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# Density effects on fish egg survival and infections depend on salinity

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ABSTRACT: Across taxa, egg survival is a critical component of reproductive success. Eggs in aquatic environments can be strongly influenced by both extrinsic and intrinsic factors, of which egg density in particular has been subjected to a limited body of research. In this study, we experimentally manipulated both egg density and water salinity — a key environmental condition — and assessed egg infection rate and survival in a small marine fish with paternal egg care, the sand goby *Pomatoschistus minutus*. We controlled for the potential confounding effect of parental care by rearing the eggs artificially. We found that both high salinity and reduced egg density treatments were associated with a higher proportion of eggs surviving to the eye-spot stage. Furthermore, the positive survival effect of reduced density was more pronounced (in relative terms) in low salinity. In contrast, a reduced egg density was actually negatively associated with the proportion of healthy-looking, uninfected eggs in high (but not low) salinity. The first signs of infection, in turn, appeared quicker under low salinity, independent of density. Together, these results demonstrate context-dependent effects of density on egg performance, and identify a potentially significant role for parental care (especially filial cannibalism) in suppressing the spread of egg infections. Furthermore, the benefits of a low egg density are not only related to infections, but they can also vary depending on the physical environment. Overall, the results highlight the role of egg performance in the face of changing environmental conditions.

KEY WORDS: Egg survival · Density · Salinity · Adaptation · Baltic Sea · Infection · Water mould · Environmental change · Filial cannibalism

## INTRODUCTION

For oviparous animals, survival of fertilised eggs is a critical component of reproductive success. Egg survival, in turn, can be strongly affected by a range of different factors, especially in animals that have their eggs in constant contact with the surrounding water. Aquatic eggs are, for example, susceptible to predation (Leggett & Deblois 1994) and pathogens, such as bacteria, fungi and water moulds (Phillips et al. 2008). In terms of the latter, particular attention has been focused on *Saprolegnia* and other oomycetes (water moulds) that can cause high levels of mortality in a wide range of temperate invertebrates, fish and amphibians, including economically valuable species, such as salmonids, sturgeons, shrimp and crayfish (Ramaiah 2006, van West 2006, Phillips et al. 2008).

The development and survival of eggs is also heavily dependent on abiotic physiochemical parameters, especially temperature and salinity (Fonds & Van Buurt 1974, Hart & Purser 1995, Karås & Klingsheim 1997), as well as gas exchange with the surrounding water (Zoran & Ward 1983, Rombough 1988). In this respect, a slightly different set of conditions may be optimal for egg development rate, as opposed to egg survival, as shown for example in the greenback flounder *Rhumbosolea tapirina* (Hart & Purser 1995).

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Nevertheless, both the development and survival of eggs can cause significant challenges for marine species occupying brackish water environments, such as the Baltic Sea, that have salinity levels markedly below those found in the oceans (Thorsen et al. 1996, Karås & Klingsheim 1997, Nissling & Dahlman 2010). Besides the direct effects on egg metabolism and development, abiotic factors can also have indirect effects on egg survival by influencing growth and infection capacity of egg pathogens. For example, water moulds that infect aquatic eggs grow faster in higher temperatures (Gomez-Mestre et al. 2006), but are sensitive to higher levels of salinity, with sodium chloride being sometimes used to inhibit water mould growth in egg hatcheries (Edgell et al. 1993, Marking et al. 1994, Rasowo et al. 2007). More generally, the negative effects of pathogens on eggs and larvae are likely to be context-dependent, with the impact being more severe in the presence of abiotic stressors (Kiesecker & Blaustein 1995, Kiesecker et al. 2001, Ruthig 2008, Sagvik et al. 2008).

The density at which eggs are developing can also be an important factor for their development and survival. In particular, egg density may affect infection and predation susceptibility, as well as the abiotic environment of the eggs. For example, clustered eggs of the wood frog *Rana sylvatica* can be subjected to higher temperatures and, hence, develop faster than more sparsely distributed eggs (Waldman 1982, Waldman & Ryan 1983), whereas eggs of *R. cascadae* laid within, or close to, large communal egg masses are more likely to become infected by the pathogen *Saprolegnia ferax* than those laid away from other egg masses (Kiesecker & Blaustein 1997). Nevertheless, regardless of taxa, very few studies have tried to assess egg performance when egg clustering or density has been experimentally manipulated. In one such study, Ruthig (2008) did not find an effect of cluster manipulation on egg mortality in southern leopard frogs *R. sphenocephala*. In contrast, Green (2003) found that a markedly higher proportion of eggs of the smooth newt *Triturus vulgaris* hatched when they had been separated from each other by 1 cm compared to when the eggs were in physical contact. Furthermore, all eggs that failed to hatch in Green's (2003) experiment were infected with water moulds. One possible mechanism for the latter pattern of egg survival (and infection rate) is that fungi and oomycetes readily infect infertile, damaged or dead eggs and then 'vegetatively' grow hyphae (filaments) that can suffocate and kill healthy eggs within reach of the hyphae (Smith et al. 1985, Robinson et al. 2003, Morreira & Barata 2005). In other words, at lower densities, viable eggs of both fish (Smith et al. 1985, Thoen et al. 2011) and amphibians (Robinson et al. 2003, Morreira & Barata 2005) may be less prone to being attacked by pathogen growth from adjacent, unviable eggs. Finally, we also note that parental care of eggs, when present, can modify the immediate surroundings of the eggs, for instance in terms of predation rate (Mappes et al. 1995, Klug et al. 2005), infection risk (Tilley 1972, Knouft et al. 2003, Boos et al. 2014), improved exchange of gases and metabolic products (Zoran & Ward 1983, Payne et al. 2002, 2004), and possibly even egg density (Klug et al. 2006, Lehtonen & Kvarnemo 2015a).

In this study, we focused on a brackish water population of a small fish, the sand goby *Pomatoschistus minutus*. Sand gobies are commonly found under a variety of different salinities across their geographic range (Miller 1986), from oceanic conditions to salinities below 3 ppt in the Northern Baltic Sea (Wiederholm 1987). Sand goby males provide egg care, which is relevant because the patterns of egg infection and survival in such species may help to explain the common but poorly understood aspect of parental behaviour, filial cannibalism (i.e. consumption of own offspring, see Lehtonen & Kvarnemo 2015a). Interestingly, the level of filial cannibalism differs between goby populations experiencing different environmental conditions, such as salinity (Lehtonen & Kvarnemo 2015a and references therein).

We addressed gaps in current knowledge — as well as contradictory previous findings — with regard to the effects of egg density on egg performance. In particular, we experimentally manipulated egg density (as opposed to assessing only naturally occurring variation in density) and measured its effect on both egg infections and egg survival (until the eye-spot stage) under different environmental conditions, namely low and high salinity. The chosen salinities represent levels that are found within the geographical range of our model species, and are known to influence the occurrence and spread of pathogenic water moulds. We also controlled for the potentially confounding effect of male care effort by rearing the eggs artificially. Finally, to better understand the spreading patterns of egg infections, the eggs were assessed on multiple occasions during the egg development time.

## MATERIALS AND METHODS

The study was carried out in June 2014 at the Tvärminne Zoological Station on the Finnish coast of the Baltic Sea (59° 50.7′ N, 23° 15.0′ E). To acquire fertilised eggs for the experiment, we first collected male and female sand gobies near the field station. This was done using dip nets and a hand-trawl, i.e. a small bottom trawl with 4 mm mesh size that 2 wading persons dragged at slow walking speed in shallow water to catch small benthic fish, such as sand gobies. After gobies were transported back to the field station, they were separated by sex and maintained in holding aquaria of various sizes between 45 and 100 l (length: 60–80 cm, width: 25–40 cm, water depth: 25–35 cm). The aquaria were kept under natural light conditions and supplied with natural brackish water (salinity: 5.5 ppt). Water was pumped from a nearby bay and then flowed through the aquaria. An adjustable overflow system kept the water level constant. Before the onset of the experiment, the gobies were fed ad libitum with live mysid shrimp *Neomysis integer* and occasionally supplemented with frozen chironomid larvae. Each male used for fertilising eggs for the experiment (see following paragraphs) was then placed individually in a flow-through tank measuring 70 × 25 × 25 cm (length × width × water depth) with a 4 cm layer of fine sand as substrate. To offer the male a resource for nest-building (i.e. excavating sand under an object and covering it with sand; see Japoshvili et al. 2012), we also added into each tank a halved clay flowerpot (diameter 6.5 cm), the inner surface of which was covered with a transparent plastic film (Fig. 1A). After the male had completed building a nest, 2 females that were ripe with eggs were added into the tank. During spawning, sand goby females attach their adhesive eggs in a monolayer on the ceiling of the nest, and hence in our set-up, the eggs were attached to the film we had placed in the nest (Fig. 1A). Once the females (in some cases only 1 of them) had spawned, they were both removed. Within 36 h after the first eggs had been laid, the film with the eggs was removed from the nest and manipulated according to the following experimental treatments.

To assess how egg density affects egg survival and susceptibility to infections under different salinities, we included 2 treatments in the experiment, both with 2 levels: (1) egg density (levels: non-manipulated and reduced density) and (2) salinity (levels: low and high salinity). The total number of replicates in each of the 4 treatment combinations was as follows: non-manipulated density + low salinity, n = 17; reduced density + low salinity, n = 18; non-manipulated density + high salinity, n = 19; reduced density + high salinity, n = 20. We started new replicates in a fashion that kept the number of concurrent replicates of each treatment roughly the same and the difference between any 2 treatments in the count of concurrent replicates never exceeded 2.

To manipulate egg density, we made 20 to 25 parallel cuts, at 2.5 to 3 mm intervals, in the plastic film (Fig. 1A), before it was used to cover the inner surface of the nesting resource (i.e. halved flowerpot), as described above. After the eggs had been laid on the film, it was removed and cut into 2 pieces, with the larger of the 2 having approximately (according to a visual estimation) twice the number of eggs compared to the smaller one. In the reduced density treatment, every second pre-cut thin strip of the larger piece was removed (Fig. 1B), resulting in a reduced egg density but a similar number of eggs (~1000) as on the smaller piece. In the non-manipulated egg density treatment, the smaller piece was handled in an otherwise similar fashion, but the egg density was left unaltered (Fig. 1C). In all but the first 12 replicates (2 to 4 per treatment combination; $n_{total} = 74$), both of the 2 pieces of film that originated from the same egg clutch were used in the experiment (by exposing them to the same salinity level), giving a more powerful paired design. This arrangement was accounted for in the statistical analyses (see section below). In all replicates, the piece of egg-bearing film, prepared in the above fashion, was then hooked to a string. This allowed it to be positioned submerged, but close to the surface, in a 1 l container

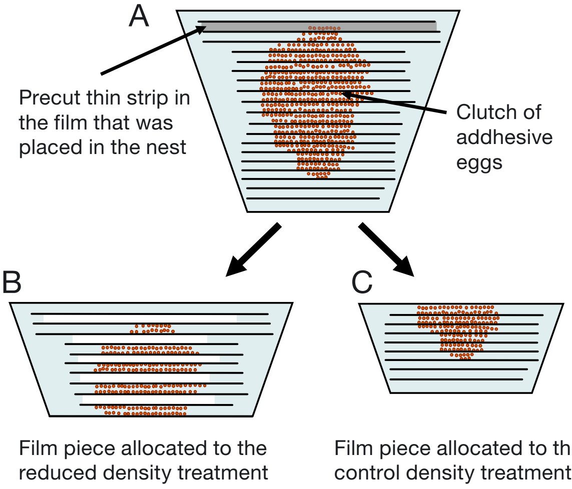

Fig. 1. Schematic presentation of egg density manipulation. (A) Adhesive eggs were laid onto a thin plastic film, pre-cut into ≤3 mm strips, covering the inner surface of the nest (i.e. the ceiling of a halved clay flowerpot placed on fine sand at the bottom of 45 l flow-through aquaria). After this 'primary' film was removed from the nest following spawning, it was cut into 2 pieces, one of which was assigned into (B) the reduced density treatment, and the other one into (C) the non-manipulated (i.e. control/higher) density treatment. A similar number of 'head' and 'tail' secondary pieces were assigned to the 2 treatments over the course of the experiment

filled with water. Only 1 piece of film was placed into each container. The containers, in turn, were placed into larger tanks, with 6 containers in each tank. The tanks had a water level slightly below the rim of the containers and were provided with continuous through flow of water from the sea (see above for details). With this design, the water in the tanks and containers did not mix, but the temperature in both followed the natural temperature variation in the sea (ca. 11 to 15°C during this study).

To manipulate salinity, each 1 l container with eggs on a piece of film had either natural brackish water from the sea (low salinity treatment, salinity: ~5.5 ppt) or natural water from the sea to which 12 g $l^{-1}$ of commercially available sea salt ('Instant Ocean', Spectrum Brands) had been added (high salinity treatment, salinity: ~15 ppt). The eggs were then incubated in the containers until they were 8 d old and, hence, in an advanced stage of development (Fonds & Van Buurt 1974, Kvarnemo 1994, Lehtonen & Kvarnemo 2015a). During this time, water in the incubation containers was replaced every second day, with the clean replacement water matched to the salinity level of the treatment.

We photographed the eggs (using an Olympus XZ-1 digital camera) first immediately prior to the start of incubation in containers (see above), and then 2, 4, 6 and 8 d after the eggs had been laid. We used the photographs to assess the following 3 response variables: (1) the proportion of eggs developing to the eye-spot stage, (2) the proportion of healthy-looking eggs, and (3) the emergence of visible signs of infection.

To measure variable 1, the proportion of eggs that had developed until the eye-spot stage, we counted the total number of eggs in the replicate and the number of eggs that had developed to the eye-spot stage from the photograph that was taken at the end, i.e. on Day 8, of each trial. To visually present the result (see Fig. 2), the number of eggs with eye-spots was divided by the total number of eggs, giving the proportion of eggs that had developed to the eye-spot stage. For the statistical analysis, however, the numbers of eggs with and without eye-spots were used as a combined response variable. The presence of 2 eye-spots in an egg was used as an indicator of its successful development to an advanced stage, because the eyes of a sand goby embryo become clearly visible only a couple of days before the egg is ready to hatch (Fonds & Van Buurt 1974, authors' pers. obs.). We note that water mould infections are known to induce early hatching in some taxa (Morreira & Barata 2005, Gomez-Mestre et al. 2006). However, we did not observe any hatching before the end of the experiment in any of the treatments.

To measure variable 2, the proportion of eggs that appeared healthy (i.e. without visible signs of impairment) at the end of the experiment, we counted the number of eggs that appeared infected or damaged (sensu Lehtonen & Kvarnemo 2015a) from the photograph taken on Day 8. Subtraction of this number from the total number of eggs, in turn, gave the number of healthy-looking eggs. As above, the proportion of healthy eggs (the number of healthy-looking eggs divided by the total number of eggs) was used to illustrate the result (see Fig. 3), whereas the actual numbers of infected and healthy eggs were used in the analysis.

Lastly, to measure variable 3, the number (count) of days from the start of the incubation to the finding of visible signs of egg infection (i.e. milky overgrowth and/or hyphae of water moulds; see Lehtonen & Kvarnemo 2015a), we visually inspected all photographs taken during the egg development period (Days 2, 4, 6 and 8). Water moulds found growing on the eggs were likely to be from the genus *Saprolegnia* (as indicated by visual inspection), and they have earlier been shown to be important pathogens of sand goby eggs (Lehtonen & Kvarnemo 2015a,b). The date of the earliest photograph with infected eggs was noted and used both to illustrate the result (see Fig. 4) and in the analysis.

### Statistical analyses

The 3 response variables, 1, 2 and 3, were analysed separately. In each of the 3 cases, we applied a generalised mixed model with the density treatment (reduced vs. non-manipulated) and salinity level (low vs. high) as fixed effects, and 'egg clutch ID' as a random effect to account for the fact that 1 egg clutch was, in most cases (see above section), used for 2 different replicates (1 per density treatment). To account for overdispersion (see e.g. Zuur et al. 2013) in variables 1 and 2, we translated the models from a binomial distribution to lognormal-Poisson distribution by including an individual-level random variable (Elston et al. 2001) into each model. For the number of days before the first signs of infection (3), we applied a generalised mixed model with Poisson distribution, after checking that the data was not overdispersed (Zuur et al. 2013). In all 3 cases, we assessed whether the complete model could be refitted without its interaction term, using $\chi^2$ tests with $p > 0.05$ as the cutting point. We used R 3.1.0 software (R Development Core Team) for the analyses and the 'lme4' statistical package for running the generalised mixed models.

## RESULTS

The survival of eggs until the eye-spot stage varied between 0.1 % for eggs with non-manipulated density in low salinity and 31 % for eggs with reduced density in high salinity (Fig. 2). We found that the high salinity and reduced density treatments resulted in a higher egg survival until the eye-spot stage, with a more pronounced density effect (in relative terms) in low salinity (Fig. 2), as indicated by a significant interaction between salinity and density (generalised mixed model: $\chi^2 = 5.854$, df = 1, p = 0.015).

The proportion of healthy-looking eggs that did not have any signs of infection ranged from 4.5 % in low salinity with non-manipulated density to 80 % in high salinity with non-manipulated density (Fig. 3). The interaction between salinity and density was significant (generalised mixed model: $\chi^2 = 4.170$, df = 1, p = 0.041): reduced density was associated with a lower number of healthy eggs in high salinity, whereas the effect of density acted in the opposite direction in low salinity (Fig. 3).

Regarding the first signs of infection, we simplified the model by removing the interaction between salinity and density (generalised mixed model: $\chi^2 = 0.387$, df = 1, p = 0.53) and then the density effect ($\chi^2 = 1.075$, df = 1, p = 0.30). The final model showed a highly significant effect of salinity ($z = 4.525$, p < 0.001). The first signs of infection were visible earlier in low than in high salinity, independent of density (Fig. 4).

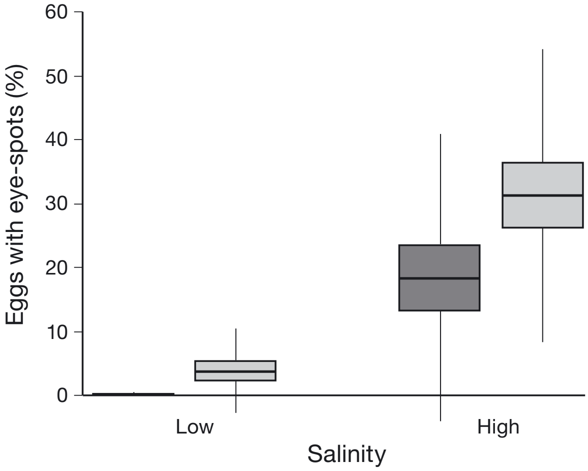

Fig. 2. Percentage of *Pomatoschistus minutus* eggs that had developed to the eye-spots stage at the end of the experiment (Day 8) in relation to egg density and water salinity. Dark grey: non-manipulated (higher) egg density; light grey: reduced density treatment (see Fig. 1). Low salinity: 5.5 ppt; high salinity: ~15 ppt. Box margins: SE; central horizontal line: mean; whiskers: SD. Sample sizes (left to right): 17, 18, 19 and 20

## DISCUSSION

The results of this study suggest that the effect of density on egg performance is context-dependent. In particular, we found that egg density interacted with salinity to impact egg performance. First, egg survival was higher in the reduced egg density treatment and this density effect was (in relative terms)

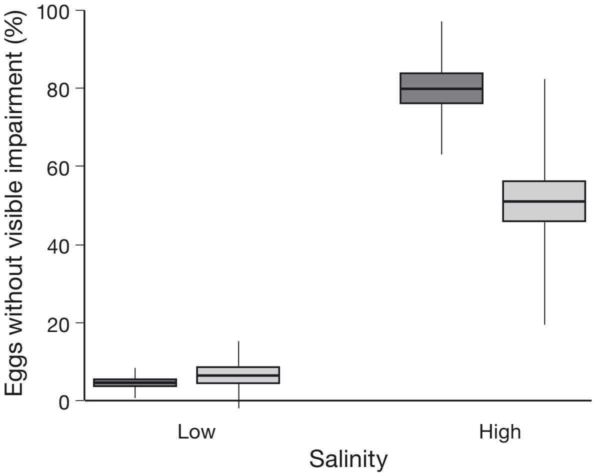

Fig. 3. Percentage of healthy *Pomatoschistus minutus* eggs, i.e. not appearing infected or damaged when visually inspected at the end of the experiment, in relation to egg density and water salinity. Box plots and treatments as in Fig. 2

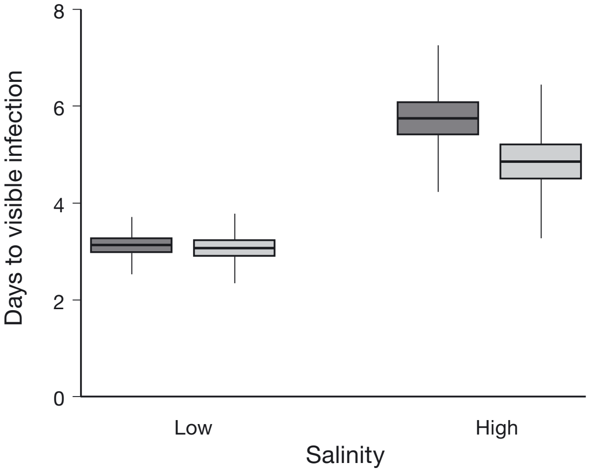

Fig. 4. Number of days from the start of *Pomatoschistus minutus* egg incubation until the visual detection of infection in relation to egg density and water salinity. Box plots and treatments as in Fig. 2

more pronounced in low salinity compared to high. As with egg survival, we expected the level of infections to be lower under reduced egg density (see Lehtonen & Kvarnemo 2015a). However, while the difference between the 2 density treatments was towards the anticipated direction under low salinity, the pattern was actually the opposite under high salinity conditions, with the proportion of healthy-looking, non-infected eggs being highest in the treatment that combined non-manipulated (i.e. high) egg density and high salinity.

The context-dependency of density effects can help to explain earlier conflicting results on the effects of egg density, such as those seen with regard to egg clustering in amphibians (Kiesecker & Blaustein 1997, Green 2003, Ruthig 2008). Similarly, in fish, especially in sand gobies, earlier studies have yielded results on the effects of egg density that, at first, may seem contradictory (Klug et al. 2006, Norevik Andrén & Kvarnemo 2014, Lehtonen & Kvarnemo 2015a). In the light of the current results, however, these previous assessments have probably provided correct views of egg density effects under their particular sets of conditions, such as under specific salinity environments (Klug et al. 2006, Norevik Andrén & Kvarnemo 2014), in the presence (as opposed to absence) of parental care (Klug et al. 2006, Norevik Andrén & Kvarnemo 2014), and when only natural, non-manipulated variation in egg density had been considered (Norevik Andrén & Kvarnemo 2014, Lehtonen & Kvarnemo 2015a). In contrast, the results of the current study (which manipulated both salinity and egg density levels in the absence of parental care) predict that any inhibitory effects of a reduced egg density on susceptibility to egg infections can even be reversed in environments that inhibit microbial infections (e.g. *Saprolegnia*), as here represented by a higher salinity level. In particular, under such conditions, factors other than infections are likely to be important drivers of egg survival, as will be discussed below. Before that, however, we will consider the potential reasons for why the extent of infection was actually higher in the reduced egg density under high salinity conditions.

We propose that one important reason for the variability in density effects could have been a difference in the spreading mechanisms of key egg pathogens (i.e. water moulds and fungi) under different environments, such as low and high salinity. In particular, spores of pathogenic water moulds are expected to colonise mainly infertile, dead or damaged eggs (Robinson et al. 2003, Morreira & Barata 2005, Thoen et al. 2011). In environments that are favourable for the growth of these pathogens, they spread predominantly by the growth of hyphae (filaments) that are also able to kill healthy eggs (as described by Smith et al. 1985, Thoen et al. 2011). Especially under such conditions, a lower egg density is expected to reduce the spread of infection (Lehtonen & Kvarnemo 2015a). In contrast, in conditions that suppress the growth of water moulds and fungi, as in the high salinity treatment of our experiment, vegetative growth of the pathogens is inhibited, and the dominant infection mode is likely to be a direct infection by spores attacking the weakest eggs. A difference in the infection mechanism is also supported by our observation that the first visible signs of infection were apparent markedly earlier in low than in high salinity. Overall, our results suggest that in high salinity conditions, a higher egg density may actually result in a lower infection risk per egg. Perhaps an increased exposure to water movement between eggs in the reduced density treatment may have resulted in a more effective infection by spores of pathogenic water moulds and fungi, which only became detectable when the conditions inhibited the growth of hyphae. Besides water moulds and fungi, the succession and spread mechanisms of other pathogens, such as bacteria and viruses, may conceivably also differ between environments that differ in conditions affecting colonisation and local spread or growth.

As argued above, a decreased potential for local growth of infections in high salinity (and reduced density) is likely to provide a partial explanation for the observed patterns of egg survival (see also Lehtonen & Kvarnemo 2015a). However, the reversed pattern for the proportion of infected (versus healthy) eggs in the high salinity environment suggests that there are also additional mechanisms that are important drivers of egg survival. One such potential mechanism is that a lower egg density is likely to increase the availability of oxygen for individual eggs, resulting in their higher survival, as suggested by Klug et al. (2006). In our study, egg survival in many replicates was at its highest on the edges of egg batches, which is in accordance with this possibility. However, it seems likely that oxygen demand can, at best, provide yet another partial explanation for the observed survival differences, as neither Klug et al. (2006) nor Lissåker et al. (2003) found manipulated oxygen levels to affect egg survival. Furthermore, in the current study, we found a strong positive effect of salinity on egg survival (see also Lehtonen & Kvarnemo 2015a), although solubility of oxygen in water is lower in high than in low salinities (Benson & Krause 1984). Other mechanisms that could also conceivably contribute to the increased survival under a reduced egg density, especially on the edge of egg batches, include more efficient removal of carbon dioxide and other waste products, and a lower rate of debris accumulation. This should be true for all sparsely distributed eggs, as compared to more tightly packed ones. In many respects, eggs on the edge were subject to the lowest 'density' in all of our treatments. Hence, the highest egg survival on the edges of egg batches may be attributed to any of the general density effects discussed above.

Although we excluded the effects of parental behaviour in the current study, our results nevertheless have important implications in the context of parental egg care. In this respect, in addition to maximising egg and juvenile survival, decreasing the spread of infections can also be an important component of parental care, as demonstrated by species that excrete antimicrobial substances to protect their eggs (Gomez-Mestre et al. 2006, Giacomello et al. 2008, Little et al. 2008). In the current study, the increased egg survival associated with the reduced egg density suggests that egg density alterations, resulting from egg removal by a parent, may be beneficial for the remaining eggs, as earlier suggested by Klug et al. (2006) and, more recently, by our own research (Lehtonen & Kvarnemo 2015a). In addition to such density effects, selective removal of dead or damaged eggs is likely to result in a lower infection rate among the remaining eggs, as has earlier been suggested, for example, for the mountain dusky salamander *Desmognathus ochrophaeus* (Tilley 1972, Forester 1979). Furthermore, besides the effect of density on infections being the opposite in the 2 salinities, we also found that the overall egg survival was much higher in the high salinity treatment. The results therefore suggest that the role of selective filial cannibalism is likely to be more important in environments that are favourable for pathogen growth (here: low salinity) than when the growth of pathogens is suppressed (here: high salinity). Such an interpretation is in accordance with earlier goby studies that have consistently found lower levels of filial cannibalism when the study has been conducted in high salinity environments (Lehtonen & Kvarnemo 2015a and references therein). Similarly, in the flagfish *Jordanella floridae*, nest preparation behaviour by the egg-tending male had a greater positive effect on hatching success in freshwater compared to brackish water (i.e. higher salinity) conditions (Hale 2008). More generally, such environment-dependent differences in the optimal parental care strategy suggest that species adapting to environments of lower, or decreasing, salinity levels — such as those in the Baltic Sea (Wikner & Andersson 2012) — also need to be able to adjust their parental care behaviours (e.g. filial cannibalism) in relation to changes in the environment. The extent to which individuals within populations facing significant environmental challenges are flexible in parental (and other key) behaviours is still poorly known (for some initial considerations on the topic, see e.g. St Mary et al. 2001, Järvi-Laturi et al. 2008, Wong & Candolin 2015).

To conclude, the results of the current study are in accordance with the prediction (Lehtonen & Kvarnemo 2015a) that suppression of the spread of egg infections can be an important aspect of parental care activities, especially of filial cannibalism, which reduces egg density. However, the current results also suggest that a lower density per se is probably not efficient in slowing the spread of infections. A low egg density, nevertheless, provides additional benefits to the developing eggs, with these density effects being context-dependent, as made evident by the different degrees of density effects on survival in low and high salinity conditions. The findings can also help to understand the challenges that many populations face in changing environmental conditions, especially with regard to salinity and nesting opportunities. In this respect, a rapidly increasing number of populations need to adjust to such changing environmental conditions, for instance when animals are relocated to new areas (e.g. invasive goby species; Kornis et al. 2012) or as a consequence of climate change (Stigebrandt & Gustafsson 2003).

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