the shore with circulating sea water until being transported to Älvkarleby in a fish transportation tank filled with sea water. In 2021, the fish (n=155) was kept in a corf for a few days before being transported to the station. In both years, round goby was separated based on sex upon arrival at the station. In 2019, the females (n=183) were euthanized, while in 2021, all round goby was kept to compensate for the poorer catch this year. After being separated based on sex, the round goby was placed in holding tanks of dimensions 97 × 97 × 28.5 cm (water level approximately 23 cm). The tank system had recirculating water where inorganic nitrogen was removed using UV-filters and biofilters, and we manually cleaned out excess food and excrement on a daily basis. The fish was allowed to acclimatize for three days in river water from Dalälven that had been salinized to correspond to the brackish water of the capture locations (6-7 psu). The fish was then gradually exposed to an increasing proportion of freshwater in the tanks, salinity decreasing with 1 psu per day until freshwater conditions (0.1-0.2 psu) were reached. We provided the tanks with dark net lids to ensure minimal disturbance of the fish and used rocks, branches, plastic pipes and artificial seagrass to mimic natural conditions and create shelter in the tanks. The maximum number of round goby per tank during the acclimatization to freshwater was 78, which corresponds to lower than maximum natural densities (>100 individuals/m^2 reported from the Great Lakes; Chotkowski and Marsden 1999; Steinhart et al. 2004). The round goby was fed defrosted krill, amphipods and chironomids *ad lib* once every day, starting when acclimatization to freshwater began. At the onset of the experiment, we withheld food for the fish used in the experiment. Temperature was allowed to vary with indoor conditions, with a maximum temperature of 16.4 °C in 2019. Sick or dead fish were removed continuously; mortality was low (3%) in the tanks in 2019 but higher (13%) in 2021. The round goby was transferred from the holding tanks to the experimental flume the day before the onset of the experiment (October 16th 2019 and October 25th 2021).

Salmon males and females were held separated in tanks (200 × 95 × 48 cm) with flowing river water. In 2019, salmon in spawning mode was delivered continuously by the hatchery at the station for use in the experiment the following day, but in 2021 the salmon was delivered in bulk and kept for a maximum of three days before use in the experiment. The tanks were covered with dark net lids, secured with clamps and weights, to minimize disturbance and prevent escape. Nonetheless, salmon was able to escape twice in 2019 and once in 2021, and in those trials we had to use salmon delivered from the hatchery the same day. The salmon was euthanized after use in the respective trials by physical trauma to the head and subsequent decapitation to prevent potential spread of parasites or diseases from round goby. The round goby was euthanized by physical trauma to the head after the trials were finished in 2019 and 2021, respectively.

Experiment setup

In order to study salmon behaviour in the presence vs. absence of round goby, we used a 35 000 L stream water aquarium, or flume, which is designed to induce spawning in salmonids (Hellström et al. 2019; Fig. 1). The flume has two identical sides, separated by removable net gates. Each side has a 12 m^2 gravel section to mimic salmon spawning habitat. Water current is generated by turbines at the upstream section of each side. Two replicated trials can be run at the same time. As concluded in previous studies using the flume, spawning salmonids do not seem to be affected by the presence of an observer (Olsén et al. 1998; Jaensson and Olsén 2010). Above the flume, warm and cold light sources can be used to simulate a diurnal light regime, including sunrise and sunset (see Olsén et al. 1998 for a technical description of the flume). The flume was filled with aerated groundwater to enhance visibility, as turbid river water may decrease visibility (Hellström et al. 2019). During the trials, one observer per flume side recorded the behaviour and position of the salmon. The observers switched sides every other trial to minimize observer bias.

On the treatment side of the flume salmon and round goby were allowed to interact, while only salmon was kept on the control side. The same round goby was used throughout all trials in the respective years (n_{2019}=200, n_{2021}=92-115), but the salmon pairs were replaced between each trial. One pair of salmon spawners (a male and a female) was used per side per day, and each day represented one experimental trial. The light regime was set to mimic natural light conditions for the season. A trial started in the morning 30 minutes after the salmon spawners were introduced into the flume where the round goby was held. During the 30 minutes acclimatization period, we mimicked sunrise. The observations lasted until one hour after the first spawning event, or 10 hours in case of no spawning events in 2019. In 2021 the observation time varied between 9 and 9.5 hours but was always the same for treatment and control of each trial.

The availability of round goby differed between years, which led to the use of different round goby densities in the treatments; 16.67 ind/m^2 in 2019 and 9.58 ind/m^2 in 2021 (Table 1). Both densities were

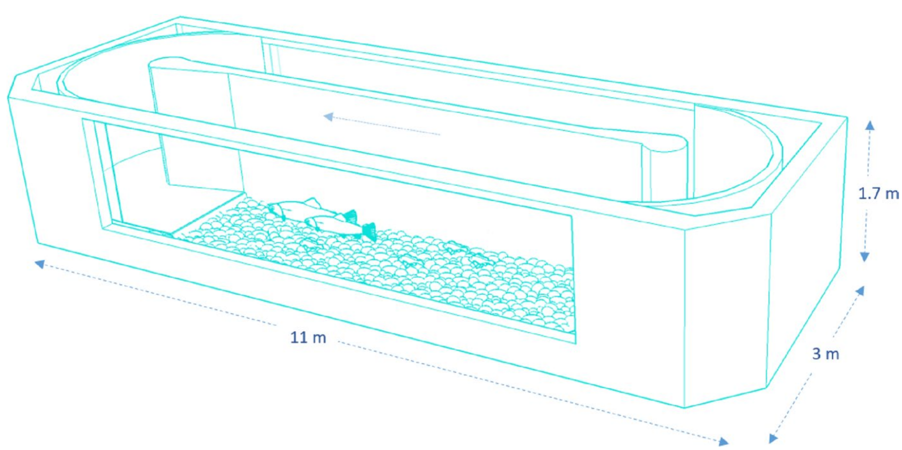

Fig. 1 Schematic drawing of the flume (Trimble Sketchup 3D design software)

Table 1 Metadata for the respective experiment rounds in 2019 and 2021

Year	Trials (n)	Flume temperature (°C)	Water velocity (m/s)	Round goby (n)	Round goby density/m^2	Round goby size (mm, mean, sd)	Round goby sex	Round goby mortality in the flume (%)
2019	8	8.1-8.6	0.25-0.8	200	16.67	158.85 (15.03)	Male	6
2021	9	8.3-8.7	0.75	92-115	7.67-9.58	150.48 (24.54)	Mixed	20

deemed high but ecologically relevant, as maximum natural adult densities in the Baltic Sea are ~20 ind/m^2 (Puntila et al. 2018). In 2019, round goby that occasionally died in the flume during the trials were replaced to keep the density constant, however in 2021, we were not able to replace perished individuals and the density hence decreased slightly during the trials to 7.7 ind/m^2 at the end of the last trial. Fungus infections and salmon attacks were the two major causes of death of round goby in the flume. In 2019, only male round goby was used as they are more aggressive and territorial (reviewed by Kornis et al. 2012) and hence believed to be more prone to interact with the salmon. However, in 2021, round goby of both sexes was used (63% males) to increase the density despite poor catches. Maximum water velocity in the first three trials in 2019 (0.25 m/s) was deemed to be insufficient to cue salmon spawning and hence increased from the fourth trial (0.8 m/s; Table 1) to match the velocity used in Hellström et al. (2019). The mean size of round goby used in the experiment differed slightly between years, as it was 159 mm in 2019 (range 131-194 mm) and 150 mm in 2021 (range 90-213 mm).

Data collection

Salmon spawning behaviour and spawning events

A mature female exhibits spawning behaviour when she is digging a redd by repeatedly whipping her tail in the gravel ("digging"; Supplement 1a). The female displays digging both before the spawning event, to prepare the redd, and after, to cover the deposited eggs in gravel. She also occasionally probes the gravel with her erect anal fin ("probing"; Supplement 1b). A dominant male in spawning condition courts the female by quivering against her body ("courting"; Supplement 1b). A spawning event occurs when eggs and milt are deposited simultaneously (Fleming and Einum 2011). The behavioural data recorded was the time of every courting event (male), as well as the time of every probing and digging event prior to spawning (female), and the time of spawning events.

Salmon movement patterns

The distance between the male and the female salmon was calculated using their recorded positions. The position was determined by dividing the flume into 3-dimensional grids, each grid representing a volume of 0.32 m^3 which generated 54 grids on each side of the flume (see Hellström et al. 2019). The observer noted in which grid the salmon resided every five minutes, and the Euclidean distance between the female and male was then calculated using the Pythagorean theorem extended for three dimensions, under the assumption that the salmon resided in the center of the grid.

Interactions with the round goby

We timestamped every direct attack by the male on the round goby. An attack was defined as a targeted, often swift, movement towards a round goby, often resulting in the round goby bursting away, or occasionally being physically hurt.

Statistical analysis

The experimental setup and data collection generated four timestamped datasets: 1) observations of spawning behaviour (male courting, female probing and digging), 2) positional data of male and female salmon, 3) observations of male attacks on round goby, and 4) observations of salmon spawning events. Separate models were run for the respective years to account for the variation in trial lengths between 2021 and 2019. We also made a slight change in methodology between years for the observations of spawning behavior. In 2019, we did not have a fixed interval regarding the ending of a spawning behaviour and the onset of a new one for a behaviour to count as a separate one, while in 2021 spawning behaviours counted as separate ones if they were separated in time by at least 20 seconds. All analyses were conducted in R, version 4.3.2, using the packages *lme4* (Bates et al. 2015) and *survival* (Therneau 2023).

To test whether presence of round goby affected salmon spawning behaviour a generalized linear model was used, where treatment was modeled as a two-level fixed effect (presence/absence of round goby). Separate models were used for the three response variables and for the respective years (2019, 2021), and the total number of courting, probing or digging events per trial was used.

To test whether presence of round goby affected salmon movement patterns a general linear mixed effect model was used, treating distance as a normally distributed response variable, treatment as a two-level fixed effect (presence/absence of round goby) and trial as a random effect to account for pseudo-replication due to repeated measurements per trial.

To test if time affected the distance between salmon male and female in the presence vs. absence of round goby a general linear mixed model was used, treating distance as the response variable, time as a continuous fixed effect (minutes of the experiment, 0-600) and treatment as a two-level fixed effect (presence/absence of round goby). An interaction term between time and treatment was included, and trial was treated as a random effect.

To investigate the pattern of salmon male attacks on round goby over time a general linear mixed effect model was used, where number of attacks per 30 minutes were modeled as a function of time (minutes of the experiment, 0-600), treating trial as a random effect.

To evaluate the effect of presence of round goby on probability and time until spawning in salmon, a survival analysis using the Cox proportional hazards model was performed where time until spawning (minutes from the start of the trial to the first spawning event) was used as response variable and treatment as explanatory variable. Data from 2019 to 2021 was pooled to achieve sufficient sample size of spawning events.

Results

In 2019, two salmon pairs out of eight spawned in the control and one out of eight in the treatment, while in 2021, five salmon pairs out of nine spawned in both the control and treatment. Pooled, this equaled seven salmon pairs spawning in the control and six in the treatment.

No effect of the presence of round goby on the courting, probing and digging behaviour of salmon could be detected, neither in 2019 nor 2021 (generalized linear model, $p>0.05$, Fig. 2).

In 2019, the male and female salmon spawners resided closer to each other in the presence of round goby compared to in the control (general linear mixed model, $F_{1,1730}=10.6$, $p = 0.001$), however no effect could be seen in 2021 ($p>0.05$; Fig. 3).

In 2019, the effect of time on the distance between male and female depended on treatment (interaction effect GLMM, Chi-square = 4.4850, df = 1, $p = 0.034$, Fig. 4), and as time progressed, the distance decreased less in the presence of round goby compared to the control. A similar trend was found for 2021, although not significant (interaction effect GLMM, $p = 0.07$, Fig. 4).

We found a significant increase in the number of salmon male attacks on round goby over time in 2019 (generalized linear mixed model, Wald type II, Chi-square = 6.47, df = 1, $p = 0.01$), and a similar but not significant trend could be observed for 2021 (Fig. 5).

The presence of round goby significantly delayed the time until spawning in salmon ($p = 0.0217$, Wald test) and the hazard ratio for the salmon exposed to round goby (i.e. the treatment group) was 0.20 (95% CI 0.04827–0.7872). Thus, the chance of spawning was approximately 80% lower at any given time for salmon in the presence of round goby compared to in the absence of round goby (Fig. 6).

Discussion

In this study, we detected indications that round goby under experimental conditions can negatively affect salmon spawning throughout the density range of 7-16 ind/m^2. We found no differences in the expression of salmon spawning behaviour (male courting and female probing and digging) between treatment and control, indicating that round goby is

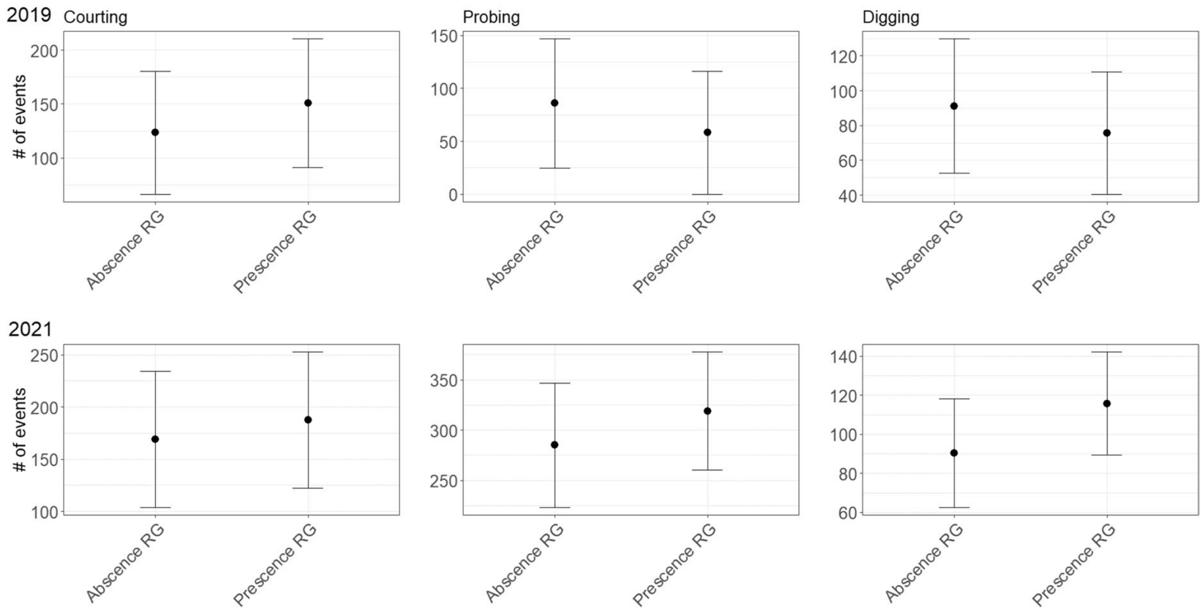

Fig. 2 Salmon spawning behaviours (courting, probing and digging) in the presence and absence of round goby over two years (2019 and 2021). Dots and whiskers represent mean and 95% CI, respectively

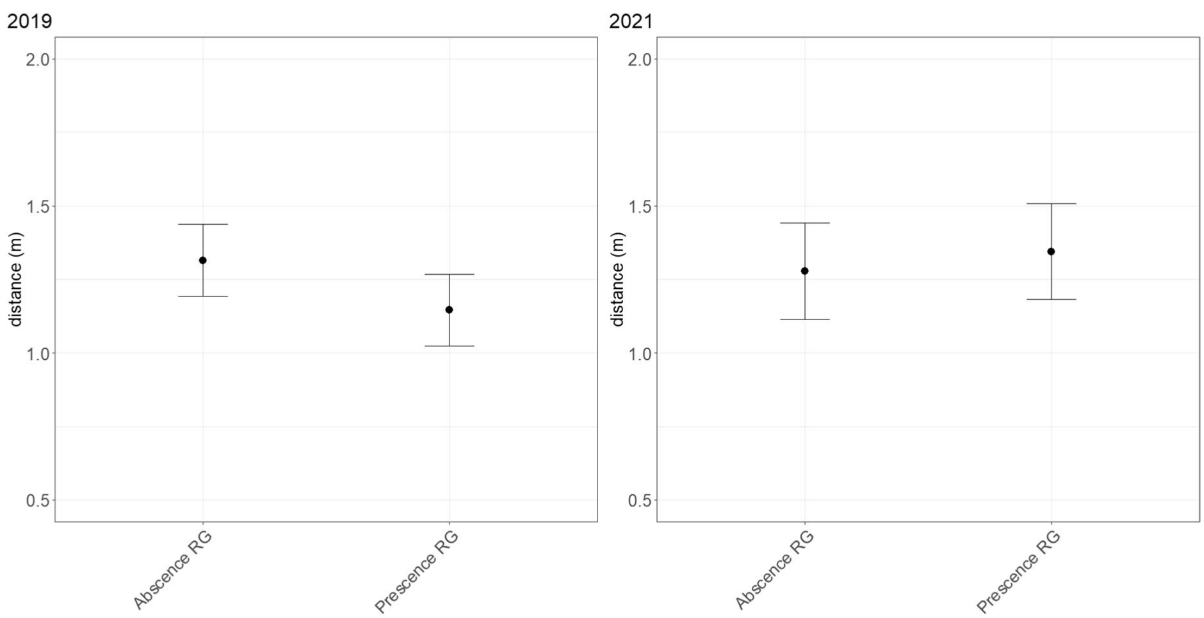

Fig. 3 Distance between male and female salmon spawners in the presence and absence of round goby over two years (2019 and 2021). Dots and whiskers represent mean and 95% CI, respectively

not a nuisance in this aspect. We did, however, detect a difference in distance between male and female salmon between treatment and control in 2019. We further found a significant increase in the number of salmon male attacks on round goby over time in 2019, and a similar but not significant trend in 2021.

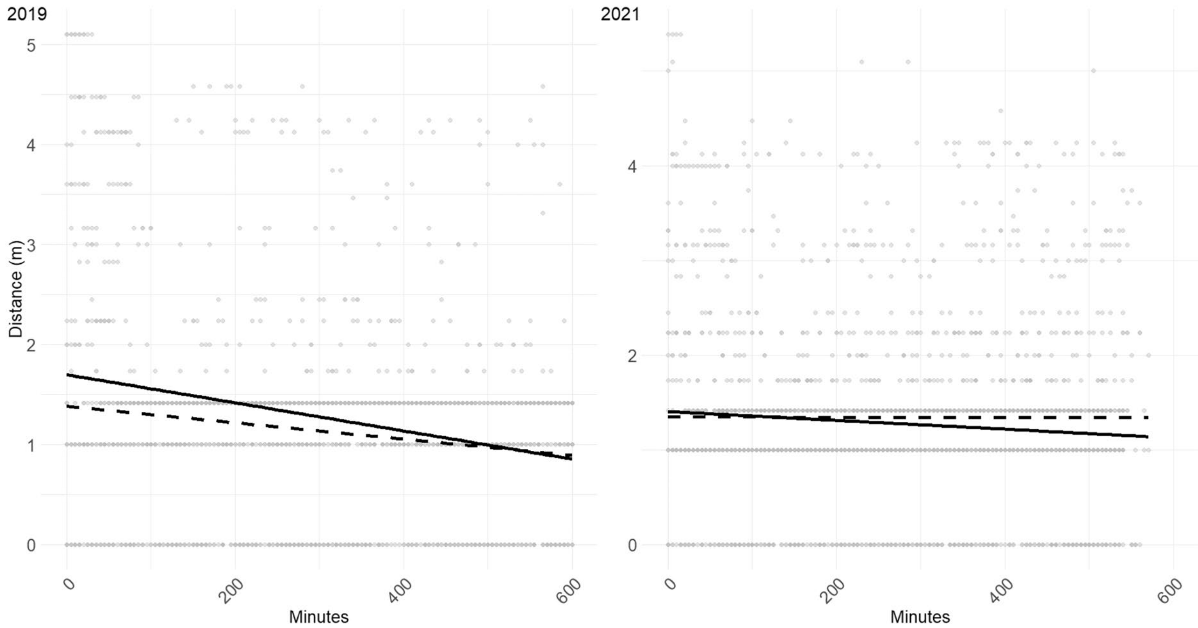

Fig. 4 Change in distance over time between male and female salmon in the presence (dashed line) and absence (solid line) of round goby in the respective years. Each grey point represents a raw data point

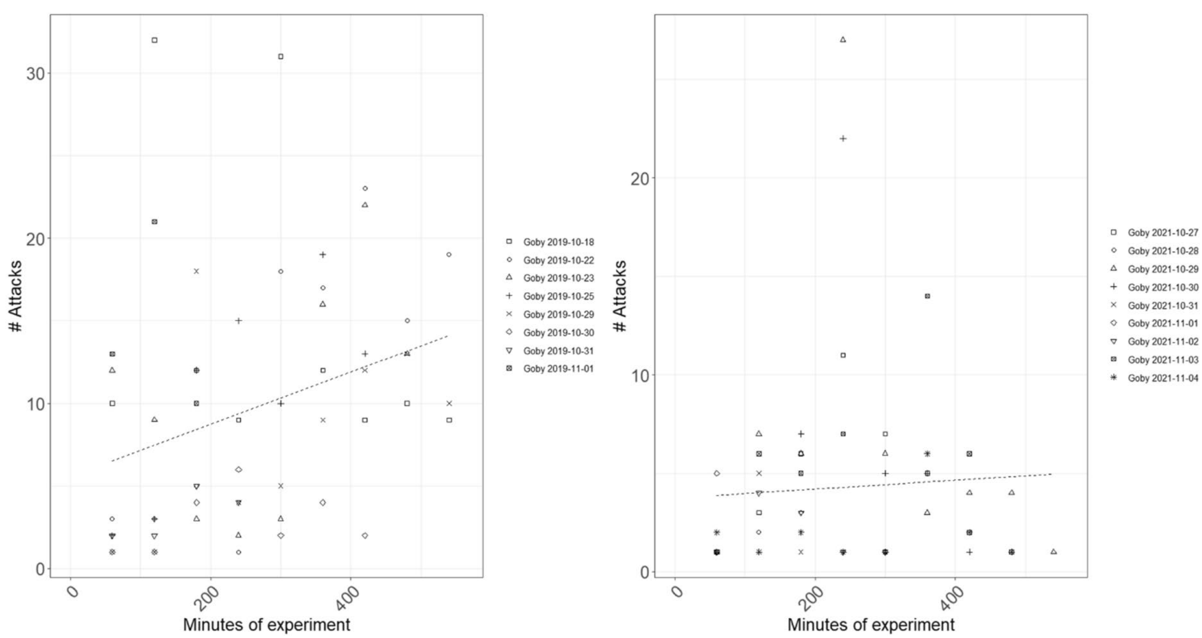

Fig. 5 Number of attacks per 30 minutes by salmon males on round goby over the duration of the trials in the respective years. Dots represent observations from the different trials (separated by symbols)

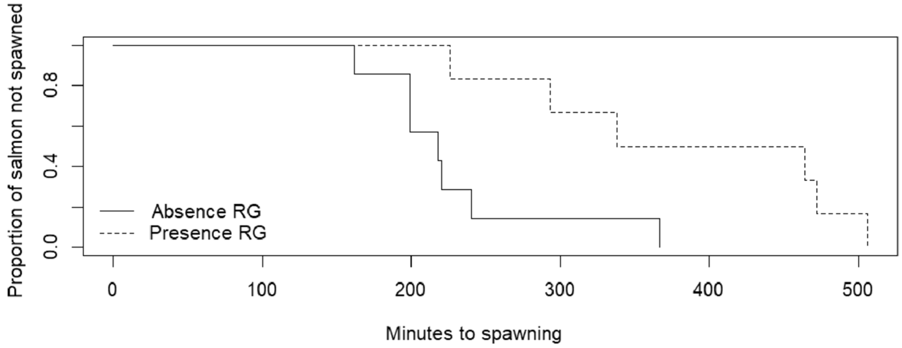

Fig. 6 Kaplan-Meier survival curves comparing the time until spawning between salmon in the presence (dashed line) and absence (solid line) of round goby. The probability of spawning represents the proportion of salmon that have not spawned at each time point

Finally, we found time until onset of spawning in salmon to be significantly longer in the presence of round goby.

Salmon resided significantly closer to each other in the treatment compared to in the control in 2019, while no such difference was evident in 2021. One explanation for this could be that the salmon would likely have resided closer to each other if they were prone to spawn, but as only one salmon pair out of eight spawned on the treatment side in 2019, there is little support for this explanation. The close proximity could instead potentially be explained by the relatively high round goby densities used in 2019. If high round goby densities, especially of males, induce a protective behaviour in salmon, this could make them stay closer together. This implies that there could be a density threshold of round goby that needs to be passed before this effect on salmonid spawning is seen. The distance between males and females decreased less over time in the presence of round goby compared to the control in 2019. This may likely be because the salmon resided closer together already in the beginning of the trials in the treatment in 2019, indicating also here a protective behaviour that appears to be dependent on round goby densities. The differences in flow velocity (Table 1) could in theory have affected the outcome between years; however, the distance between males and females was observed also between control and treatment in 2019, carried out under the same flow velocity conditions. Hence, flow velocity does not appear to be a major source of error in this case.

The trend in aggression, expressed as attacks, may indicate increasing frustration in salmon males over time. It may seem contradictory that we observed this trend in 2019, while at the same time the salmon male and female resided closer together in the round goby treatment in 2019 and also that the distance between them decreased over time. However, salmon position was noted every five minutes while attacks were recorded continuously, meaning that there was plenty of time to carry out attacks in between the observations of position. The confined space of the flume represents a limitation, as both salmon and round goby are spatially restricted and may behave differently under natural conditions where they can move freely. Salmonids under natural conditions may choose to move to suboptimal spawning grounds in order to avoid round goby, and round goby under natural conditions could choose to move away from spawning salmonids to avoid attacks. The attacks on round goby by salmon during spawning have similarities with chases of round goby by smallmouth bass during nest-guarding, which were reported to lead to impaired condition and lower energy reserves in males (Steinhart et al. 2005). Indirect effects, such as increased energy expenditure of salmon males due to attacks during spawning, need to be quantified but could potentially lead to lower survival rates of salmon males (reviewed by Jonsson and Jonsson 2009) or future reduction in fitness.

The detected delay and lower probability of salmon spawning in the presence of round goby indicate reproductive disturbance by round goby on salmonids. Salmon frequently attacked round goby, while salmon spawning behaviour was not affected by the presence of round goby. This could indicate that the delayed spawning in the treatment could be an effect of male aggression rather than disrupted spawning behaviour. However, it is also possible that the delayed spawning is a consequence of other behavioural effects that we did not quantify in this study.

For the analysis of probability and time until salmon spawning, the data was pooled for the two years to achieve sufficient sample size. However, the trials in 2019 and 2021 were conducted in different flow velocities, round goby densities and sex (Table 1), which introduced potential sources of error. Flow velocities were, however, very similar between years at the dates when the salmon were spawning (0.8 and 0.75 m/s), i.e. no spawning events took place in the lowest velocity used in the first three trials in 2019 (0.25 m/s). We therefore expect the very low variation in flow velocity during spawning to be of low to negligible importance. Regarding the variation in round goby densities and sex between 2019 and 2021, the majority of the salmon pairs in the treatment spawned in 2021 (five pairs, compared to one pair in 2019). Thus, the dataset is mainly based on data from 2021, when round goby densities were lower and the sexes were mixed (Table 1). Yet, the results were significant, which, in our opinion, strengthens our conclusion that it is, in fact, the presence of round goby that negatively affects probability and time until spawning in Baltic salmon.

The potential ecological consequences of the significant, albeit rather small, delay in salmon spawning in the presence of round goby are unclear. A small delay in itself may have a limited effect on salmonid reproduction. However, if the delay increases with, for example, density of round goby, salmonid reproductive success would be more likely to be negatively influenced at higher round goby densities. In addition, effects of climate change, such as high temperatures and low water levels in rivers and streams, have the potential to delay salmonid migration and spawning (reviewed by Jonsson and Jonsson 2009). The cumulative effects of these pressures could, in theory, cause substantial delay in salmonid spawning, potentially affecting time of hatching and lead to mismatch with suitable prey. Since salmon and sea trout have similar reproductive behaviour, we expect our results to be representative also for round goby interaction with sea trout. Salmon typically spawns in faster flowing larger rivers, often high up in the system, while sea trout spawns in smaller creeks and often closer to the coast (cf Thorstad et al. 2011, 2016). This means that sea trout may be even more at risk since round goby seems to prefer less turbulent water (Kotta et al. 2016) and lacustrine environment (McAllister et al. 2022). The indicated negative effect on salmonid reproduction by round goby has management implications as it underlines the importance to try to hinder further spread and subsequent establishment of round goby to freshwater rivers and streams around the Baltic Sea. In addition, the majority of the salmon pairs spawned in 2021 when the round goby densities were lower (7-9 ind/m^2, Table 1), but we still detected an effect as time until spawning was delayed in the treatment. The total effect needs to be quantified, but our findings suggest that round goby densities in salmonid holding freshwaters should be kept as low as possible to minimize the risk of negative effects on salmonids.

We suggest several follow-up studies to investigate the scale of impact as well as the underlying causes for change in salmon behaviour in the presence of round goby. The impact of variation in round goby densities on salmon spawning should be further studied, as we see indications of threshold effects. Further, in order to mimic natural conditions as much as possible sneaker salmon males could be added, as this common reproductive tactic may also cause disturbance for the spawning salmon pair (Fleming and Einum 2011). Round goby is territorial and aggressive (reviewed by Cerwenka et al. 2023; Vivó-Pons et al. 2023), and display of such behavioural traits could modify its interactions with native salmonids compared to functionally similar species in freshwater. We therefore see the need to study salmon spawning in the presence of native demersal freshwater species like European bullhead (*Cottus gobio*) at environmentally relevant densities, to be able to draw conclusions about whether the reproductive disturbance observed in salmon is species-specific or e.g. density specific. In addition to disturbance during spawning, other types of interactions with potential effects on salmon reproductive success should also be investigated, like round goby predation on salmon eggs or newly hatched alevins, or interspecific competition between round goby and salmon fry or parr.

Altogether, our results imply that reproductive success of the vulnerable Baltic Sea salmonids may be impaired under a scenario where round goby migrates upstream and establishes in rivers and streams emerging along the Baltic Sea coastline. We conclude that presence of the invasive round goby may lead to sub-lethal effects, such as reproductive disturbance, in salmonids, which could have species-specific and potentially ecosystem-wide consequences, adding to the many threats for salmonid populations in freshwater (Smialek et al. 2021).

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Author contributions Isa Wallin Kihlberg, Ann-Britt Florin, Tomas Brodin and Gustav Hellström contributed to the study concept and design. Isa Wallin Kihlberg and Ioannis Efstathiadis performed material preparation and data collection. Data analysis was performed by Gustav Hellström. Interpretation of the results was performed by Isa Wallin Kihlberg, Ann-Britt Florin and Gustav Hellström. The first draft of the manuscript was written by Isa Wallin Kihlberg and Gustav Hellström and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

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Data availability The datasets generated and analyzed during the study, as well as the code, are available at: https://doi.org/https://doi.org/10.5281/zenodo.10820804.

Declarations

Conflicts of interests The authors have no relevant financial or non-financial interests to disclose.

Research involving animals All fish handling and experimental work was carried out under the ethical permits 5.8.18-07747/2018 and 5.8.18-09363/2021 (Swedish Board of Agriculture, the Uppsala region Ethics Committee on Animal Experiments).

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