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Abstract

Native fauna in species poor communities, such as those of the Baltic Sea, may be particularly vulnerable to the effects of species invasions. However, the interspecific interactions that result in the negative impacts on native species tend to be poorly understood. One contributing factor to this knowledge gap may be that the vulnerability of native species can vary between different life-history stages. For example, the parental care phase is often risky both for the developing offspring and care-giving parents. Accordingly, we investigated the interactions between invasive mud crabs, *Rhithropanopeus harrissii*, and native nest-building littoral fish, with a special focus on the sand goby, *Pomatoschistus minutus*. Firstly, our field survey demonstrated that while the recently established mud crab and native nest-building fish have different habitat and depth optima, their distributions also have a considerable overlap, with a high potential for interactions between them. Secondly, our laboratory experiments indicate that the crabs are
likely to impact the fish negatively, especially by taking over occupied nests, as well as pre-occupying
nesting resources that are of very limited supply in the studied population. We did not find evidence for
successful predation on eggs guarded by male sand gobies naive to the mud crabs. Collectively, the results
suggest that the invasive crabs have high potential to negatively impact native fish. Furthermore, the
results support the scenario that the parental phase can be a particularly vulnerable life-history stage in face
of novel selection pressures, such as species invasions.

Keywords (separated by ‘-’) Baltic Sea - Benthic fish - Competition - Mud crab - Nest - Reproductive behaviour

Footnote Information
The impact of an invasive mud crab on brood success of nest-building fish in the Northern Baltic Sea

Topi K. Lehtonen · Outi Vesakoski · Juho Yli-Rosti · Anniina Saarinen · Kai Lindström

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Abstract Native fauna in species poor communities, such as those of the Baltic Sea, may be particularly vulnerable to the effects of species invasions. However, the interspecific interactions that result in the negative impacts on native species tend to be poorly understood. One contributing factor to this knowledge gap may be that the vulnerability of native species can vary between different life-history stages. For example, the parental care phase is often risky both for the developing offspring and care-giving parents. Accordingly, we investigated the interactions between invasive mud crabs, Rhithropanopeus harrissii, and native nest-building littoral fish, with a special focus on the sand goby, Pomatoschistus minutus. Firstly, our field survey demonstrated that while the recently established mud crab and native nest-building fish have different habitat and depth optima, their distributions also have a considerable overlap, with a high potential for interactions between them. Secondly, our laboratory experiments indicate that the crabs are likely to impact the fish negatively, especially by taking over occupied nests, as well as pre-occupying nesting resources that are of very limited supply in the studied population. We did not find evidence for successful predation on eggs guarded by male sand gobies naive to the mud crabs. Collectively, the results suggest that the invasive crabs have high potential to negatively impact native fish. Furthermore, the results support the scenario that the parental phase can be a particularly vulnerable life-history stage in face of novel selection pressures, such as species invasions.

Keywords Baltic Sea · Benthic fish · Competition · Mud crab · Nest · Reproductive behaviour

Introduction

Invasive species are one of the leading global threats to biodiversity (Sakai et al. 2001; Clavero and García-Berthou 2005). For instance, invasive predators have been found to have a greater negative impact on prey populations than similar native predators (Salo et al. 2007). However, the vulnerability of native species is likely to vary depending on their life-history stage (Ficetola et al. 2011, 2012; Siesa et al. 2014),
contributing to the difficulty of predicting the impacts of interactions between invaders and natives. The phase of parental care (when present) may be a particularly susceptible life-history stage, in terms of affecting the fitness of two generations. For example, parents of native species may react incompetently towards novel predators of offspring due to a lack of joint evolutionary history with them (Cox and Lima 2006; Freeman and Byers 2006; Sih et al. 2010; Wong and Candolin 2015). Such suboptimal responses are seen in a biparental Neotropical cichlid fish, *Amphilophus zaliosus*, when the parents are facing an introduced, potential predator of their offspring (Lehtonen et al. 2012). Similarly to predators, novel competitors could conceivably decrease the fitness of the parents, e.g. by interfering with their reproductive behaviours.

Native fauna in communities with low species diversity are thought to be particularly vulnerable to the effects of species invasions (Stachowicz et al. 1999; Leppäkoski and Olenin 2000; Strauss 2014). Such communities can also provide excellent opportunities to assess novel species interactions and, in the longer term, evolutionary change in traits such as anti-predator behaviours. One of the most recent invaders in the species poor community of the Northern Baltic Sea (as described by Paavola et al. 2005) is the mud crab *Rhithropanopeus harrissii* (Fowler et al. 2013), also known as the ‘white-fingered mud crab’, ‘the estuarine mud crab’, ‘Harris mud crab’, and ‘dwarf mud crab’. From hereon, we refer to the species simply as ‘mud crab’. This mud crab species is native to the Atlantic coast of North America (Williams 1984), from where it has recently proliferated around the globe, mostly via the ballast waters of ships (Roche and Torchin 2007).

The mud crab was first found on the coast of Finland, in the Northern Baltic Sea, in 2009, and has since been expanding its local range, and as a result, now occupies multiple habitats (Fowler et al. 2013). Regarding their preferred habitat, mud crabs have previously been found to select vegetated boundary areas over more bare substratum, cover provided by the bladder wrack, *Fucus vesiculosus*, over cover by other local algae (Aarnio et al. 2015; Nurkse et al. 2015), and cavities provided by stones over shelter provided by vegetation (Riipinen et al. 2017). The mud crab also has a high potential for impacting native species within the invaded habitats, as it has been found to predate on native immobile and mobile fauna (Forsström et al. 2015; Lokko et al. 2016), and isotope analyses suggest that the diet of larger mud crabs (> 12 mm) corresponds to a relatively high position (that of a secondary consumer) in the food web (Aarnio et al. 2015). Recently, mud crabs were also found to have a drastic effect in the community associated with the habitat forming brown alga bladder wrack, with a decrease in local species diversity, as well as decline in the growth of the bladder wrack, during a 3-year rapid increase of mud crab numbers (Jormalainen et al. 2016). Given the mud crab’s preference for crevices amongst rocks as shelters (Riipinen et al. 2017; personal observations), the species also has a high potential to interact with native species that may have a period of stationary egg defence in a nest or crevice along the seafloor. Not only might this omnivorous crab species predate on fish eggs, but it may also interfere with parental care strategies (e.g. egg fanning), in addition to competing for suitable cavities and other potential nesting resources with species such as gobies (e.g. the common goby, *Pomatoschistus microps*), sticklebacks (e.g. the three-spined stickleback, *Gasterosteus aculeatus*) and sculpins (e.g. the European bullhead, *Cottus gobio*). In this regard, we expect that in cases when suitable hiding cavities are in short supply, as is the case on most sandy substrate bottoms in the Northern Baltic Sea (Forsgren et al. 1996; Lehtonen and Lindström 2004), mud crabs should have the potential to have negative impacts on such fish species by expelling the egg-guarding male from the nesting cavity it is occupying.

One particularly well-studied nest-building fish, with a wide distribution range along the European coasts (Miller 1986), is the sand goby, *Pomatoschistus minutus*. It is the most common species of littoral fish in many parts of the Baltic Sea (Jansson et al. 1985; Zander 1990; Sundell 1994; Parmanne and Lindström 2003) and an important node in the food web of the littoral zone (Fonds 1973), as demonstrated by the wide array of parasites that have adapted to exploit it as an intermediate host (Zander et al. 1993). In many areas, sand gobies compete intensively for suitable nesting resources, such as mussel shells, which are small and sparsely distributed in the Northern Baltic Sea (Forsgren et al. 1996; Singer et al. 2006; Wong et al. 2008), or flat stones lying on fine sand (Lehtonen and Lindström 2004). Similarly to e.g. common gobies, the male sand goby first prepares a nest by displacing sand from under a nesting resource.
The impact of an invasive mud crab on brood success

(typically < 100 cm² in surface area). It attracts females to lay eggs in a mono-layer onto the roof of the nesting chamber, and then guards the nest and cares for the eggs until they hatch. Nesting behaviours of gobies (e.g. black gobies, Gobius niger) in areas of rocky, hard substrate are similar, except that the male needs to use existing crevices as there is no fine sand to displace (Lehtonen and Lindström 2004; Järvi-Laturi et al. 2008). In the case of the sand goby, the use of the rocky substratum type appears to be a unique, local adaptation to a low abundance of nest predators (Lehtonen and Lindström 2004; Järvi-Laturi et al. 2008). In particular, sand gobies seem to be using such cavities under and amidst stones for breeding only in the Northern Baltic Sea (Zander 1990; Lehtonen and Lindström 2004), where formidable predators of goby eggs, marine crabs and dogwhelks, Nucella lapillus, have thus far been absent, not withstanding the rare occurrence of non-breeding individuals of the Chinese mitten crab, Eriocheir sinensis (see Bonsdorff 2006).

Given the above, we expected that the invasive mud crab may impact the breeding of nest-building and cavity-breeding fish, if the fish and crabs co-occur within the same areas and habitats.

The aim of the current study was to use the recent invader, the mud crab R. harrisi, as a model to investigate the influence of newly established crabs on local nest-building fish. In this context, 'nest-building fish' are broadly defined as any fish species that uses a crevice or other nesting resource on the seafloor during parental care of eggs or juveniles. We first surveyed multiple habitat types and water depths in the field to identify the species of fish that co-occur with the mud crab, particularly focusing on the overlap between mud crabs and nest-building fish. We also conducted two laboratory experiments to investigate (i) whether mud crabs compete with sand gobies for the type of structures that sand gobies use for nesting, (ii) whether the presence of mud crabs influences nest-building of sand gobies naïve to crabs, and (iii) whether the presence of mud crabs affects survival of sand goby eggs.

Methods

Field study

The field study was carried out in June–August in 2012, in the surroundings of the Archipelago Research Institute of the University of Turku, on the Finnish coast of the Baltic Sea (lat. 60°14.2′N; long. 21°57.5′E; Fig. 1). Here, the aim was to investigate the extent to which the invasive mud crab co-occurs with native nest-building fish. For this purpose, we used data from a field study by the Finnish Environment Institute’s Marine Research Centre, with other aspects of that study, i.e. assessment of methods for monitoring marine invasive species, being presented elsewhere (Forssström et al., unpublished data).

The approach in the field study was to catch small, seafloor-oriented species, such as benthic fish and invasive mud crabs, using traps (see below for trap details). The traps were placed in three different habitats, each represented by three distinct ‘locations’ (Fig. 1), with three different depth categories sampled at each location. Hence, in total we had 3 × 3 × 3 = 27 sampling sites. Each sampling site, in turn, had either two slightly different traps (n = 22 sampling sites, see below for information about the two trap types) or only one trap (n = 5 sampling sites with an ‘American trap’, see below). Hence, n = 49 traps were included in the study. The distance between the nearest traps was always ≥ 3 m, whereas the distances between the nine locations (three per habitat type) are shown in Fig. 1.

The three habitat types sampled for the survey—soft substrate, sandy substrate and Fucus habitat—represent typical benthic environments in the area, with different animal and macrophyte communities inhabiting each of them. Previous findings suggest that both mud crabs and breeding individuals of nest-building fish can be found in most, if not all, of these habitats (Lehtonen and Lindström 2004; Fowler et al. 2013; Gagnon and Boström 2016; Riipinen et al. 2017). Soft substrate habitat had a layer of organic material and silt on the bottom, typically combined with low water clarity and the common reed, Phragmites australis, as the dominant macrophyte. Sandy substrate habitat was characterised by relatively fine-grained sand with patches of vascular plants, such as the eelgrass, Zostera marina, and low densities of stones and rocks. Finally, hard substrate habitat, from hereon Fucus habitat, was covered with coarser gravel or rock substrate and had a moderate to high density of the bladder wrack, F. vesiculosa, growing on the seafloor, forming an important perennial environment for benthic fauna. While no detailed underwater maps of the region are available, underwater habitats are...
likely to be highly affected by the fragmented island mosaic of the archipelago (Fig. 1), with soft substrate areas being more common than the other two habitat types within the area covered by the survey (personal observations).

The three sampling depth categories, 1, 1.5–2 and 3–4 m, covered the typical depth range in which all three habitat types can be found, and in which many littoral fish have been found to occur during the reproductive season (Lappalainen and Urho 2006).

The traps, placed on the seafloor, were filled with pieces of clay flowerpots and lava rocks to provide a network of cavities, with animals being able to move in (and out of) the traps through holes in the walls and ceiling. The dimensions of the traps were 30 cm × 30 cm × 30 cm. They had either both the ceiling and walls made of 1 cm × 1 cm plastic mesh with 2–3 larger holes of approximately 2 cm × 2 cm in each wall (‘mesh trap’), or the 1 cm × 1 cm mesh ceiling was combined with plastic walls with 2 cm × 2 cm holes (9 holes per wall; ‘American trap’). Crabs of various sizes, including those having carapace width of > 2 cm, commonly enter such traps, because their movement is limited by the narrowest dimension of their carapace (rather than its width), while the distance between the opposite corners of a 1 cm × 1 cm grid is $\sqrt{2}$ cm. The bottom of all traps was covered with plastic sheets. Because such traps capture aquatic fauna by providing attractive places of shelter, they are also sometimes called ‘habitat traps’ (sensu Fowler et al. 2013).

Each trap was checked approximately once a week (in total 2–7 times, except for 1 trap that got lost after the first check). When we checked traps, they were lifted quickly to a boat and their contents were emptied into a bucket with seawater. Because the traps were filled up with pieces of clay pots, and had their bottom parts covered with plastic sheets, the traps caught a

![Diagram](image)

**Fig. 1** The 9 locations of the field survey cover 3 types of habitats, as indicated by the star symbols. In addition, black dots show observations of mud crabs on the Finnish coast between 2011 and 2014, as reported by the general public (data collected and provided by the Finnish Environment Institute).
wide range of aquatic animals, including mud crabs and fish, even without wrapping at the time they were lifted out of the water. After carefully removing the trap filling materials from the bucket, the catch was immediately filtered through a 1 mm sieve. All animals discovered with this method were then moved to labelled sample containers and identified later.

Experiment 1: nest building behaviour

The laboratory experiments were conducted at the Tvärminne Zoological Station of the University of Helsinki (lat. 59°50.7′N; long. 23°15.0′E), in June 2012, during the breeding season of the local sand goby population. Sand gobies were chosen as the model system because the species readily exhibits the full range of breeding behaviours within a short timeframe in aquarium conditions (see e.g. Lehtonen 2012), and occurs in high numbers across multiple habitats, making it readily available for experimental set-ups. The aim of experiment 1 was to investigate whether the presence of the invasive mud crab affects the motivation of fish naive to its presence, in terms of investment in nest building or adjustment of nest characteristics. For this purpose, we used male sand gobies from the proximity of the Tvärminne field station, where mud crabs do not yet occur (as of 2016). The fish were exposed to the following three treatment levels: presence of a mud crab, presence of a brown shrimp, Cragon cragon (also known as the ‘sand shrimp’, ‘common shrimp’ and ‘bay shrimp’), which is a native shrimp that is known to predate at least on unguarded goby eggs (Chin-Baarstad et al. 2009), and a control treatment in which intruders were absent.

Sand gobies, as well as brown shrimp used in this study, were collected in shallow sand bottom areas using a hand-trawl (see Lehtonen and Kvarnemo 2015) and dip nets. At the station, both gobies and shrimp were housed in several aquaria (50–100 l), with the gobies being separated by sex. All aquaria were kept under natural light conditions and were supplied with continuous flow-through of seawater. Before the commencement of the experiment, sand gobies were fed twice a day with live mysid shrimp, whereas brown shrimp were fed with frozen chironomid larvae. Mud crabs used in our laboratory experiments were caught ~100 km northwest on the Finnish coast (lat. 60°24.5′N; long. 22°26.4′E) using traps similar to those of the field study, and then transported to the field station. The mud crabs were housed in separate sex stock tanks similar to those used for housing gobies and brown shrimp, and were fed with frozen chironomid larvae.

Immediately prior to the onset of the experiment, gobies, mud crabs and shrimp were weighed using an electronic balance. The carapace width of mud crabs was also measured using a calliper. At the onset of each trial, one sand goby male was placed into an experimental arena measuring 18 cm × 25 cm × 20 cm (length × width × water depth), with a 4 cm layer of fine sand as the substratum. In each arena, a ceramic tile (measuring 10 cm × 10 cm) was placed on top of the substratum in the middle of the tank as a nesting resource. Such tiles are similar in size to flat stones that sand gobies often use for nest construction in the wild (Lindström 1992; Lehtonen and Lindström 2004; Wong et al. 2008), and have previously been used successfully as sand goby nesting resources both in the wild and laboratory (Wong et al. 2008; Lehtonen et al. 2013, 2015). Each male sand goby was haphazardly assigned to one of the three treatments. In the mud crab treatment, one crab (n = 38 replicates; mean carapace width ± SD: 17.6 ± 3.5 mm; weight ± SD: 2.66 ± 1.07 g) was released in the arena at the same time with the male goby (n = 38; weight: 0.97 ± 0.21 g, corresponding to ~ 5 cm of total length). Crabs of that size are not large enough to predate on adult gobies (Lissäker and Kvarnemo 2006). In the brown shrimp treatment, one brown shrimp (n = 38; weight: 1.32 ± 0.32 g, corresponding to ~ 5 cm body length) was released into the arena at the same time with the male goby (n = 38; weight: 1.06 ± 0.34 g). In the control treatment, only the male goby was introduced (n = 36; weight: 1.11 ± 0.29 g). One individual of a species in each tank corresponds to natural densities of these animals in the wild. In particular, it is uncommon that a comparable area of seafloor area is occupied by more than one nesting sand goby male (e.g. Lehtonen and Lindström 2004). One brown shrimp within a comparable area of seafloor is also very typical on sandy substrata (personal observations). Densities of the invasive mud crabs, in turn, are highly site dependent (Fowler et al. 2013; Gagnon and Boström 2016).

We checked all tanks every 3–4 h between 09:00 and 21:00 for signs of nest building. Nets were not checked between 21:00 and 9:00. A male was deemed to have initiated nest building when it started to pile sand on top of, and excavate under, the tile (Wong
et al. 2008; Japoshvili et al. 2012; Lehtonen et al. 2013). If a male did not start to build a nest within 48 h, the replicate was terminated. Nest characteristics were assessed for replicates in which the male did start to build a nest within 48 h. After the first signs of nest building, the male was left in the tank for another 24 h to complete his nest (nest building typically takes from one to a few hours). We then measured the following two ecologically relevant and commonly used measures of nest construction in gobies (see Japoshvili et al. 2012): (1) the amount of sand the male piled on top of the tile, and (2) the width of the nest entrance (also called ‘nest-opening’ sensu Svensson and Kvarnemo 2003). To measure the amount of sand piled on top of the tile, we carefully collected the sand by lifting the tile into a tray. The sand was later dried for 36 h at 60 °C, after which its dry weight was measured using an electronic balance (Lehtonen and Wong 2009; Lehtonen et al. 2015). The width of the nest entrance was measured using a ruler.

Experiment 2: egg survival

The aim of this experiment was to quantify the survival of sand goby eggs when the eggs were tended by a male (1) in the presence of a mud crab, (2) in the presence of a brown shrimp or (3) in the absence of any intruders. In all treatments, one male sand goby was placed into a tank, which measured 18 cm × 25 cm × 20 cm (length × width × water depth) and had a 4 cm layer of fine sand on the bottom. The male was provided with two identical nesting resources of halved clay flowerpots (diameter: 6.5 cm), lined with a piece of transparent acetate onto which eggs were attached during spawning. Such artificial nesting resources are readily accepted by sand gobies in both laboratory conditions and the field (Forsgren et al. 1996; Lehtonen and Lindström 2007; Japoshvili et al. 2012). The purpose of the second clay pot was to function as a potential shelter for the intruder (mud crab or shrimp). Egg survival was assessed by photographing the acetate film as soon as eggs were laid on it and then again after 7 days, which is close to a typical hatching time (Fonds and Van Buurt 1974; Lehtonen and Lindström 2007). The numbers of eggs were later counted from the two photographs. The most important sources of egg losses were likely to be filial cannibalism by the focal goby male and egg predation by the intruder (Lissåker and Kvarnemo 2006; Lehtonen and Lindström 2007; Chin-Baarstad et al. 2009).

All focal males were given at maximum 48 h to initiate nest building. If the goby did not initiate nest building within 48 h, the replicate was restarted with another individual. When a male had finished building the nest, a female goby that had her belly distended with eggs (n = 53; weight: 1.03 ± 0.30 g) was added into the tank. As soon as the fish had spawned, the female was removed and the transparent film with the eggs was photographed (Olympus XZ-1 digital camera), and the eggs were then carefully placed back into the nest (Lehtonen and Lindström 2007). At this point, an intruder was introduced: a mud crab (n = 19, carapace width: 17.3 ± 2.0 mm, weight: 2.27 ± 0.77 g; sand goby weight: 0.94 ± 0.30 g) in the crab treatment, a brown shrimp (n = 17, weight: 1.58 ± 0.50 g; sand goby weight: 1.02 ± 0.28 g) in the shrimp treatment. To control for the potential disturbance of introducing the intruder, the same amount of water but without an intruder was poured into control treatment replicates (n = 17; sand goby weight: 1.03 ± 0.32 g). After 48 h, the tanks were again disturbed with a hand net and the intruders in the crab and shrimp treatments were removed. This was done to replicate invader densities in which they are not constantly present throughout the brood cycle of the fish, and also to allow the focal male goby to be fed without an intruder, as described below.

During the nest building phase, the males in all treatments were fed 4 frozen chironomid larvae daily. Feeding was suspended when the female was added in the tank and then resumed after the intruder presence period had ended (the same schedule was followed in the control replicates). The trials were completed after 7 days of egg care and gobies and brown shrimp were then released back to the sea, whereas the invasive mud crabs were euthanized. Statistical analyses

When analysing the field survey data, we considered whether or not a trap, when checked, had caught any individuals of the species of interest, i.e. we considered binomial absent/present data. The occurrence of mud crabs and nesting fish at the different depths and habitats was analysed using a generalized mixed model with a binomial distribution and the species type (mud crab/nesting fish), depth category (1 m/
1.5–2 m/3–4 m) and habitat (soft/sand/Fucus) as fixed effects, and trap type (‘American trap’/‘mesh trap’) as a covariate. The covariate was added to account for 5 of the 27 sampling sites (location × depth combinations) having only one of the two trap types. Similarly, to account for the procedure of counting both mud crabs and fish captured by the same 1–2 traps at each sampling site, ‘sampling site ID’ was added as a random effect. We then proceeded with stepwise refits of the model, each time excluding the least significant, highest order interaction term, using $\chi^2$-tests with $p = 0.05$ as the cut-off point (Crawley 2007). We used R 3.2.2 software (R Development Core Team) for the field survey analysis.

The effect of the intruders on the time until the start of nest building in experiment 1 was analysed using a Cox proportional hazards model, ‘right censoring’ (Lagakos 1979) the 60 replicates in which a nest was not built within 48 h. Treatment and male weight were used as explanatory factors. To investigate nest characteristics, we ran a linear model for the amount of sand on nest (i.e. nest elaboration) and nest entrance width separately. In both cases, we had the treatment and male weight as fixed effects. Sand weight was log-transformed for improved normality. We then assessed whether the models could be refitted without the treatment × weight interaction by using a $\chi^2$-test (with $p = 0.05$ as the cut-off point).

For experiment 2, egg survival was assessed by using both the number of eggs present at the end of the experiment and the number of ‘failures’, i.e. the difference between initial and final number of eggs in the nest. As the data did not meet assumptions of a binomial distribution due to over-dispersion, we applied a generalized model with quasi-binomial distribution. Treatment and male weight were used as explanatory variables and model simplification was conducted as above. Experiments 1 and 2 were analysed using R 3.0.2 software (R Development Core Team).

### Results

#### Field study

Our traps caught in total of 561 mud crabs and the following nest-building fish: 98 individuals of black gobies, 3 sand gobies, 1 common goby, 1 three-spined stickleback, and 1 ninespine stickleback, _Pungitius pungitius_. The traps also caught 6 individuals of other litoral fish species that do not build nests.

The generalized mixed model indicated that nest-building fish and mud crabs significantly differed in their habitat and depth preferences, with both species category × habitat ($\chi^2 = 36.1, df = 2, p < 0.001$) and species category × depth ($\chi^2 = 9.14, df = 2, p = 0.010$) interactions being significant. In particular, nesting fish were the most common in shallow water and sandy habitats, whereas mud crab catches were higher in the other two habitat types (Fig. 2) and deeper water (Fig. 3). Nevertheless, there was considerable overlap between the distribution of mud crabs and nesting fish across the different depths and habitats, with 96% (25 out of 26) of the traps that had nesting fish also having mud crabs, either at the same time or another time those traps were checked (Figs. 2, 3).

#### Experiment 1: nest building behaviour

Neither intruder treatment (Cox proportional hazards model, treatment effect, $\chi^2 = 3.11, df = 2, p = 0.21$; Fig. 4), male weight ($\chi^2 = 1.40, df = 1, p = 0.24$), nor their interaction ($\chi^2 = 1.36, df = 2, p = 0.51$) had a significant effect on time until the gobies started nest building (Likelihood ratio test on the full model).
The number of males that built a nest within 48 h was 22 out of 36, 15 out of 38, and 17 out of 38 in the brown shrimp, mud crab, and control treatments, respectively. The probability of a nest take-over in the presence of a mud crab was 0.103–0.560 (assessed by a binomial test). The probability for a nest take-over in the control treatment (without an intruder) was 0, and we are also not aware of the brown shrimp taking over sand goby nests. The 5 take-overs with a mud crab present resulted in the treatments to differ in the rate of nest destructions ($G$ test of independence with Williams’ correction, $G^2 = 11.17$, $df = 2$, $p = 0.004$). In two additional mud crab replicates, in which the male was not recorded to have built a nest, the mud crab was using the nesting resource (i.e. tile) as a hiding place at the end of the trial. We do not know whether the mud crab occupied the tile before any nest-building attempts by the goby male or whether the crab took over the tile during initial stages of a nesting attempt.

The amount of sand on those nests that were not taken over during the first 24 h after the onset of nest building was independent of the treatment (after exclusion of a non-significant treatment $\times$ male weight interaction, linear model, $F_{2,46} = 0.003$, $p = 1.0$). The males piled $38 \pm 38$ g ($n = 22$), $32 \pm 25$ g ($n = 14$) and $45 \pm 36$ g ($n = 12$) of sand on the nest ridge in the control, brown shrimp and crab treatments, respectively. There was a non-significant tendency for heavier males to pile more sand on the nest ($F_{2,46} = 3.89$, $p = 0.055$). In the model with a non-significant interaction removed, nest entrance width did not significantly differ among the treatments (linear model, $F_{2,42} = 0.165$, $p = 0.85$), with the entrance widths of 23 $\pm 10$ mm ($n = 22$), 23 $\pm 8$ mm ($n = 14$) and 21 $\pm 5$ mm ($n = 10$), in the control, shrimp and crab treatments, respectively. There was a non-significant tendency for larger males to have wider nest entrances ($F_{1,42} = 2.89$, $p = 0.096$). In two crab and one shrimp replicate, the goby had completely closed the nest entrance, resulting in nest entrance measures to be missing in these replicates.

**Experiment 2: egg survival**

In the model without the non-significant interaction, neither intruder treatment (generalized linear model, $F_{1,49} = 0.663$, $p = 0.52$) nor male size ($F_{1,49} = 0.723$, $p = 0.40$) had an effect on the proportion of eggs surviving over the 7 day period (Fig. 5). The mud crab took over the goby nest with eggs in one replicate, indicating that the 95% confidence interval for the probability of a nest take-over in the presence of a mud crab was 0.103–0.560 (assessed by a binomial test). The probability for a nest take-over in the control treatment (without an intruder) was 0, and we are also not aware of the brown shrimp taking over sand goby nests. The 5 take-overs with a mud crab present resulted in the treatments to differ in the rate of nest destructions ($G$ test of independence with Williams’ correction, $G^2 = 11.17$, $df = 2$, $p = 0.004$). In two additional mud crab replicates, in which the male was not recorded to have built a nest, the mud crab was using the nesting resource (i.e. tile) as a hiding place at the end of the trial. We do not know whether the mud crab occupied the tile before any nest-building attempts by the goby male or whether the crab took over the tile during initial stages of a nesting attempt.

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crab was 0.001–0.260 (binomial test). This means that the rate of nest takeovers did not significantly differ between experiments 1 and 2 (Fisher’s exact, \( p = 0.081 \)). As above, no signs of nest take-overs were expected, or recorded, in the two other treatments.

Discussion

Our results show that the invasive mud crab co-occurs with several species of littoral fish, especially gobies, along the coastal areas that it has invaded. In the laboratory, mud crabs took over and destroyed between 5% (1 out of 19 in experiment 2) and 29% (5 out of 17 in experiment 1) of the nests built by male sand gobies. Despite this threat to the nests, motivation of the goby to build a nest did not seem to be affected by the presence of a mud crab, as there was no significant difference between the proportion of males that built a nest (and hence initiated a breeding attempt) in the three treatments.

For assessing the potential ecological impact of invasive species to natives, it is important to know how likely the two are to encounter each other. In this respect, the field survey indicated a considerable overlap in the distribution of nest-building fish and mud crabs, as demonstrated by 52% of the traps capturing both nesting fish and mud crabs at the same time at least once (Figs. 2, 3). However, the peaks of fish and mud crab distributions differed. It is possible that the presence of mud crabs in a trap (or sampling site) affected the likelihood of nesting fish to seek shelter in the same trap. This, in turn, could have inflated the differences in the distributions of mud crabs and fish. More generally, the presence of individuals of other species in the traps may have affected subsequent accumulation of individuals of other species. Our statistical analyses, however, were not designed to detect the presence (or absence) of such patterns. It is also important to note that the traps were optimised to catch a wide array of benthic invasive animals, and their efficiency in catching fish was likely to be species specific. For example, our fish catch was heavily dominated by black gobies, although at least a subset of the sampled sandy substrate and Fucus (i.e., rocky substrate with bladder wrack) areas were inhabited by dense populations of sand gobies and three-spined sticklebacks, respectively (personal observations). In this respect, black gobies may have been particularly prone to being caught by the traps we used, or their presence may have negatively affected the occurrence of other nesting fish in the same traps. Even if this was the case, our results show considerable overlaps in the small scale distributions of invasive mud crabs and native nest-building fish. In particular, the results indicate that at each of the assessed habitat type and depth, there is a high potential for encounters between mud crabs and nest-building fish, despite the differences in their peak distributions.

Nest-building behaviour (see below for details) was not affected by the presence of a mud crab or brown shrimp, and although the proportion of surviving eggs was the lowest in the crab treatment, the difference between treatments in hatching success was not significant. Hence, our results suggest that, unlike crabs of similar size that are native on the Swedish west coast (Lissåker and Kvarnemo 2006), mud crabs do not seem to be effective predators on sand goby eggs while the eggs are guarded by the male. Similarly, the lack of treatment difference probably indicates that the presence of a mud crab did not significantly compromise the efficiency of male parental behaviours, such as egg fanning (see Lissåker and Kvarnemo 2006; Järvi-Laturi et al. 2008). Mud crabs may nevertheless have important cumulative effects on nesting fish. To demonstrate this, we may consider the results of the crab treatments over the two lab experiments: in these, 15 of the 38 gobies successfully started nesting, on average 17.3% of nests were destroyed over the two experiments, and

![Fig. 5](image_url) Egg survival in the three treatments in experiment 2. Means of proportional survival with standard errors and sample sizes are shown.
700 species have the capacity to adapt the design of their nests, presumably to use them as a shelter. As suitable nesting structures are a common limiting resource for reproduction in sand gobies, and potentially other nesting fish, in the Northern Baltic Sea (Forsgren et al. 1996; Lehtonen and Lindström 2004), an abundant mud crab population could be expected to negatively affect breeding attempts of such fish.

Previous studies suggest that at least some nesting species have the capacity to adapt the design of their nests according to the local environmental conditions. For example, three-spined sticklebacks built smaller and more streamlined nests in flowing than still water (Rushbrook and Barber 2008). In the context of egg predation, sand gobies on the western coast of Sweden reduced the size of their nest entrances in the presence of shore crabs, Carcinus maenas (Lissåker and Kvarnemo 2006). In the current study, male sand gobies did not adjust the size of nest entrance (or the amount of sand on top of the nest) in the presence of mud crabs. This difference between the populations in the response to (relatively similar) crabs might imply that because sand gobies in the Northern Baltic (including the study population) are naive to the presence of crabs, and they do not adequately adjust their behaviour in crabs’ presence (see Cox and Lima 2006; Freeman and Byers 2006; Sih et al. 2010; Wong and Candolin 2015). Besides such naivety of local sand gobies, other factors may have also contributed to the lack of observed adjustments of nesting behaviours in the presence of mud crabs. For example, behavioural adjustments might have been too minute to be detected by our sample sizes, sand goby males in the Northern Baltic Sea may show an overall low level of responsiveness to potential egg predators, and the adjustments of nest building in experiment 1 could also have been larger if there had been eggs present in the nest.

In this respect, an interesting scope for future studies is to assess whether prolonged presence of mud crabs in the community will select for changes in nesting (behaviour) of sand gobies and other nesting fish.

Successful nest take-overs by mud crabs, and the potential cumulative effects of their presence, imply that the species might have a negative effect on an array of native species of nest-building fish. Due to the significance of these small fish in food webs (Fonds 1973; Zander et al. 1993), even a mere decline in their abundance could induce unpredictable changes in the functions of local communities (see also Forström et al. 2015; Jormalainen et al. 2016). Furthermore, the highly unusual, novel pattern of habitat use by sand gobies in the Northern Baltic Sea—reproduction using crevices amidst stones on rocky bottom habitats—may be attributed to the absence of marine egg predators such as crabs and dogwhelks in this low salinity environment (see Lehtonen and Lindström 2004; Järvi-Laturi et al. 2008, 2011). Therefore, it is possible that the arrival of mud crabs will impact individuals displaying this unique behaviour particularly strongly. However, there are also more nesting opportunities for gobies in the rocky habitat (Lehtonen and Lindström 2004), potentially diluting the effects of crevice competition with mud crabs. In this respect, the conditions in our experiment 1 (nest take-over rate 29%) may have corresponded to a high crab density, as the mud crab was present at the onset of nest building and there was no extra shelters in the tank. In contrast, during experiment 2 (5% take-overs), the interactions with the intruder took place only after egg-laying in a tank with an additional shelter structure. Hence, the conditions in the latter experiment could be reminiscent of lower crab densities, or higher nesting structure availability. Our setup was not designed to detect differences in the rate of destroyed nesting efforts between the experiments and, indeed, no such difference was found. Therefore, more investigations are needed to assess potential density-dependent impacts of mud crabs.

Many crab species are considered to be habitat engineers in the sense that they alter the availability of resources for other species (Jones et al. 1994). For example, their burrows can change the structure of the sediment or substrate, in other words, cause ‘bioturbation’ (Wallentinus and Nyberg 2007; Smith and Green 2015). Such habitat alterations can negatively...
impact other species that use cavities on seafloor for nesting and/or shelter. In this respect, our results indicate that crabs may affect benthic fish either by occupying suitable nesting and shelter cavities, by taking over nest cavities already occupied by fish, or by disturbing individuals that are in the parental phase. Hence, the interaction between nesting fish and mud crabs (in relation to shelter use) can be considered amensalistic: mud crabs can affect fish negatively when seeking cavities and especially by destroying nests, while not directly benefiting from the destruction of the nests. In the Baltic Sea, the most notable fish species that may be affected in such a way are gobies (such as the common goby, the black goby and the two-spotted goby, Gobiusculus flavescens) and sticklebacks (such as three-spined stickleback and ninespine stickleback). It is worth noting that black gobies, which were most commonly caught with mud crabs, are typically larger than sand gobies and are therefore potentially highly capable of defending their shelters and nesting crevices against mud crabs. More generally, our results support the idea that one reason contributing to the vulnerability of species poor communities to the effects of species invasions (Stachowicz et al. 1999) could be the lower ability of species in such communities to cope with invaders claiming key resources (here: shelters and nesting cavities). To conclude, our study demonstrates that there is a potential for interactions between the invasive mud crab and nest-building fish, as mud crabs and fish overlap extensively in their habitat use. The mud crabs may impact these littoral fish by accumulation of multiple negative effects over the reproductive cycle of the latter, and especially by taking over nests and occupying crevices that fish would otherwise use for nesting. However, we did not find significant predation by mud crabs on guarded sand goby eggs. Despite the lack of demonstrated direct predation, the results support the hypothesis that the parental phase can be a particularly vulnerable life-history stage in face of novel selection pressures, such as those caused by the invasive mud crab. We suggest that future research should assess whether mud crabs have additional impacts on littoral fish (whether or not these build nests), such as competition for the same food resources or indirect cascade effects by reduction of the abundance of herbivores.

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