

Egg density and salinity influence filial cannibalism in common gobies

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Abstract

Filial cannibalism, i.e. the consumption of own offspring, has fascinated animal ecologists for many decades but is still not fully understood. Often assumed to happen primarily due to energetic needs of the cannibalizing parents, we here address a more recent notion that suggests an interplay between egg density, salinity, egg infections and filial cannibalism in fish. Previous evidence indicates that (a) filial cannibalism may be related to egg density that (b) egg pathogens such as water moulds spread more easily on high density clutches and are (c) generally suppressed in high salinity conditions and that (d) parents selectively cannibalized infected eggs, suggesting cannibalism to maximise in high density clutches in low salinity as a response to egg infections. We thus tested if egg density, salinity and their interaction directly affect filial cannibalism using the common goby (*Pomatoschistus microps*) as a model system. We additionally recorded male brood care behaviour and weight to account for other potentially salinity-related effects. While males unexpectedly cannibalized more eggs in low density instead of high density clutches, we found that egg consumption was higher in low salinity conditions in agreement with our prediction. Neither male behaviour nor metabolism did adequately explain this finding, indicating that

variation in filial cannibalism under different environmental conditions such as salinity may indeed be driven by a differential prevalence of egg infections.

Significance statement

During parental care, parents may forgo taking care of their offspring but rather invest in future reproduction. Filial cannibalism, the consumption of one's own offspring, has often been considered to occur for energetic reasons or as a strategy to reduce investment in less valuable offspring. In this study we specifically test how male brood care behaviour, i.e. egg fanning and filial cannibalism is affected by salinity and egg density (how tightly females decide to place their eggs) in a small marine fish. Our experimental approach is to assess environmental influences and to show their relevance for the evolution and persistence of such a seemingly maladaptive behavioural trait with drastic consequences for fish reproduction. We conclude that variation in filial cannibalism under different environmental conditions such as salinity may indeed be driven by a differential prevalence of egg infections.

Keywords Selective filial cannibalism · Egg infection · Paternal care · Egg fanning · Water mould · Clutch size

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Introduction

Many animals care for their offspring, e.g. by providing food or protection from predators (Clutton-Brock 1991; Royle et al. 2012). Parental care is often costly but can be seen as an investment to increase offspring survival and reproductive success (Clutton-Brock 1991; Smith and Wootton 1995). However, some animal parents simultaneously show filial cannibalism (FC), i.e. they regularly cannibalize some or even all of their current offspring (Polis 1981; Klug and Bonsall 2007). This behaviour is surprisingly widespread despite its

counterintuitive nature (e.g. Anthony 2003; Gilbert et al. 2005; Miller and Zink 2012) and is particularly common in fish with male brood care (Manica 2002). It is often assumed that the cannibalized offspring are used as an additional or alternative source of energy that can be reinvested into care for the remaining offspring or future reproduction (Rohwer 1978; Sargent 1992). However, the empirical evidence for this hypothesis is mixed and energetics alone cannot adequately explain all occurrences and patterns of FC (Manica 2002; Klug and St Mary 2005; Klug et al. 2006; Mehliis et al. 2009; Vallon et al. 2016b).

Addressing FC in beaugregory damselfish (*Stegastes leucostictus*), Payne et al. (2002, 2004) proposed that the cannibalizing parent actively reduces egg density to enhance oxygen availability and thus the survivorship of the remaining eggs in the nest. Other studies indeed indicate a general influence of egg density per se on FC (Klug et al. 2006), but question the importance of oxygen in this context (Lissåker et al. 2003; Klug et al. 2006). In particular, they found no (Lissåker et al. 2003) or only inconsistent effects (Klug et al. 2006) of oxygen manipulation on egg survivorship and FC in sand gobies (*Pomatoschistus minutus*), a species where males oxygenate their eggs via fanning. Such egg fanning is common in teleost fish with male brood care (Blumer 1982), possibly limiting the broader relevance of oxygen-mediated FC. Hence, while the apparent effect of egg density on FC remains intriguing, the underlying mechanisms remain unclear.

Recent work shows a strong influence of mould infections on FC in fish and suggests that parents selectively cannibalize infected offspring to inhibit the spread of the disease (Bandoli 2016; Vallon et al. 2016a). Such microbial infections are a frequent threat in fish and can damage and kill eggs or entire clutches (van West 2006; Brown and Clotfelter 2012). Preventing infections thus likely constitutes an important part of parental care in many fish species (Bronstein 1982; Côté and Gross 1993; Knouft et al. 2003; Giacomello et al. 2008). Water moulds (oomycetes) of the genus *Saprolegnia*, common pathogens in aquatic systems (van West 2006), are known to spread within egg clutches primarily by hyphal growth from egg to egg and less so by release of zoospores (Smith et al. 1985; Thoen et al. 2011). Accordingly, one may expect that not only the specific removal of infected eggs by parents but also a general reduction of egg density (and thus less direct contact between individual eggs) would impede spreading of *Saprolegnia* and other pathogens (as proposed by Lehtonen and Kvarnemo 2015b). Hence, density-dependent FC could represent a measure to contain egg infections.

Lehtonen and Kvarnemo (2015b) indeed found that artificially reared sand goby clutches had a lower prevalence of *Saprolegnia* and a higher egg survivorship when egg density was low, suggesting that creating a lower density via FC may be beneficial for parents. Notably, this effect was only present

when clutches were raised in low salinity water but not when salinity was high, where mould growth was generally reduced. Such a susceptibility of *Saprolegnia* water moulds to high salinity levels has also been observed in many other studies (e.g. Marking et al. 1994; St Mary et al. 2004; Ali 2005; Vallon et al. 2016a). While discussing their findings, the authors note that mould infections and their sensitivity to salinity might in fact explain why average FC levels tend to be higher in sand goby studies conducted in low salinity environments (Finnish coast of the Baltic Sea) compared to high salinity environments (Swedish west coast), where conditions are less favourable for the pathogen (Lehtonen and Kvarnemo 2015b and references therein). Likewise, they argue that this relationship could explain why Klug et al. (2006), who used low salinity conditions, found higher egg survival at low egg densities, while a recent study conducted in higher salinity did not (Andrén and Kvarnemo 2014). However, it remains impossible to disentangle the potential direct effect of salinity from other confounding factors that differ between all these studies.

In the present study, we further pursue the ideas of Lehtonen and Kvarnemo (2015b) and directly assess FC in relation to egg density and salinity using the common goby (*Pomatoschistus microps* Krøyer), a small fish closely related to sand gobies with male brood care and regular FC (Vallon et al. 2016b). Specifically, by manipulating both factors simultaneously while allowing male access to eggs, we tested the prediction that FC increases when egg density is high, but only in conditions favourable for mould growth such as low salinity. In other words, we predicted an interaction between egg density and salinity with a positive effect of egg density on FC in low but not in high salinity. To further assess potential effects of salinity per se on male brood care or metabolism, we additionally recorded fanning behaviour and male weight.

Material and methods

Study species

The common goby is widespread along the European coast from the Mediterranean to the Baltic Sea including estuaries (Miller 1975) and thus naturally occurs in a very broad salinity range spanning from nearly freshwater to full marine conditions (0.5–35 ppt; Fonds and van Buurt 1974). Common gobies usually live only for 1 year and reproduce during several consecutive breeding cycles in summer (Miller 1975). Adults live and mate in shallow soft-bottom areas close to the shore and rely on suitable hard structures (e.g. mussel shells) as a nest substrate (Borg et al. 2002). After courtship, the female attaches its eggs to the ceiling of the nest, but abandons the clutch afterwards. Brood care (e.g. cleaning and ventilating the eggs) is thus done exclusively by the male (Nyman 1953), which can care for several clutches of different

females simultaneously (Magnhagen and Vestergaard 1993). After 1 to 2 weeks, depending on water temperature, larvae hatch and leave the nest and the male (Rogers 1988).

Animal maintenance

We conducted the experiment in July 2014 at Tvärminne Zoological Station near Hanko, Finland. Common gobies were collected close to the shoreline at Henriksberg either by using a beach seine or from previously deployed artificial nests (ceramic tiles measuring 5×5 cm) using a hand net while snorkelling. All fish were measured for body size (total length to the nearest mm) prior to use. Males were additionally weighed to the nearest mg. To increase sample size, we conducted two consecutive rounds of all experimental procedures using two different cohorts of males (mean \pm SE total length [TL]; cohort 1 34.8 ± 0.4 mm; cohort 2 35.3 ± 0.4 mm). Forty-eight experimental tanks (35 l) were each fitted with a halved flowerpot of 4.5 cm diameter as an artificial nesting site, which was placed upside-down on sandy substrate and faced the front window. Each nest contained a removable plastic sheet at the ceiling for females to spawn on. While all tanks were covered with black plastic foil to prevent interactions between neighbouring males, the front cover was easily detachable to enable behavioural recordings. Individual males received a standardised amount of frozen chironomid larvae (two small ones in the morning and evening) as food during the experiment. All fish experienced a 19:5-h day:night light regime. The natural salinity level at the site of capture is typically between 6.2 and 6.4 ppt (Vallon et al. 2016a).

Salinity treatments

To achieve two different, stable salinity treatments, experimental tanks were connected to one of two closed flow-through systems. Each system was connected to a large water basin (120 l) which could be used to add or remove water and salt without disturbing fish and which was heavily aerated via air stones to provide aeration for the whole system. The low salinity (mean \pm SE 6.14 ± 0.02 ppt) and high (18.22 ± 0.01 ppt) salinity treatments were chosen based on previous results showing drastic differences in growth of water moulds on common goby clutches under these conditions (Vallon et al. 2016a). We created both salinities by adding the corresponding amount of sea salt (commercially available mix for marine aquaria from Instant Ocean, Aquarium Systems, Sarrebourg, France) to a mix of 50% natural Baltic Sea water and 50% purified water (Milli-Q). We refrained from using only natural Baltic Sea water from the local inflow to keep the procedure similar for both treatments, considering that otherwise the salinity would have been already high enough for our low salinity treatment without adding salt.

Approximately 18% of the total water volume in each system was exchanged daily. For this, we stopped the water flow-through, removed all water from the water basin, added a fresh water-salt mix (often with lower salinity to account for water evaporation) and then restarted the system. Water temperature and salinity in individual tanks were monitored on a daily basis. Since there was no permanent water inflow from the outside, water temperature was primarily determined by the room temperature but was similar in both salinity systems (mean \pm SE; low salinity 20.23 ± 0.05 °C; high salinity 20.29 ± 0.03 °C).

Acclimatisation and spawning procedures

We gradually acclimatised males of the first cohort to the treatment salinity conditions in a stepwise fashion. To make sure that both treatment groups experienced a similar change in salinity, we first put all males in a stock tank with intermediate salinity (12 ppt) for 8 h. Afterwards, individual males were moved to their respective experimental tanks and kept at 9 ppt (low salinity group) or 15 ppt (high salinity group) overnight. After an additional day at 8 or 16 ppt, the final salinities were set and the experiment started. Previous evidence suggests that much shorter time periods should be sufficient to allow for metabolic readjustment after even larger changes in salinity (von Oertzen 1984). Since we also did not observe prolonged signs of stress in the first cohort, we shortened the total acclimatisation time for the second cohort of males to 1 day, while still gradually adjusting salinity. Females were also kept in a stock tank with intermediate salinity (12 ppt) for several hours before being used for spawning.

All males received a female to spawn with after acclimatisation, which was inserted in the early evening and removed the next afternoon (if spawning had happened). Although females were larger than males on average, we assigned pairs according to body length and obtained a similar average female size in both salinity (mean \pm SE TL; low salinity 36.1 ± 0.3 mm; high salinity 35.9 ± 0.4 mm) and egg density treatments (see below; low egg density 36.1 ± 0.3 mm; high egg density 35.9 ± 0.3 mm). Nests were visually checked for the presence of eggs using an electric torch, which allowed us to determine if eggs were present without disturbing the nest. Males without eggs were left with the same female for another day, then provided with a new female the day after if necessary, but were not considered further if still unsuccessful after the next 2 days.

Egg density manipulation

Seventy-eight males successfully acquired a clutch during the course of the experiment. Individual clutches were removed, photographed and manipulated to fit in one of two egg density

treatments. There was no significant difference in initial egg number between clutches laid in low (mean \pm SE eggs 892.9 ± 41.6) and high (954.8 ± 40.3 ; t test; $t = -1.07$, $df = 76$, $P = 0.290$) salinity. Egg density was manipulated by manually scraping off eggs using scissors. While we cannot fully exclude procedural damage on eggs caused by this manipulation, we consider it unlikely that our results have been significantly influenced by this. Scraping off eggs did not seem to affect adjacent eggs in a previous study in common gobies (Vallon et al. 2016a). Please see the discussion section for further considerations on this topic.

To create a low egg density, we removed eggs from within the clutch in a grid-like fashion. Specifically, we traced several thin diagonal stripes (actual number depended on the size of the clutch) with the tip of the scissors, followed by additional stripes perpendicular to the first ones (Fig. 1a). For high densities, we removed eggs only from the edge of the clutch (Fig. 1b).

While we aimed at removing similar proportions of eggs in both treatments, we ended with an unexpectedly large difference in egg number after the density manipulation (mean \pm SE eggs; low egg density 512.2 ± 27.3 ; high egg density 699.9 ± 28.4). Although our main analysis was based on proportional data and thus accounted for differences in clutch size, we decided to sacrifice some sample size in order to achieve a more similar baseline between groups. For this, all males whose manipulated egg number was more than 0.4 times lower or higher than the overall mean were excluded from the analysis ($n = 21$), leading to a reduced data set with a much smaller, non-significant difference in egg number between groups (mean \pm SE eggs; low egg density 585.4 ± 23.9 ; high egg density 639.4 ± 23.3 ; t test; $t = -1.61$, $df = 55$,

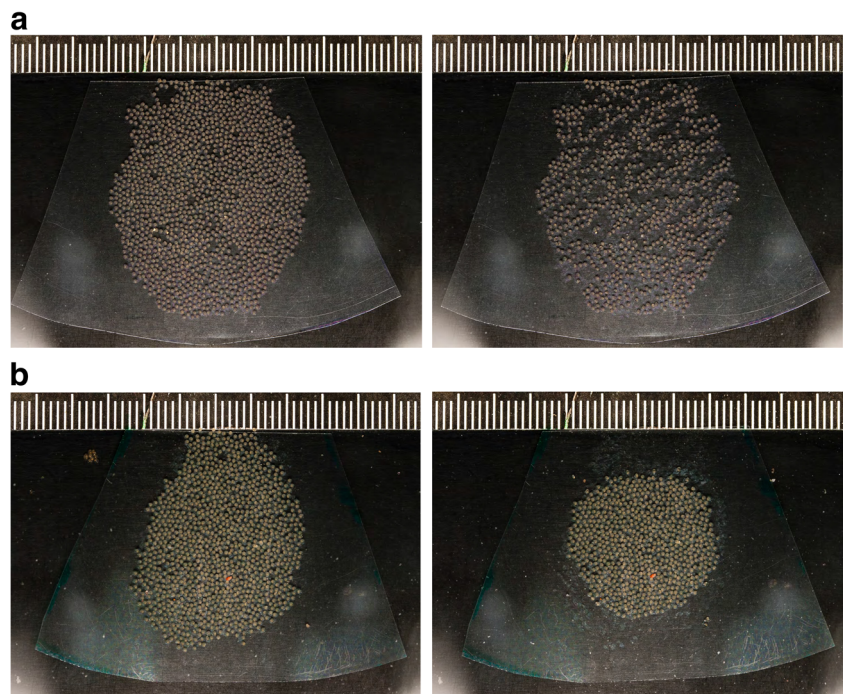
$P = 0.112$). Please note that using the full data set with all males for analysis still led to the same qualitative main results (not shown).

Data collection

Immediately following manipulation, clutches were photographed to have a baseline for the assessment of filial cannibalism and then returned to the males, which were left to care for their respective clutch for 3 days in total. On the last day, clutches were removed, photographed again and discarded. FC was determined by comparing egg numbers between those two pictures. All eggs were counted manually using the Cell Counter plugin (K. de Vos, University of Sheffield, UK) in ImageJ version 1.47v (W. Rasband, NIH, USA). It is well established in the literature that the concept of FC in fact includes two distinct phenomena, which are typically analysed separately (reviewed in Manica 2002): partial FC (parents eat some of their offspring) and total FC (parents eat all of their current offspring). In our study, total FC was rare ($n = 5$) and spread evenly among treatment groups, preventing meaningful inference. We thus excluded all corresponding males and one individual that was found dead on the last day from statistical analysis.

In addition to FC, we measured male egg fanning behaviour using video recordings 2 days after the egg density manipulation. Recordings were made in a randomised order with a digital video camera through the front windows of the experimental tanks. Individual males were given 10 min with the front cover of the tank removed before the actual 10 min recording started. To further reduce potential disturbance (e.g.

Fig. 1 Sample photographs of clutches before (left) and after (right) egg density manipulation for the **a** low egg density treatment and **b** high egg density treatment. See main text for details on the procedure



by turning the camera on and off), only the central 5 min of the video recording were used for analysis. To minimize observer bias, behavioural data collection based on the video recordings was carried out blind to the actual treatment. We evaluated three different aspects of egg fanning behaviour using JWatcher version 1.0 (D.T. Blumstein et al., University of California, USA and Macquarie University, Australia): overall time spent fanning eggs within 5 min, number of distinct fanning bouts and egg fanning rate (fin flaps per second). Males that were never visible on the recording were excluded from this analysis.

All males (except one which was accidentally forgotten) were weighed once more at the end of the experiment and released to the wild 1 day after the clutches had been removed. We calculated the difference between final weight and initial weight to assess weight change. In total, we obtained data on FC, egg fanning and weight for 51, 49 and 50 males, respectively.

Statistical analysis

FC was analysed using a generalised linear mixed model (GLMM) with binomial error distribution, which incorporated a measure of initial clutch size (i.e. eggs present after density manipulation) by using the number of eggs cannibalised out of the initial number of eggs. We preferred this method over simply comparing proportions, as we here keep the information on absolute numbers the proportions are based on (Crawley 2007; Zuur et al. 2009; Korner-Nievergelt et al. 2015; see also Vallon et al. 2016a; Vallon and Heubel 2016). Overdispersion is present when there is more variance in the real data than expected by the binomial distribution, or more specifically, the residual deviance is higher than the residual degrees of freedom (Crawley 2007; Korner-Nievergelt et al. 2015). As a result of overdispersion, the probability we aim to model is not constant but behaves like a random factor. Thus, as a commonly applied approach to deal with overdispersion, we added an observation-level random factor, where each data point receives a unique level of a random effect to account for the additional binomial variation present in our data (Gelman and Hill 2007; Harrison 2015; Korner-Nievergelt et al. 2015). This allowed us to correct overdispersion by accounting for the extra variance in our data and substantially improved the model fit. Fixed factors included the main treatments *salinity* (low or high), *egg density* (low or high) and their interaction. We further added *cohort* (1 or 2) and male *length* to check for potential confounding effects.

We analysed all remaining response variables in separate models with corresponding error distributions using the same fixed factors, except additionally including clutch size (after density manipulation) as a covariate. However, since there was a small difference in clutch size between egg density groups even in the reduced data set (see “Egg density

manipulation” section), we decided to centre clutch size around its group-wise density mean to avoid confounding in the models, i.e. we subtracted the mean value for a respective density group from each observation (Korner-Nievergelt et al. 2015; see also Vallon et al. 2016b). Time spent egg fanning and weight difference (end weight – start weight) both followed a normal distribution and were analysed using linear models. Number of fanning bouts was analysed as count data in a GLMM (including an observation-level random factor) with Poisson error distribution. For egg fanning rate, we used a similar Poisson GLMM but with the total number of fin flaps as response variable and the time a male actually spent fanning as an offset (see analysis of rates in Korner-Nievergelt et al. 2015).

All models were fitted using the ‘lme4’ package (Bates et al. 2014) in R v. 3.0.3 (R Core Team 2014). We used the Bayesian information criterion (BIC) for backward model selection, and individual factors were removed when their inclusion did not reduce the BIC by at least two (Zuur et al. 2009), while salinity and egg density were always retained as our main treatment factors. Ultimately, all models except the ones for time spent fanning and weight development contained only those two factors, since we found no significant contribution to model fit of either their interaction or any of the additional factors (exceptions detailed in the “Results” section).

Results

The mean proportion of eggs eaten was significantly higher in the low salinity treatment compared to the high salinity treatment (salinity; Table 1; Fig. 2). We also found a significant effect of egg density on FC, but contrary to our predictions males cannibalized more on low density than on high density clutches (Table 1; Fig. 2). Irrespective of these overall differences, there was no evidence for an interaction between both treatments on FC (Fig. 2), and the interaction term dropped out early during model selection (removal of interaction improved model BIC by 3.59).

Our analysis of male egg fanning behaviour revealed that neither time spent fanning (Fig. 3a) nor number of egg fanning bouts (Fig. 3b) nor egg fanning rate (Fig. 3c) were affected by salinity (Table 1). Similarly, there was no effect of egg density on any of the measured aspects of egg fanning (Table 1). However, we found that males spent more time fanning eggs when they had more eggs in their nest (clutch size; Table 1; Fig. 4). At the end of the experiment, high salinity males had lost on average $8.9 \pm 1.2\%$ (mean \pm SE) of their body weight while low salinity males had only lost $4.0 \pm 0.9\%$, which is reflected in a significant effect of salinity on weight difference (Table 1; Fig. 5). A similar but much weaker and non-significant trend was observed for egg density (Table 1). In

Table 1 Test results for the analysis of FC ($n = 51$), different aspects of egg fanning behaviour ($n = 49$) and weight ($n = 50$). Note that estimates for the binomial model are on the logit scale and for the Poisson models on the log scale. Test statistics are given as either t -values (Gaussian error distribution) or z -values (binomial or Poisson error distribution). Bold-type P values indicate significant effects

	Estimate \pm SE	Test statistic	P value
Filial cannibalism			
Binomial GLMM			
(Intercept)	-1.07 ± 0.28		
Salinity	-0.67 ± 0.30	$z = -2.20$	0.028
Egg density	-0.78 ± 0.30	$z = -2.55$	0.011
Time spent egg fanning			
Linear model			
(Intercept)	111.79 ± 18.28		
Salinity	26.21 ± 19.66	$t = 1.33$	0.189
Egg density	-0.03 ± 19.66	$t = -0.002$	0.999
Clutch size (centred)	0.17 ± 0.08	$t = 2.17$	0.036
Number of egg fanning bouts			
Poisson GLMM			
(Intercept)	2.37 ± 0.12		
Salinity	0.01 ± 0.13	$z = 0.04$	0.966
Egg density	0.03 ± 0.13	$z = 0.24$	0.808
Egg fanning rate			
Poisson GLMM			
(Intercept)	0.97 ± 0.04		
Salinity	0.02 ± 0.04	$z = 0.35$	0.729
Egg density	0.05 ± 0.04	$z = 1.06$	0.291
Weight difference			
Linear model			
(Intercept)	91.94 ± 42.25		
Salinity	-22.33 ± 6.24	$t = -3.58$	0.001
Egg density	-11.24 ± 6.42	$t = -1.75$	0.086
Length	-2.92 ± 1.18	$t = -2.48$	0.017

addition, weight loss was higher in larger individuals (length; Table 1).

Discussion

Contrary to our prediction, we found no significant interaction effect of salinity and egg density on FC. Although there seems to be a weak trend that egg density was more important under low salinity conditions (as predicted), the effect of egg density was directly opposite to what we expected. Irrespective of salinity, males cannibalized a higher proportion of their eggs when egg density was low. This is particularly surprising, as many studies highlight the potential positive effects of lower egg densities on offspring survival or disease inhibition, not only in fish (Payne et al. 2002; Klug et al. 2006; Lehtonen and

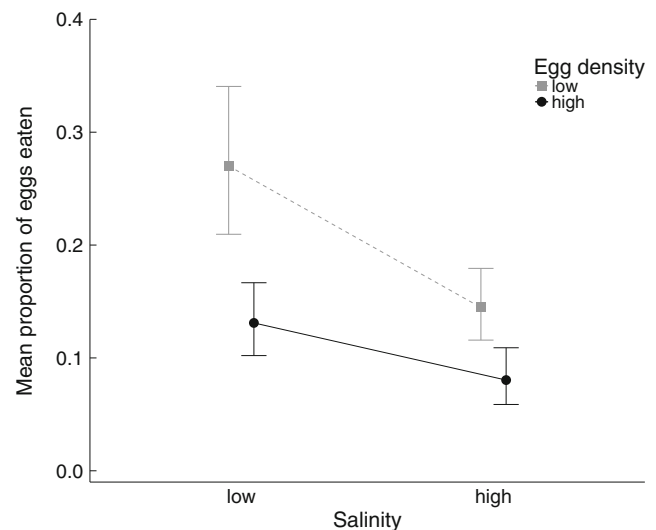


Fig. 2 Interaction plot based on model estimates for the mean proportion of eggs cannibalized after 3 days (excluding cases of TFC). Presented are group means and standard errors for each factor combination of salinity ($n_{\text{low}} = 24$, $n_{\text{high}} = 27$) and egg density ($n_{\text{low}} = 27$, $n_{\text{high}} = 24$)

Kvarnemo 2015b) but also in amphibians (Kiesecker and Blaustein 1997; Green 1999), suggesting that parents should benefit more from FC on high density clutches. Intriguingly, Lehtonen and Kvarnemo (2015a) found that mould growth was higher in low density sand goby egg clutches and argued that the possibly increased water movement between eggs could have facilitated infection by spores. However, contrary to our study, that effect was only present in high (~15 ppt) but not in low salinity (~5.5 ppt) conditions.

To our best knowledge, ours is one of only two studies that tested whether experimentally manipulated egg densities affect levels of FC. While Klug et al. (2006) could show that FC was indeed increased in the high egg density group, it was only true for total FC (a conceptually different mechanism not analysed in our study due to the low number of occurrences; see “Material and methods” section), but not partial FC. The different egg densities in that study were created by letting females spawn in differently sized nests, inducing more densely packed eggs when nest size was small (Klug et al. 2006). In contrast, spawning conditions were identical in our treatments, and we afterwards carefully removed eggs using scissors from either within the clutch or along the edge. As indicated earlier, we cannot fully exclude procedural damage on eggs that later were cannibalized (to a possibly greater extent in the low density treatment because there were more surrounding eggs to be touched). However, we think it is unlikely that this happened on a scale large enough to explain our results. Visually assessing our clutch pictures revealed rather random patterns of FC. For example, we could not observe FC in the high density treatment to happen mostly along the margin with its possibly damaged eggs. Correspondingly, scraping off eggs did also not seem to affect

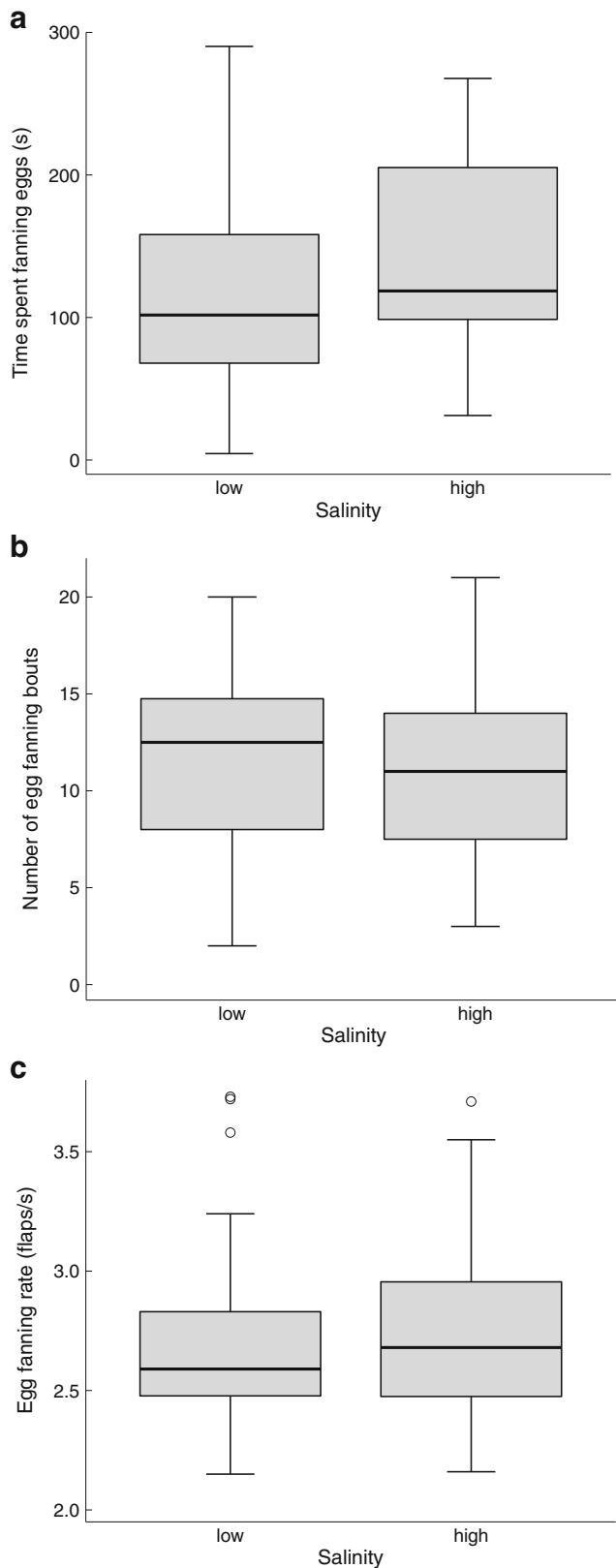


Fig. 3 Comparison of egg fanning behaviour shown by low ($n = 22$) and high salinity males ($n = 27$). Evaluated were **a** time spent egg fanning within 300 s, **b** number of egg fanning bouts and **c** egg fanning rate as fin flaps per second. Box plots represent the medians and the first and third quartiles. Whiskers represent the most extreme data point ≤ 1.5 times the interquartile range from the box. Outliers are shown as separate data points

FC on adjacent eggs in a previous study in common gobies (Vallon et al. 2016a).

While clutch size was similar between the two egg density treatments, the actual egg density in our low egg density treatment, however, could have possibly been too low or the egg distribution too patchy. We generally aimed at maximising the difference between treatments. Particularly in respect to the potential function of preventing the spread of infections, a high mean distance between eggs seemed appropriate. Similarly patchy clutches regularly occur in the lab (and have been observed in the field), but typically only after males had already removed eggs via FC (MV and KUH, personal observation). Although this is the situation we were trying to simulate with our manipulation, males may reject such clutches when received directly after spawning. Specifically, patchy or very low density clutches are potentially of low quality and are gradually consumed while the male tries to attract additional mates to fill the currently free space.

Intriguingly, we found that males cannibalized a greater proportion of their clutch in low compared to high salinity. In contrast to the puzzling outcome of the egg density manipulation, this supports the predictions of Lehtonen and Kvarnemo (2015b) and ourselves. In principle, one may argue that salinity could have influenced egg development and thus possibly FC in other ways than only via mould growth. For

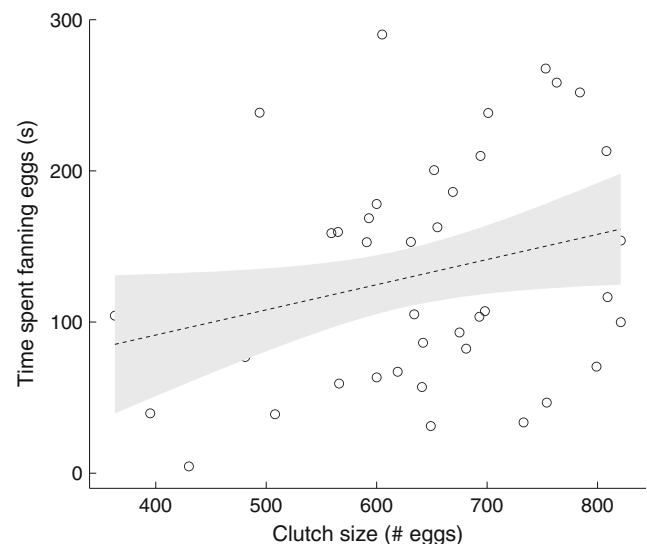


Fig. 4 Time spent fanning eggs as determined by behavioural recordings in relation to the number of eggs present after density manipulation ($n = 49$). The grey area depicts the 95% confidence interval of the regression line

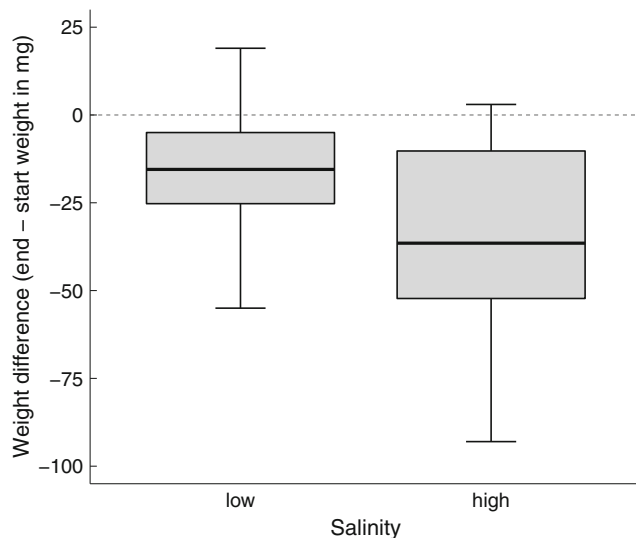


Fig. 5 Difference in weight development between males that were kept in low salinity ($n = 24$) versus high salinity ($n = 26$) conditions. Negative values indicate a weight loss over the course of the experiment. Box plots represent the medians and the first and third quartiles. Whiskers represent the most extreme data point ≤ 1.5 times the interquartile range from the box. Outliers are shown as separate data points

instance, there is evidence from other brackish water fish species that salinity can affect egg developmental rate (Brooks et al. 1997; Karas and Klingsheim 1997). However, a previous study in common gobies shows that eggs raised under similar salinity and temperature conditions as in our experiment do not differ in time until hatching or size of larvae after hatching, indicating no negative effect of the lower salinity in that respect (Fonds and van Buurt 1974).

Alternatively, salinity could have influenced the behaviour or metabolism of adult fish directly. While we cannot rule out potential effects on other aspects of behaviour, our results clearly indicate that salinity did not affect egg fanning, an important part of paternal care and thus potentially very relevant in the context of FC. Effects of salinity on behaviour often vary strongly with the behaviour measured, rendering generalisations difficult. In sand gobies, salinity influenced male aggression but not courtship (Lehtonen et al. 2016a) and only some aspects of nest-building behaviour (Lehtonen et al. 2016b). Notably, male flagfish (*Jordanella floridae*) decreased cleaning and fanning of eggs at high salinity while other, non-egg-directed behaviours remained unaffected (St Mary et al. 2001). The authors argue that this happened likely due to reduced egg needs compared to low salinity conditions (St Mary et al. 2001) and specifically highlight the potential impact of egg diseases in this context (St Mary et al. 2004).

There is generally mixed evidence regarding how parents should adjust fanning behaviour if conditions are challenging. For example, while breeding, convict cichlids (*Cichlasoma nigrofasciatum*) spent less time fanning eggs when on low food rations (Townshend and Wootton 1985) and upland bullies (*Gobiomorphus breviceps*) spent more time fanning

when heavily infected with parasites (Stott and Poulin 1996). Similarly, a previous study in common gobies showed that males increase fanning rate and duration under low oxygen conditions (Jones and Reynolds 1999). This is interesting because oxygen availability is known to decrease with increasing salinity (Kinne and Kinne 1962; Fonds and van Buurt 1974), and one may thus have expected egg fanning to also increase in our high salinity treatment. However, since we did not measure dissolved oxygen levels in our setup, we lack the data to assess the actual differences between treatments.

Neither salinity nor egg density influenced any of the egg fanning measures in our study, but the time a male spent fanning increased with the number of eggs in its nest. This may seem surprising at first, as parental care in fishes is typically assumed to be sharable among all offspring ('non-depreciable' sensu Clutton-Brock 1991) and thus theoretically independent of offspring number (Blumer 1982; Smith and Wootton 1995). Yet, although the cost of care is similar, a large clutch has a greater reproductive value and thus offers a higher potential benefit of care (Sargent and Gross 1985, 1986). Our results are indeed consistent with previous studies showing higher parental effort for larger clutches in terms of egg fanning (St Mary et al. 2001; Suk and Choe 2002; Karino and Arai 2006) or other parental care behaviours (Sargent 1988; Lindström 1998).

In contrast to behaviour, male weight loss varied with salinity. Although fed regularly, males of both salinity treatments lost weight on average over the course of the experiment with weight loss being higher in the high salinity group. In principle, this corresponds to a previous finding that common gobies lose more weight during paternal care when levels of dissolved oxygen are low (Jones and Reynolds 1999), conditions that might have also been present in our high salinity treatment (as discussed earlier). However, increased weight loss was likely connected to more energy spent on paternal care in that study (Jones and Reynolds 1999), which cannot explain our result.

Unrelated to behaviour, three-spined sticklebacks (*Gasterosteus aculeatus*) from a low native salinity regime subjected to high salinity in a long-term common garden experiment were smaller and in worse condition than fish subjected to mid or low salinities (DeFaveri and Merilä 2014). Such potential local adaptation may also be relevant in our study, since the low salinity treatment corresponded to the native salinity of our study population. While a reduced osmoregulatory efficiency may be important in this context in sticklebacks (DeFaveri and Merilä 2014), it does not seem to be a likely factor in common gobies: Several studies highlight the very efficient osmoregulatory capabilities of this species under a broad range of salinities irrespective of the origin of the studied fish (Tolksdorf 1978; von Oertzen 1984; Rigal et al. 2008). Intriguingly, the standard metabolic rate

(Tolksdorf 1978; von Oertzen 1984) and the routine metabolic rate (defined as the oxygen consumption during a feeding or digestion phase with normal locomotor activity (von Oertzen 1984)) vary only slightly between salinities similar to our experiment (at similar temperature conditions), suggesting that energy demands for osmoregulation are negligible.

Nevertheless, Tolksdorf (1978) found that males consumed significantly more food in the high salinity treatment and although they also gained more weight compared to males in the lowest salinity, their food conversion rate was lower. This might explain why male weight loss differed between treatments in our study although all males received the same amount of food. Likewise, larger individuals with likely higher energy demands lost more weight. In principle, these findings also correspond to our main FC result, as additional energy gained via increased cannibalism in low salinity could have led to less weight loss. Yet, this effect was much less pronounced between the egg density groups despite even larger differences in FC. Considering the reverse situation speaks against FC to be driven by energetic needs, the potentially higher food requirements in high salinity did not induce more FC.

Although we could not directly confirm our predictions of an interacting effect between salinity and egg density on FC as well as overall higher FC under low density, our results clearly demonstrate that FC is influenced by salinity as well as egg density. Furthermore, our combined findings suggest that differences in FC between salinities are caused by differential mould growth. This is concordant with our own previous work, where males preferentially cannibalized eggs that had been raised (and developed mould) in low compared to high salinity conditions, although males themselves were housed in low salinity (Vallon et al. 2016a). Brackish water conditions as found in the Baltic Sea can thus drastically influence reproductive decisions of aquatic organisms and may have an even larger impact in the future, as salinity levels in the Baltic Sea are predicted to decrease further (Meier 2006; Neumann 2010).

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Compliance with ethical standards

Data availability Original datasets associated with this paper are available from the corresponding author on reasonable request.

Ethical note All applicable international, national and/or institutional guidelines for the care and use of animals were followed. The study complies with all the relevant laws of Finland and was approved by Finnish authorities. All procedures were declared as class 0 experiments and inspected and approved by ELLA, Animal Experimental Board in Finland on site at Tvärminne Zoological Station in Hanko, Finland.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Ali EH (2005) Morphological and biochemical alterations of oomycete fish pathogen *Saprolegnia parasitica* as affected by salinity, ascorbic acid and their synergistic action. *Mycopathologia* 159:231–243
- Andrén MN, Kvamemo C (2014) Filial cannibalism in a nest-guarding fish: females prefer to spawn in nests with few eggs over many. *Behav Ecol Sociobiol* 68:1565–1576
- Anthony CD (2003) Kinship influences cannibalism in the wolf spider, *Pardosa milvina*. *J Insect Behav* 16:23–36
- Bandoli JH (2016) Filial cannibalism in spottail darters (*Etheostoma squamiceps*) includes the targeted removal of infected eggs. *Behav Ecol Sociobiol* 70:617–624
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1–6, <http://CRAN.R-project.org/package=lme4>
- Blumer LS (1982) A bibliography and categorization of bony fishes exhibiting parental care. *Zool J Linn Soc* 75:1–22
- Borg ÅA, Forsgren E, Magnhagen C (2002) Plastic sex roles in the common goby—the effect of nest availability. *Oikos* 98:105–115
- Bronstein PM (1982) Breeding, paternal behavior, and their interruption in *Betta splendens*. *Anim Learn Behav* 10:145–151
- Brooks S, Tyler C, Sumpter J (1997) Egg quality in fish: what makes a good egg? *Rev Fish Biol Fish* 7:387–416
- Brown AC, Clotfelter ED (2012) Fighting fish (*Betta splendens*) bubble nests do not inhibit microbial growth. *J Exp Zool A* 317:481–487
- Clutton-Brock T (1991) The evolution of parental care. Princeton University Press, Princeton
- R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, <http://www.r-project.org>
- Côté IM, Gross MR (1993) Reduced disease in offspring: a benefit of coloniality in sunfish. *Behav Ecol Sociobiol* 33:269–274
- Crawley MJ (2007) The R book. John Wiley & Sons, Chichester
- DeFaveri J, Merilä J (2014) Local adaptation to salinity in the three-spined stickleback? *J Evol Biol* 27:290–302
- Fonds M, van Buurt G (1974) The influence of temperature and salinity on development and survival of goby eggs (Pisces, Gobiidae). *Hydrobiol Bull* 8:110–116
- Gelman A, Hill J (2007) Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge
- Giacomello E, Marri L, Marchini D, Mazzoldi C, Rasotto MB (2008) Sperm-duct gland secretion of the grass goby *Zosterisessor ophiocephalus* exhibits antimicrobial activity. *J Fish Biol* 73:1823–1828
- Gilbert WM, Nolan PM, Stoehr AM, Hill GE (2005) Filial cannibalism at a house finch nest. *Wilson Bull* 117:413–415
- Green AJ (1999) Implications of pathogenic fungi for life-history evolution in amphibians. *Funct Ecol* 13:573–575
- Harrison XA (2015) A comparison of observation-level random effect and beta-binomial models for modelling overdispersion in binomial data in ecology & evolution. *Peer J* 3:e1114

- Jones JC, Reynolds JD (1999) Costs of egg ventilation for male common gobies breeding in conditions of low dissolved oxygen. *Anim Behav* 57:181–188
- Karas P, Klingsheim V (1997) Effects of temperature and salinity on embryonic development of turbot (*Scophthalmus maximus* L.) from the North Sea, and comparisons with Baltic populations. *Helgolander Meeresun* 51:241–247
- Karino K, Arai R (2006) Effect of clutch size on male egg-fanning behavior and hatching success in the goby, *Eviota prasina* (Klunzinger). *J Exp Mar Biol Ecol* 334:43–50
- Kiesecker JM, Blaustein AR (1997) Influences of egg laying behavior on pathogenic infection of amphibian eggs. *Conserv Biol* 11:214–220
- Kinne O, Kinne EM (1962) Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations. *Can J Zool* 40:231–253
- Klug H, Bonsall MB (2007) When to care for, abandon, or eat your offspring: the evolution of parental care and filial cannibalism. *Am Nat* 170:886–901
- Klug H, St Mary CM (2005) Reproductive fitness consequences of filial cannibalism in the flagfish, *Jordanella floridae*. *Anim Behav* 70:685–691
- Klug H, Lindström K, St Mary CM (2006) Parents benefit from eating offspring: density-dependent egg survivorship compensates for filial cannibalism. *Evolution* 60:2087–2095
- Knouft JH, Page LM, Plewa MJ (2003) Antimicrobial egg cleaning by the fringed darter (Perciformes: Percidae: *Etheostoma crossopetrum*): implications of a novel component of parental care in fishes. *Proc R Soc Lond B* 270:2405–2411
- Korner-Nievergelt F, Roth T, von Felten S, Guélat J, Almasi B, Korner-Nievergelt P (2015) Bayesian data analysis in ecology using linear models with R, BUGS, and Stan. Academic Press, London
- Lehtonen TK, Kvarnemo C (2015a) Density effects on fish egg survival and infections depend on salinity. *Mar Ecol-Prog Ser* 540:183–191
- Lehtonen TK, Kvarnemo C (2015b) Infections may select for filial cannibalism by impacting egg survival in interactions with water salinity and egg density. *Oecologia* 178:673–683
- Lehtonen TK, Svensson PA, Wong BBM (2016a) The influence of recent social experience and physical environment on courtship and male aggression. *BMC Evol Biol* 16:18
- Lehtonen TK, Wong BBM, Kvarnemo C (2016b) Effects of salinity on nest-building behaviour in a marine fish. *BMC Ecol* 16:7
- Lindström K (1998) Effects of costs and benefits of brood care on filial cannibalism in the sand goby. *Behav Ecol Sociobiol* 42:101–106
- Lissåker M, Kvarnemo C, Svensson O (2003) Effects of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, *Pomatoschistus minutus*. *Behav Ecol* 14:374–381
- Magnhagen C, Vestergaard K (1993) Brood size and offspring age affect risk-taking and aggression in nest-guarding common gobies. *Behaviour* 125:233–243
- Manica A (2002) Filial cannibalism in teleost fish. *Biol Rev* 77:261–277
- Marking LL, Rach JJ, Schreiber TM (1994) American Fisheries Society evaluation of antifungal agents for fish culture. *Progr Fish Cult* 56:225–231
- Mehlis M, Bakker TCM, Frommen JG (2009) Nutritional benefits of filial cannibalism in three-spined sticklebacks (*Gasterosteus aculeatus*). *Naturwissenschaften* 96:399–403
- Meier HEM (2006) Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. *Clim Dynam* 27:39–68
- Miller PJ (1975) Age structure and life span in the common goby, *Pomatoschistus microps*. *J Zool* 177:425–448
- Miller JS, Zink AG (2012) Parental care trade-offs and the role of filial cannibalism in the maritime earwig, *Anisolabis maritima*. *Anim Behav* 83:1387–1394
- Neumann T (2010) Climate-change effects on the Baltic Sea ecosystem: a model study. *J Mar Syst* 81:213–224
- Nyman KJ (1953) Observations on the behaviour of *Gobius microps*. *Acta Soc Fauna Flora Fenn* 69:1–11
- Payne AG, Smith C, Campbell AC (2002) Filial cannibalism improves survival and development of beaugregory damselfish embryos. *Proc R Soc Lond B* 269:2095–2102
- Payne AG, Smith C, Campbell AC (2004) A model of oxygen-mediated filial cannibalism in fishes. *Ecol Model* 174:253–266
- Polis GA (1981) The evolution and dynamics of intraspecific predation. *Annu Rev Ecol Syst* 12:225–251
- Rigal F, Chevalier T, Lorin-Nebel C, Charmantier G, Tomasini JA, Aujoulat F, Berrebi P (2008) Osmoregulation as a potential factor for the differential distribution of two cryptic gobiid species, *Pomatoschistus microps* and *P. marmoratus* in French Mediterranean lagoons. *Sci Mar* 72:469–476
- Rogers SI (1988) Reproductive effort and efficiency in the female common goby, *Pomatoschistus microps* (Kroyer) (Teleostei, Gobioidae). *J Fish Biol* 33:109–119
- Rohwer S (1978) Parent cannibalism of offspring and egg raiding as a courtship strategy. *Am Nat* 112:429–440
- Royle NJ, Smiseth PT, Kölliker M (eds) (2012) The evolution of parental care. Oxford University Press, Oxford
- Sargent RC (1988) Paternal care and egg survival both increase with clutch size in the fathead minnow, *Pimephales promelas*. *Behav Ecol Sociobiol* 23:33–37
- Sargent RC (1992) Ecology of filial cannibalism in fish: theoretical perspectives. In: Elgar MA, Crespi BJ (eds) *Cannibalism: ecology and evolution among diverse taxa*. Oxford University Press, Oxford, pp 38–62
- Sargent RC, Gross MR (1985) Parental investment decision rules and the Concorde fallacy. *Behav Ecol Sociobiol* 17:43–45
- Sargent RC, Gross MR (1986) Williams' principle: an explanation of parental care in teleost fishes. In: Pitcher TJ (ed) *The behaviour of teleost fishes*. Croom Helm, Beckenham, pp 275–293
- Smith C, Wootton RJ (1995) The costs of parental care in teleost fishes. *Rev Fish Biol Fish* 5:7–22
- Smith SN, Armstrong RA, Springate J, Barker G (1985) Infection and colonization of trout eggs by Saprolegniaceae. *T Brit Mycol Soc* 85:719–723
- St Mary CM, Noureddine CG, Lindström K (2001) Environmental effects on male reproductive success and parental care in the Florida flagfish *Jordanella floridae*. *Ethology* 107:1035–1052
- St Mary CM, Gordon E, Hale RE (2004) Environmental effects on egg development and hatching success in *Jordanella floridae*, a species with parental care. *J Fish Biol* 65:760–768
- Stott MK, Poulin R (1996) Parasites and parental care in male upland bullies (Eleotridae). *J Fish Biol* 48:283–291
- Suk HY, Choe JC (2002) The presence of eggs in the nest and female choice in common freshwater gobies (*Rhinogobius brunneus*). *Behav Ecol Sociobiol* 52:211–215
- Thoen E, Evensen Ø, Skaar I (2011) Pathogenicity of *Saprolegnia* spp. to Atlantic salmon, *Salmo salar* L., eggs. *J Fish Dis* 34:601–608
- Tolksdorf W (1978) Der Einfluß von Salzgehalt und Temperatur auf Wachstum, Stoffwechsel und Letaltemperatur der Strandgrundel *Pomatoschistus microps* K. *Meeresforschung* 26:15–29
- Townshend TJ, Wootton RJ (1985) Adjusting parental investment to changing environmental conditions: the effect of food ration on parental behaviour of the convict cichlid, *Cichlasoma nigrofasciatum*. *Anim Behav* 33:494–501
- Vallon M, Heubel KU (2016) Old but gold: males preferentially cannibalize young eggs. *Behav Ecol Sociobiol* 70:569–573
- Vallon M, Anthes N, Heubel KU (2016a) Water mold infection but not paternity induces selective filial cannibalism in a goby. *Ecol Evol* 6:7221–7229

- Vallon M, Grom C, Kalb N, Sprenger D, Anthes N, Lindström K, Heubel KU (2016b) You eat what you are: personality-dependent filial cannibalism in a fish with paternal care. *Ecol Evol* 6:1340–1352
- van West P (2006) *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: new challenges for an old problem. *Mycologist* 20: 99–104
- von Oertzen JA (1984) Influence of steady-state and fluctuating salinities on the oxygen consumption and activity of some brackish water shrimps and fishes. *J Exp Mar Biol Ecol* 80:29–46
- Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York