Filial Cannibalism in a Fish
with Paternal Care

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ZUSAMMENFASSUNG

Brutkannibalismus (das Fressen des eigenen Nachwuchses durch Elterntiere) ist erstaunlich weit verbreitet im Tierreich und besonders häufig bei Fischen mit väterlicher Brutfürsorge. Obwohl an sich ein gut bekanntes Phänomen, hat diese augenscheinlich sonderbare Verhaltensweise Wissenschaftler jahrzehntelang vor Rätsel gestellt und ihr adaptiver Nutzen ist bis heute nicht vollständig geklärt. Die bisherige Forschung zu diesem Thema hat sich hauptsächlich auf die Hypothese konzentriert, dass Eltern ihren Nachwuchs fressen um ihren eigenen Energiebedarf zu decken. Allerdings deuten die verfügbaren Ergebnisse daraufhin, dass noch mehr dahinter steckt. Im Rahmen dieser Doktorarbeit habe ich verschiedene alternative Hypothesen zum adaptiven Nutzen von Brutkannibalismus untersucht. Dazu habe ich eine Reihe umfangreicher Laborexperimente durchgeführt mit einem kleinen marinen Fisch, der Strandgrundel (*Pomatoschistus microps*), als Modellsystem. Indem ich brutpflegenden, männlichen Strandgrundeln gleichzeitig Eier unterschiedlichen Alters zur Verfügung gestellt habe, konnte ich zeigen, dass vorzugsweise die jungen, weniger wertvollen Eier kannibalisiert werden (Kapitel I). Dieses Ergebnis bestätigt die Vorhersage, dass Brutkannibalismus ein Mechanismus sein könnte um selektiv Nachwuchs von „minderer Qualität" zu entfernen. Auf ähnliche Weise konnte ich zeigen, dass Eiinfektionen, aber nicht die vaterschaftliche Beziehung, solch selektiven Brutkannibalismus auslösen können (Kapitel II). Mithilfe eines anderen experimentellen Ansatzes habe ich außerdem untersucht wie sich Salinität, Dichte der Eier im Gelege, und die Interaktion beider Faktoren auf den Kannibalismus auswirken (Kapitel III). Ich konnte zeigen, dass der Brutkannibalismus bei niedriger Salinität höher ist, was den Erwartungen entspricht, weil unter solchen Bedingungen Ei-Pathogene besser wachsen. Dies war allerdings nicht der Fall bei Gelegen mit hoher Ei-Dichte, obwohl hier ebenso bessere Bedingungen für Pathogene herrschen sollten (Kapitel III). Indem ich diese einerseits unterschiedlichen aber gleichzeitig auch zusammenhängenden Faktoren untersucht habe, konnte ich in dieser Arbeit darlegen, dass Brutkannibalismus tatsächlich

durch eine Vielzahl verschiedener Umwelt-, Eltern- und Nachwuchsparameter beeinflusst zu sein scheint, wobei ich Eiinfektionen als einen Hauptfaktor hervorheben konnte.

SUMMARY

Filial cannibalism – the consumption of one's own offspring – has been described in a wide range of animal taxa, while being particularly common in fish showing paternal care. Despite being a well-known phenomenon, this seemingly odd behaviour has puzzled researchers for decades and its adaptiveness is still not fully understood. Previous research on this topic has mainly focussed on the hypothesis that parents may consume their offspring to satisfy their own energetic needs, but the available evidence indicates that there is more to filial cannibalism than energetics alone. During the course of this thesis, I investigated several alternative hypotheses on the adaptiveness of filial cannibalism. For this, I conducted a series of extensive laboratory experiments using a small marine fish, the common goby (*Pomatoschistus microps*), as a model species. By simultaneously presenting egg-guarding male common gobies with eggs of varying age, I was able to show that the young, least valuable eggs are preferentially cannibalised (chapter I). This confirms the prediction that filial cannibalism may be a mechanisms to selectively remove and consume offspring with a certain "low-quality" phenotype. Similarly, I could demonstrate that egg infections, but not paternity, trigger such selective filial cannibalism (chapter II). Using a different approach, I tested how water salinity, egg density and their interaction relate to cannibalistic behaviour (chapter III). I could show that filial cannibalism is increased in low salinity as predicted due to increased growth of egg pathogens under such conditions, while this was not the case for the presumably similarly susceptible high-density egg clutches (chapter III). By investigating these different yet connected factors, I could demonstrate in this thesis that filial cannibalism is indeed likely influenced by a wide array of environmental, parental and offspring parameters, while highlighting egg infections as a major driver at least in fish.

LIST OF PUBLICATIONS

Accepted papers

Chapter I:

Vallon M & Heubel KU (2016) Old but gold: males preferentially cannibalize young eggs. *Behavioral Ecology and Sociobiology* **178**: 673-683. DOI: 10.1007/s00265-016-2074-6

Chapter II:

Vallon M, Anthes N & Heubel KU (2016) Water mold infection but not paternity induces selective filial cannibalism in a goby. *Ecology and Evolution* **6**: 7221-7229. DOI: 10.1002/ece3.2403

Submitted papers

Chapter III:

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PERSONAL CONTRIBUTION

Martin Vallon conceptually developed the theoretical and experimental framework, conducted all experiments and data analyses, and authored this dissertation and the resulting manuscripts

Katja U. Heubel conceived and initiated the original project, supervised all studies including advice on the overall framework, experimental design and data analysis, participated in data collection, and critically revised the manuscripts

Nils Anthes gave advice on experimental design and data analysis, participated in data collection, and critically revised the manuscripts

INTRODUCTION

Parental care

From a human's point of view, it seems only natural to care for one's own offspring and many parents go to great lengths to support their children. Not surprisingly, this phenomenon is not restricted to humans, but is in fact widespread in the animal kingdom (Gross & Sargent 1985, Clutton-Brock 1991, Royle *et al.* 2012). But what exactly is parental care? First of all, it is important to note that parental care is per definition not restricted to certain behavioural traits, but rather incorporates any form of parental investment into offspring aimed at increasing offspring condition and survival, and ultimately offspring fitness (Trivers 1972, Clutton-Brock 1991, Royle *et al.* 2012). Hence, it can in principle span from the production of yolk-containing eggs over directly provisioning for offspring before and after egg laying or birth to more indirect behaviours such as cleaning or guarding offspring (Blumer 1982, Royle *et al.* 2012). Some animals even go to extremes to ensure the wellbeing of their offspring, such as females of the subsocial spider (*Stegodyphus lineatus*), which repeatedly regurgitate food and then ultimately sacrifice themselves to let their young feed upon their body (Salomon *et al.* 2015).

While there has been some debate in the past about where to draw the line between parental care and other forms of reproductive investment, a common definition is that parental care can be given both before and after the eggs are fertilized (Clutton-Brock 1991). According to other, more narrow definitions, prezygotic gamete investment such as nutritious investment into female gametes (sometimes also by males in form of nuptial gifts) is not a part of actual parental care, while other prezygotic investments like nest building behaviour are less controversial (Kvarnemo 2010).

Parental care can generally be given by either the female (maternal care), the male (paternal care), or both (biparental care)[1]. Interestingly, the predominant mode often differs between taxonomic groups (Kvarnemo 2010). In birds, biparental care is most common (e.g. incubation of eggs), while the majority of mammal species features female-only care (e.g. gestation and lactation). Likewise, maternal care is the dominant mode in insects. Reptiles and amphibians often feature maternal or biparental and maternal or paternal care, respectively (Clutton-Brock 1991, Reynolds *et al.* 2002, Kvarnemo 2010).

Notably, fishes are the only group where male-only care is clearly more common than care by females or both parents. This otherwise rather uncommon mode occurs in more than 60 % of all families of bony fishes exhibiting parental care (Gittleman 1981). Due to the fact that all three modes occur to some extend (sometimes within the same taxonomic family) and are widespread across fish phylogeny, fishes are a common model system to study evolutionary transitions in parental care (Baylis 1981, Gittleman 1981, Gross & Sargent 1985, Reynolds *et al.* 2002, Amundsen 2003).

Paternal care in fishes incorporates a wide range of different behaviours including nest building and maintenance, and egg-directed behaviours such as cleaning and guarding (Blumer 1982). While direct nutritional provisioning for hatched young is rare (but see peculiar cases such as ectodermal mucus feeding in discus fish; Buckley *et al.* 2010), larvae are sometimes also guarded, e.g. by returning them to the nest or school when straying off too far (Wootton 1984) or by directly providing shelter as in mouth-brooding species (Balshine-Earn & Earn 1998).

In addition, fishes exhibit a characteristic care behaviour related to their aquatic lifestyle; using fanning movements with one or several fins simultaneously, the caring parent (male or female) creates water movement inside the often quite sheltered nest. While not strictly unique to fish (see for example a similar behaviour in Japanese giant salamanders *Andrias*

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[1] Please note that extraordinary cases such as, for example, cooperative breeding in fishes (Taborsky 1994) or social insects (Andersson 1984) go beyond the scope of this thesis and will not be discussed further.

japonicus; Okuda *et al.* 2014), this so-called egg fanning is particularly common in this group and is thought of as primarily serving the function of improving oxygenation of the eggs (van Iersel 1953, Jones & Reynolds 1999). Alternative, non-mutually exclusive functions of egg fanning are removal of sediments from the eggs and active prevention of infections via inhibiting settlement of pathogens by creating a constant water flow (Côté & Gross 1993, Hale *et al.* 2003, St Mary *et al.* 2004).

Not surprisingly, regular egg fanning is energetically costly (Townshend & Wootton 1985, Lindström & Hellström 1993), which is – to a varying degree – also true for most other forms of parental care (Clutton-Brock 1991, Smith & Wootton 1995, Cooke *et al.* 2006, Bose *et al.* 2016b).

Infanticide

Although many animal invest heavily in the wellbeing of their offspring as described above, it is surprisingly common that parents abandon or even kill their very own young (Polis 1981, Royle *et al.* 2012). The killing of own offspring by parents is a special form of infanticide (i.e. the killing of conspecific young by mature animals irrespective of relatedness) and is typically known as filial or parental infanticide. Infanticide in general has been observed in a wide array of animal taxa (Hrdy 1979, van Schaik & Janson 2000) and is best documented in social species such as primates (Hiraiwa-Hasegawa 1988) and carnivores (Packer & Pusey 1984, Balme & Hunter 2013).

Infanticide is assumed to be often related to sexual conflict, as males can use it to improve their fitness by increasing their opportunity to sire offspring (Hrdy 1979, Palombit 2015). A well-known example is the killing of young cubs by individual male lions to be able to impregnate the now once again available female (Packer & Pusey 1983, Packer & Pusey 1984). Similarly, male subsocial spiders (*Stegodyphus lineatus*) destroy eggs sacs of females fertilized by other males to encourage them to mate again (Schneider & Lubin 1996). Another common explanation for infanticide is competition for limited physical resources such as food or nest sites (Hrdy 1979, Palombit 2015), which may be particularly relevant under high population densities (Ebensperger 1998). For instance, in the cooperatively-breeding meerkat (*Suricata suricata*) pregnant females regularly kill new-born pups of other females in the group, presumably to increase future food availability (i.e. availability of adult helpers) for their own pups (Clutton-Brock *et al.* 1998).

However, such mechanisms seem less and less suitable to adequately explain the occurrence of infanticide the closer related the killed young are to the mature animal. It is indeed initially hard to image how killing one's own offspring should increase paternal reproductive success. Notably, filial infanticide seems to be often connected to actual consumption of the killed offspring (Hrdy 1979, Polis 1981, Elgar & Crespi 1992, Manica 2002, Royle *et al.* 2012).

Filial cannibalism

Parents eating their own eggs or young is generally known as filial cannibalism (hereafter referred to as FC), while sometimes also termed 'kronism' (Schüz 1957) after the titan Kronos who – according to Greek mythology – swallowed his own children whole (Allaby 2010). This seemingly odd behaviour is in fact widespread in the animal kingdom and can be exhibited by either sex of the parents (Polis 1981, Elgar & Crespi 1992; Table 1).

Table 1. *Examples for cases of filial cannibalism from different taxonomic groups.*

Species	Cannibalising parent	Reference
Wolf spider (*Pardosa milvina*)	Female	Anthony (2003)
Maritime earwig (*Anisolabis maritima*)	Female	Miller and Zink (2012)
Assassin bug (*Rhinocoris tristis*)	Male	Thomas and Manica (2003)
Burying beetle (*Nicrophorus quadripunctatus*)	Both	Takata *et al.* (2013)
Japanese giant salamander (*Andrias japonicus*)	Male	Okada *et al.* (2015)
Three-spined stickleback (*Gasterosteus aculeatus*)	Both	Rohwer (1978), FitzGerald and van Havre (1987)
Beaugregory damselfish (*Stegastes leucostictus*)	Male	Payne *et al.* (2002)
House finch (*Carpodacus mexicanus*)	Female	Gilbert *et al.* (2005)
Chestnut-backed Sparrow-lark (*Eremopterix leucotis*)	Female	Engelbrecht (2013)
Norway rat (*Rattus norvegicus*)	Female	Boice (1972)
House mouse (*Mus musculus*)	Female	König (1989)

Nevertheless, the question remains why parents should consume their very own offspring. Several decades ago, FC was often simply considered as a maladaptive or abnormal behaviour, or even a laboratory artefact (Manica 2002). In contrast, nowadays it is typically seen as an adaptive behaviour that increases the cannibalising parent's lifetime reproductive success (FitzGerald 1992, Manica 2002), while there is no strict consensus on the actual adaptive function. However, a number of different hypotheses have been suggested and most are not necessarily mutually exclusive (FitzGerald 1992, Manica 2002, Klug & Bonsall 2007, Royle *et al.* 2012).

It is often assumed that FC is connected to energetic or nutritional needs of the cannibalising parent. According to the so-called energy-based hypothesis, the energy gained by consuming offspring may be necessary for the parent to be able to sustain care for remaining offspring or can be reinvested in future offspring, hence actually improving overall parental fitness (Rohwer 1978, Sargent 1992). In this context, it is important to distinguish between two very distinct processes when assessing the adaptiveness of FC: In many cases only a part of the eggs or young are consumed, but it also happens regularly that parents consume all of their current offspring and thereby fully terminate their current reproduction (Manica 2002). While the former (so-called partial filial cannibalism) can potentially serve as an investment in both the current and future reproductive success, the latter (total filial cannibalism), consequently can only be beneficial for future reproductive success (Sargent 1992).

Empirical and theoretical work on the adaptiveness of FC is often conducted using fishes, where this behaviour seems to be particularly common (Elgar & Crespi 1992, FitzGerald 1992, Manica 2002, Lindström & St. Mary 2008) and which will also be the main focus of this thesis. There is indeed a large number of documented cases with records from 17 different taxonomic families of teleost fish. Notably, in fish FC is often exhibited only by males in form of consuming eggs during paternal care (Manica 2002).

As described earlier, paternal care in fish is usually an energy-intensive process, often involving demanding activities such as egg fanning (Smith & Wootton 1995, Cooke *et al.*

2006, Bose *et al.* 2016b). At the same time, egg-guarding males often do not venture far from the nest and thus likely only have limited feeding opportunities (Magnhagen 1986, Marconato *et al.* 1993, Smith & Wootton 1995). Hence, sacrificing eggs, i.e. using them as an alternative energy source, may enable males to survive and provide care in the first place (Rohwer 1978, Sargent 1992). In addition, eggs are very nutritious and it has been suggested that they are eaten primarily due to specific nutrients otherwise hard to obtain for egg-guarding males (Belles-Isles & FitzGerald 1991, FitzGerald 1991).

This potential connection between FC and energetic needs of the cannibalising parent in fish has been the focus of various empirical studies during the last decades (earlier studies reviewed in Manica 2002). Specifically, those experiments were designed to test the prediction that limited food availability or bad parental condition lead to an increase in FC (Lindström & St. Mary 2008). Manica (2004) could indeed show that male scissortail sergeants (*Abudefduf sexfasciatus*) consumed significantly fewer eggs when given a supplementary feeding treatment. Likewise, starved male common gobies (*Pomatoschistus microps*) showed more FC than males given food in excess (Kvarnemo *et al.* 1998) and field observation in river bullheads (*Cottus gobio*) indicate that the frequency of FC is negatively correlated with the male's chance of getting other food items (Marconato *et al.* 1993). However, others found no such effect in other species where FC occurs regularly: FC was unrelated to food ration in threespine sticklebacks, *Gasterosteus aculeatus* (Belles-Isles & FitzGerald 1991) and to initial body condition in fantail darters, *Etheostoma abellare* (Lindström & Sargent 1997). In addition, male flagfish (*Jordanella floridae*) from a low-food treatment (Klug & St Mary 2005) and sand gobies (*Pomatoschistus microps*) in poor condition (Klug *et al.* 2006) surprisingly even cannibalised fewer eggs than their well-fed conspecifics. Related to such experiments, Bose *et al.* (2016b) did not find a difference in body energy reserves between cannibals and non-cannibals after a given care period in plainfin midshipman (*Porichthys notatus*). It thus clearly seems like there is more to FC than energetics alone.

As indicated earlier, various other ideas on the adaptiveness of FC have previously been suggested. However, those have not received the same level of attention as energy-based models and thus often have neither been fully developed theoretically nor adequately addressed in empirical studies. For instance, offspring survival may often be density-dependent. Under such circumstances, cannibalising a fraction of the eggs or young may directly increase survival of the remaining offspring and thus overall parental reproductive success (Payne *et al.* 2002, Klug *et al.* 2006). It has specifically been suggested that oxygen availability to fish eggs may be the driving factor in this case (Payne *et al.* 2002, 2004), but other factors such as reduced spread of egg diseases in low density clutches are just as likely.

Filial cannibalism may also be connected to variation in offspring phenotype. Parents could increase their reproductive success by selectively removing "unwanted", low-quality offspring (e.g. offspring that is diseased, has a low reproductive value due to long maturation time or is connected to a low certainty of paternity). Although this possibility has been given consideration already many decades ago, particularly in relation to the consumption of dead or diseased eggs (e.g. Bailey 1952, Winn 1958), actual empirical evidence is rare and not conclusive, as generally the case for the overall assessment of FC.

Using a small gobiid fish, the common goby (*Pomatoschistus microps*, Krøyer 1838) as a model system, the work for this thesis was conducted to further our understanding on the evolutionary conundrum that is filial cannibalism.

The common goby as a model system

Common gobies are small benthic fish occurring from the Mediterranean to the Baltic Sea (Miller 1975, 1986, Louisy 2002, Gysels *et al.* 2004). Adults inhabit shallow, soft-bottom coastal and estuarine habitats (Miller 1986), while larvae mostly develop in deeper waters (Jones & Miller 1966). Common gobies are short-lived and typically reproduce repeatedly only during a single, 2-3 month reproductive season (Miller 1975).

Nests are built using suitable hard structures such as mussel shells of the soft clam *Mya arenaria* (Nyman 1953, Vestergaard 1976). Occasionally also small rocks, pieces of wood (Nyman 1953) or even man-made material (e.g. plastic) found on the seafloor are used (personal observation). The male excavates underneath the hard structure and covers everything with sand, sometimes leaving only a small opening (Nyman 1953, Vestergaard 1976). During courtship, which also involves acoustic signals (Blom *et al.* 2016), the female is led into the nest to spawn. The eggs are attached to the ceiling of the nest in a single layer and spawning can take several hours (Nyman 1953). Afterwards, the female abandons the eggs and leaves the male to care for them until hatching. Males can receive several egg clutches of different females, depending on the size of the nest, and care for all eggs simultaneously during a single breeding cycle (Magnhagen & Vestergaard 1993).

Competition for mussel shells and other nest structures can be fierce (Borg *et al.* 2002) and large males often manage to obtain larger shells, which can also result in more eggs in their nest (Magnhagen & Vestergaard 1993). Small males may try to 'sneak' fertilisations while a spawning is happening in another male's nest (Magnhagen 1992, 1998, Svensson *et al.* 1998), an alternative reproductive tactic also present in numerous other fish species (see Taborsky 1994 for a review). Aggression by nest-holding males against other males that get close to their nest is thus typically strong (Magnhagen 1994, Magnhagen 1995). Other males may also try to completely take over the nest even if eggs are already present, leading to complete destruction of the current egg batch through cannibalism. In addition, eggs often have to be guarded against egg predators. Feeding possibilities for egg guarding

males are consequently limited and they feed significantly less than females during this period (Magnhagen 1986).

Filial cannibalism by male common gobies seems to occur frequently in the field (personal observation) and has been observed and quantified in various laboratory studies (e.g. Kvarnemo *et al.* 1998, Svensson *et al.* 1998, Vallon *et al.* 2016b). Those and similar previous experiments have shown that artificial nest structures such as halved ceramic flowerpots or bathroom tiles are often quickly occupied when brought out in the field (Magnhagen & Vestergaard 1991) and also readily accepted when offered in the lab, facilitating artificial spawnings and subsequent experimental work in a controlled laboratory setting. Hence, the common goby is the ideal model system for the purpose of this thesis.

STUDY GOALS

The general aim of this thesis was to explore possible alternative functions of filial cannibalism that go beyond satisfying the energetic needs of the parent. This was done based on several extensive laboratory experiments using wild-caught common gobies. Previous experience from laboratory experiments with common gobies had indicated that egg-guarding males readily re-accept egg clutches that are temporarily removed from their nests. This behavioural feature was in principle the basis for the experimental work for this thesis and has two major benefits: First, it generally greatly facilitates determination of initial egg number and subsequent reduction via FC by allowing for repeated photography of egg clutches. And second, it enabled me not only to directly manipulate individual clutches (e.g. in relation to egg density) but also to mix clutch parts of different origin to create mixed egg batches that simultaneously included differently treated (or naturally different) eggs.

Based on these methods, the first two studies both foremost addressed the general question whether the cannibalising parent is able to specifically pick out offspring of a certain phenotype (chapter I and II). In particular, I investigated in a series of experiments if eggs of low reproductive value due to young age (chapter I), egg infections (chapter II) or unrelatedness (chapter II) are preferentially eaten. For chapter II, I partly made use of indirectly manipulating microbial infections by influencing their growth via adjusting water salinity. Promising results in these earlier fundamental studies then led me to develop those considerations on putative alternative functions of FC further. Only very little is currently known about the influence of environmental conditions on FC. However, I suspected that salinity, by influencing growth of water mould and other pathogens, indirectly influences FC. More specifically, I assessed the possibility that density-dependent filial cannibalism is driven by egg infections – which are in turn influenced by salinity – because pathogens spread more easily on high-density clutches (chapter III).

While not comprehensive in covering all alternative functions of FC, the chosen topics for the three chapters forming this thesis are inherently connected with each other and aimed at shedding light on particularly promising and understudied aspects of FC. Hence, combined they may contribute significantly to our general understanding of this behaviour.

CHAPTER I

Old but gold: males preferentially cannibalize young eggs

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Abstract

Although counterintuitive at first sight, filial cannibalism is common in the animal kingdom and has been recognized as a mechanism to increase the cannibalizing parent's lifetime reproductive success. However, previous evidence is often inconclusive and the adaptiveness of filial cannibalism is still not fully understood. We here address the notion that parents do not cannibalize at random but preferably consume offspring with a particular phenotype. To assess if differences in developmental stage and thus reproductive value of eggs trigger such selectivity, we experimentally presented male common gobies (*Pomatoschistus microps*) with two differently aged egg clutches within mixed broods. We found that males consumed significantly more young than old eggs. This result indicates that parents are not only able to discriminate between eggs based on developmental stage, but might use this to reduce the cost of partial filial cannibalism by selectively removing eggs of lower reproductive value.

Significance Statement

Cannibalizing some of one's own eggs instead of caring for them can be seen as an extreme form of strategically redirecting parental investment to the remaining offspring. This is the first controlled experiment confirming the prediction that animal parents caring for mixed broods with eggs of different developmental stages should preferentially eat the younger, to the parent less valuable eggs.

Keywords

Foster care, Life history evolution, Offspring age, Paternal care, Reproductive value, Selective filial cannibalism, Kin discrimination

Introduction

Some animal parents are known to regularly consume their own offspring, a phenomenon that has been coined filial cannibalism. This peculiar behavior occurs in a large number of taxa (Polis 1981) and is particularly common in fish exhibiting paternal care (Manica 2002). While cannibalizing one's own eggs or young may seem odd at first, it is generally thought to represent an adaptive strategy, where parents sacrifice some (partial filial cannibalism) or even all of their current offspring (total filial cannibalism) to enhance survival of the remaining or possible future progeny (Rohwer 1978; Manica 2002). Total filial cannibalism (or whole-clutch termination) relates conceptually to life-history related brood abandonment strategies and mainly depends on future mating opportunities and hence the trade-off between investment in current versus future reproduction (Manica 2002; Kvarnemo 2010; Klug et al. 2012). In contrast, partial filial cannibalism is not necessarily only an investment in future reproduction, but can also be considered as an extreme form of facultative adjustment of parental investment into offspring with greater reproductive value within the brood currently cared for. Various mutually non-exclusive hypotheses on benefits of filial cannibalism for the caring parent or the remaining offspring exist (Manica 2002; Klug et al. 2012). In particular, it is often argued that the consumption of offspring provides energy needed for sustaining brood care and the cannibal's own survival (the energy-based hypothesis: Rohwer 1978; Sargent 1992; Mehlis et al. 2009). Which eggs or young are actually eaten may, however, not be random. In accordance with parental investment theory (Klug et al. 2012), given the choice, one would expect cannibals to specifically consume offspring of lower reproductive value to reduce the cost of cannibalism.

Notably, in many species showing paternal care, differently aged eggs can be present in one nest since males often accept clutches from more than one female (Baylis 1981). Long time lags between individual spawnings potentially lead to substantial differences in age and thus developmental stage between eggs. The more developed an egg is, the

more valuable it should be for the male, particularly when considering the large effort usually needed to keep eggs alive until hatching and the continuous risk of losing the whole brood. This in turn implies that young eggs should be cannibalized preferentially. While there are indications that males selectively eat younger eggs when differentially aged clutches are present, egg age in earlier studies was at least partially confounded with other variables such as position in the nest or clutch size (Sikkel 1994; Klug and Lindström 2008). In addition, previous manipulative experiments in the lab have so far failed to confirm these results (Manica 2003; Takegaki et al. 2011). Thus, an experimental confirmation of theoretical predictions on filial cannibalism based on offspring age is still missing.

We here addressed this topic with a direct experimental approach using a small marine fish, the common goby (*Pomatoschistus microps*, Krøyer), as a model system. Common gobies have a resource-based mating system and exclusive paternal care. Males often care for multiple clutches laid by different females within a single nest at the same time and frequently cannibalize their own eggs (Nyman 1953). To assess if paternal males selectively cannibalize based on egg age, we provided each male with eggs from two differentially aged clutches simultaneously while controlling for egg number, position in the nest, kinship and female size.

Material and methods

The experiment was conducted at Tvärminne Zoological Station, Finland, in July 2013. Fish were collected using either a beach seine or a hand net while snorkeling. We measured body size as total length (to the nearest mm) prior to use. Males were housed in separate aquaria (35 L), each equipped with a half-flowerpot (4.5 cm diameter) as artificial nest site. Individual nests contained a removable plastic sheet at the ceiling onto which females laid their eggs during spawning. All aquaria were continuously supplied with fresh sea water and water temperature was measured daily. All fish were fed with frozen chironomid larvae twice a day.

Males were divided into two groups: "fathers" (mean ± SE total length: 35.0 ± 0.9 mm) and "surrogates" (35.1 ± 0.9 mm). Each father spawned sequentially (time difference of 3.3 ± 0.2 days) with two similar-sized females (female 1: 37.9 ± 0.5 mm; female 2: 37.8 ± 0.8 mm), while each surrogate reared the first clutch of one father until the father acquired its second clutch to keep the two clutches as independent as possible. Surrogate males were used because rearing common goby eggs without a male and thus without brood care often leads to mold infections on eggs (MV and KUH, personal observation). All surrogates also had to spawn once (the clutch was discarded afterwards) to initiate paternal care behavior, which ensured that they accepted and cared for a foreign clutch inserted into their nest.

Following the second spawning, both clutches were cut into two halves each, while removing a central stripe with eggs from the larger clutch to achieve a similar number of eggs on all pieces (young halves: 480.3 ± 39.4 eggs; old halves: 472.3 ± 39.4 eggs; no difference in egg number between halves: paired t-test; t = 0.06, df = 14, P = 0.953). The left side of the older clutch was then recombined with the right side of the younger clutch and vice versa. One random pair was inserted into the father's nest. In addition, we inserted the other pair into the corresponding surrogate's nest to check for potential

kinship effects. Males were left to care for these mixed-age broods until the end of the experiment three days later.

To quantify original egg number and filial cannibalism, clutches (or clutch halves) were photographed at several stages throughout the experiment (after first and second spawning, after recombining the two halves, at the end of the experiment). Males always accepted eggs returned after photography without detectable changes in paternal care behavior and similar methods involving repeated nest disturbances have been successfully applied before (e.g. Jones and Reynolds 1999; Heubel et al. 2008; Andrén and Kvarnemo 2014). We analyzed all images by manually counting eggs using the Cell Counter plugin (Kurt De Vos, University of Sheffield, UK) in ImageJ version 1.44p (Wayne Rasband, NIH, USA), while being blind to the treatments.

After excluding all fish that either did not spawn twice or suffered from water mold infections (on adult fish or eggs), and one case of consumption of the entire brood (unconnected to any infection), we were able to quantify cannibalism on mixed-age broods for 15 males (fathers and surrogates). To analyze if males cannibalized differentially on young and old halves, we fitted a generalized linear mixed model (GLMM) with binomial error distribution (considering for each male number of eggs eaten and initial egg number as binomial denominator; Manica 2004) with number of eggs eaten (*'successes'*) versus eggs remaining (*'failures'*) after three days as response variables and thus incorporating a measure of initial egg number (see analyses of proportion data in Crawley 2007; Zuur et al. 2009; Korner-Nievergelt et al. 2015). Since each male provided a data point for young eggs eaten as well as for old eggs eaten, *Male ID* was included as a random factor to account for the paired measurements, while also fitting random slopes to individuals over egg age. Considering that two broods were sired by one male (the father from a father-surrogate pair), we used *Father ID* as a second random factor with nine levels. Fixed factors were *egg age* (young or old) and *group* (father or surrogate). Additionally including their interaction or total length of males did not improve model fit according to the Bayesian Information Criterion (BIC)

and was not considered further. All statistical analyses were conducted using the lme4 package (Bates et al. 2014) in R v. 3.0.3 (R Core Team 2014).

Results

Males cannibalized on average 127.3 ± 30.1 (mean ± SE) young eggs (i.e. 25.8 ± 6.0 %) and only 57.0 ± 27.4 old eggs (11.9 ± 5.4 %), which is reflected in a highly significant effect of *egg age* (Table 1). Only 3 out of 15 individuals did not consume more eggs from the younger clutch half, but two of these barely cannibalized at all (Fig. 1).

Table 1. *Fixed effect estimates from a generalized linear mixed model with binomial error structure. The model evaluated the effect of egg age (young or old; paired measurements per male) and group (fathers or surrogates; nfathers = 6, nsurrogates = 9) on the number of eggs cannibalized by common goby males (n = 30 observations of 15 individuals) while considering the initial number of eggs. Note that estimates are on the logit-scale*

	estimate	SE	z-value	*P*
(Intercept)	-1.26	0.59	-2.13	0.033
egg age	-1.16	0.29	-4.02	< 0.001
group	-0.59	0.57	-1.04	0.300

Whether a male was a father (i.e. caring for its own eggs after a phase of no paternal care) or a surrogate (i.e. continuing foster care) did not affect cannibalism levels (*group*; Table 1, Fig. 2). In addition, testing the effect of the main factor *egg age* excluding all surrogates and thus only using the six fathers still confirmed the observed difference (*egg age*; n = 12 observations of 6 individuals, $z = -2.63$, $P = 0.009$; see father group in Fig. 2).
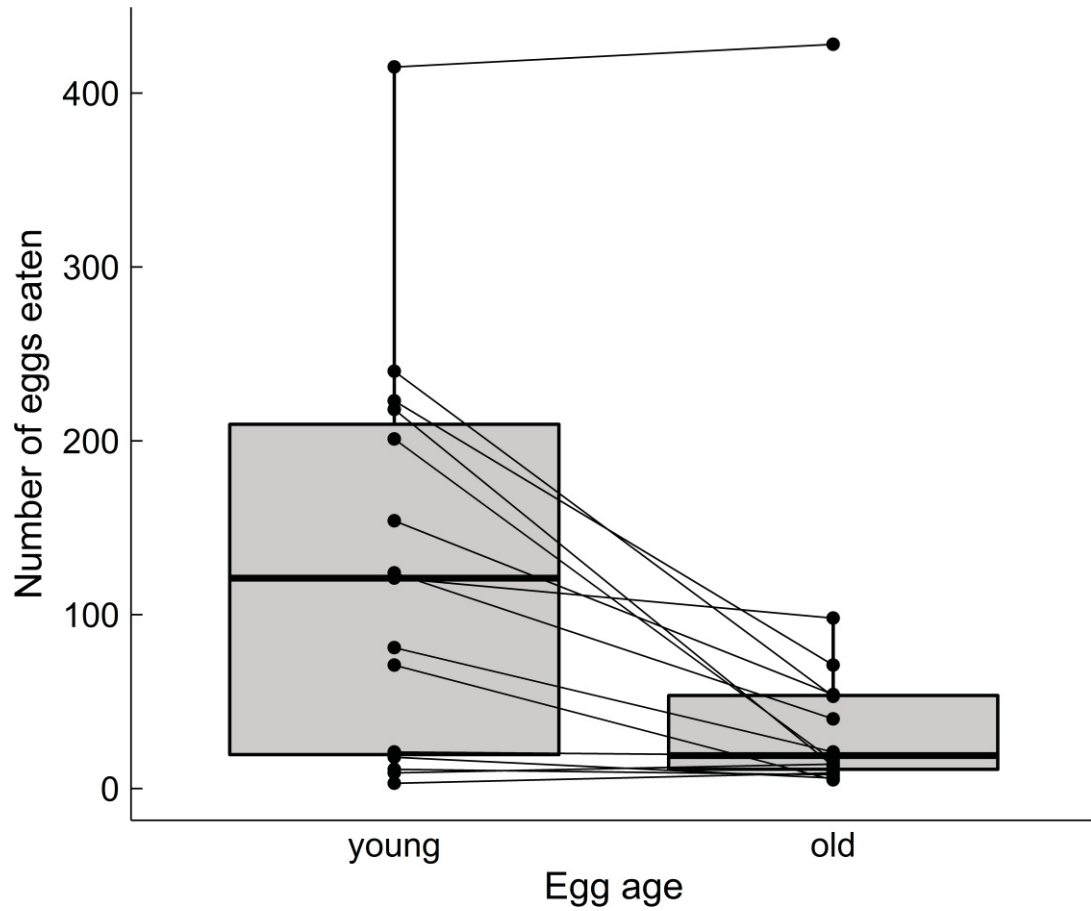
Figure 1. *Absolute filial cannibalism on young and old clutch halves after three days. Lines connect data points of individual males. Each box shows the interquartile range (IQR) divided by the median and whiskers extend to the most extreme data points still within 1.5 × IQR from the edges of the box*
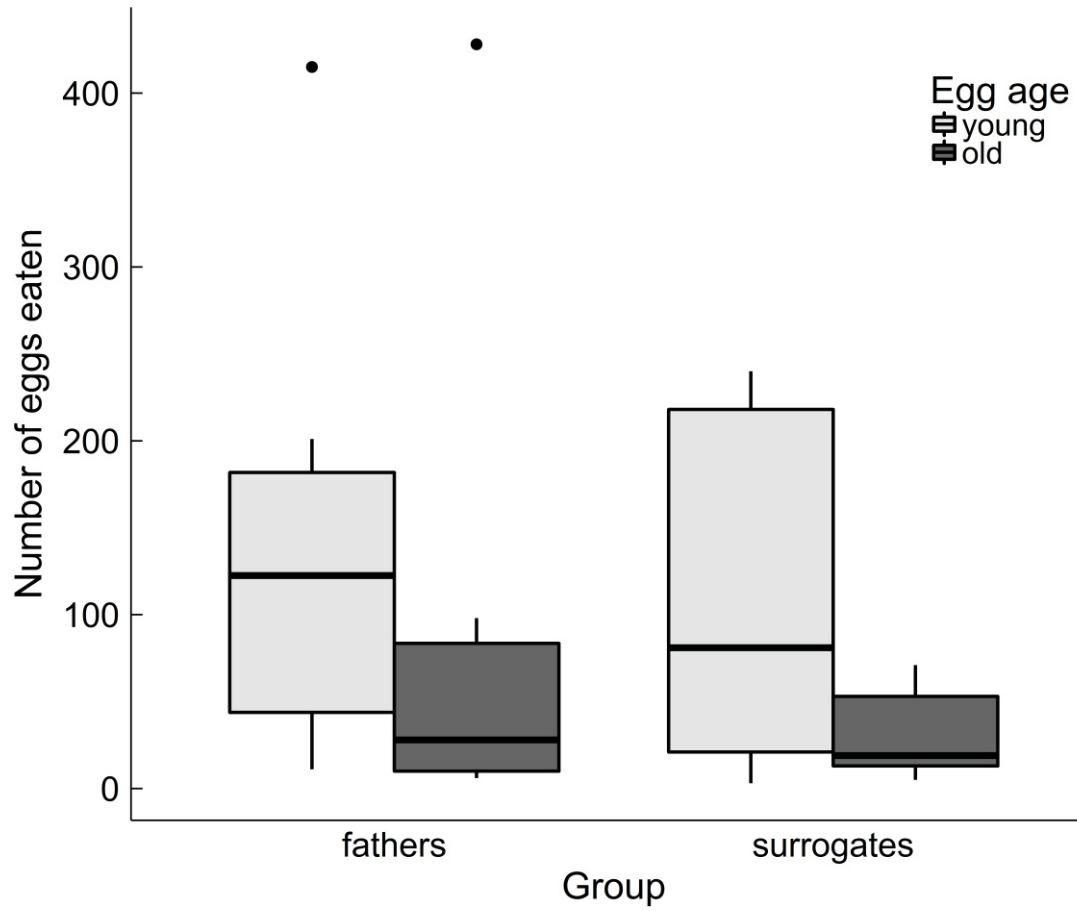
Figure 2. *Difference in filial cannibalism between groups. Values are split by egg age within each group. Each box shows the interquartile range (IQR) divided by the median. Whiskers extend to the most extreme data points still within 1.5 × IQR from the edges of the box while data beyond this range are plotted as points*

Discussion

Male common gobies showed a clear preference for younger eggs when cannibalizing their brood. This supports our prediction that paternal males selectively cannibalize based on egg age when multiple clutches are present. Filial cannibalism of younger eggs may provide several benefits. Less developed eggs are generally more nutritious (Gilbert 1985) and thus more beneficial to eat from an energetic point of view. At the same time, they still require more time and energy in terms of paternal care to reach independence, while typically facing a high risk of being lost to egg predators (Chin-Baarstad et al. 2009) or nest take-overs by conspecifics (Lindström and Hellström 1993) during each additional day. Hence, young eggs should have a lower reproductive value compared to eggs that are closer to hatching (Pressley 1981) and, following theoretical predictions on parental investment and filial cannibalism, be a preferred target for cannibalistic decisions (Klug and Bonsall 2007). Since cannibalizing eggs with lower value could also be seen as a drastic form of redirecting parental care to the remaining eggs, our result thus also fits well with the general prediction that parents should give more care to offspring with higher reproductive value (Klug et al. 2012).

The hypotheses above share the assumption that parents consume their offspring due to energetic requirements and the selectivity only arises to minimize the cost of filial cannibalism. In contrast, Klug and Lindström (2008) argue that males may consume slower developing eggs to decrease the duration of the current brood cycle and acquire a new brood more quickly, thus increasing the chance for an additional brood cycle during the breeding season. However, in common gobies, eggs from a single clutch do not vary much in developmental time and usually hatch nearly synchronously within a few hours (MV and KUH, personal observation). This would imply that males have to consume whole young clutches instead of only a subset of eggs to considerably decrease duration of care, which seems implausible and never happened in our experiment.

Previous studies that assessed selective filial cannibalism by manipulating clutches of two fish species in the lab did not find a similar preference for younger eggs (Manica 2003; Takegaki et al. 2011). The authors argue that the age difference between clutches in one nest relative to the duration of clutch development, and consequently the difference in value between eggs, may be too small for a preference to have evolved. In common gobies, however, time lags of three days between individual spawnings are regularly observed, which can constitute a substantial proportion of a full clutch cycle (5.5 days in 21.9° C, but 11.7 days in 15.8° C; MV, unpublished data).

Water temperature was comparably low in the present study (Mean ± SE: 12.7 ± 0.1 ° C), indicating that there was only a small difference in development between young and old clutches. It is striking that males were nevertheless able to discriminate between eggs. In contrast, the similarity in average cannibalism levels between fathers and surrogates suggests that foreign eggs were not recognized. However, sample size was low for this comparison and fathers and surrogates also differed in other aspects in addition to kinship (e.g. if there was a prolonged period of paternal care before receiving the mixed-age brood or not). Kin recognition of eggs is generally predicted by theory and has wide empirical support (Loiselle 1983; Frommen et al. 2007; Mehlis et al. 2010). The potential absence of kin recognition of eggs in our study thus needs further examination and offers scope for future experiments. Generally, there is only little information on how fish manage to assess egg phenotype. Visual discrimination appears rather unlikely given the low light levels that reach the eggs inside the almost fully enclosed nests in many species. In contrast, olfactory cues of eggs have been suggested to be a major trigger for kin recognition (Loiselle 1983; Frommen et al. 2007; Mehlis et al. 2010) and might also be important to assess egg age, assuming the odor of individual eggs changes during development.

We could not prevent surrogate males in our study from cannibalizing some eggs already while rearing the "old" clutches (mean ± SE eaten after 3 days: 7.0 ± 3.2 % of 1301.9 ± 109.3 eggs). However, the amount of cannibalism fathers and surrogates

showed subsequently on the same (now halved) clutches after getting the mixed-age broods back was still higher (see Results for proportion cannibalized on old halves and Methods for initial egg number). This indicates that although surrogates may have possibly removed low-quality (e.g. sick) eggs already during the rearing period, this did apparently not induce particularly low rates of cannibalism on the old eggs later in the experiment and is unlikely to explain our results.

A preferential consumption of young eggs by males may also affect female reproductive decisions. Females of various species prefer to lay eggs in nests of males that already care for eggs (e.g. Marconato and Bisazza 1986; Goldschmidt et al. 1993; Forsgren et al. 1996), possibly to lower the risk of filial cannibalism on their own eggs by diluting them with others (for alternative hypotheses, see Forsgren et al. 1996). But there are also indications that some specifically choose males with only early-stage eggs (Petersen and Marchetti 1989; Sikkel 1989), which may theoretically protect their eggs from being selectively picked out. In the present case, one might alternatively expect females to avoid being the last to spawn altogether and rather pick nests without any eggs present. More work is needed to address female choice and other fitness and population-level consequences of selective filial cannibalism and its interaction with environmental and intrinsic factors (Vallon et al. 2016).

Acknowledgments

Compliance with ethical standards

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

Ethical approval: 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.

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CHAPTER II

Water mold infection but not paternity induces selective filial cannibalism in a goby

ORIGINAL RESEARCH

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Water mold infection but not paternity induces selective filial cannibalism in a goby

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Abstract

Many animals heavily invest in parental care but still reject at least some of their offspring. Although seemingly paradoxical, selection can favor parents to neglect offspring of particularly low reproductive value, for example, because of small survival chances. We here assess whether filial cannibalism (FC), where parents routinely eat some of their own young, is selective in response to individual offspring reproductive value. We performed two independent laboratory experiments in the common goby (*Pomatoschistus microps*) to test whether caring fathers preferentially cannibalize eggs of a given infection history and paternity. While males did not discriminate kin from nonkin eggs, they consumed significantly more eggs previously exposed to water mold compared to uninfected eggs. Our findings clearly show that parents differentiate between eggs based on differences in egg condition, and thus complement the prevailing view that FC arises for energetic reasons. By preventing the spread of microbial infections, the removal of molded eggs can constitute an important component of parental care and may represent a key driver of selective FC in a wide array of parental fish.

KEYWORDS
infanticide, kin discrimination, parental care, reproductive value, selective filial cannibalism, water mold infections

1 | INTRODUCTION

Many animals invest much time and effort into the well-being of their offspring through parental care. Yet, some of these regularly eat their very own young. This filial cannibalism (FC) appears paradoxical but is widespread across a diverse range of taxa, particularly in arthropods (Anthony, 2003; Miller & Zink, 2012; Thomas & Manica, 2003) and fish (Manica, 2002). Although FC potentially imposes direct fitness costs, it has been recognized as a reproductive strategy by which the cannibal can ultimately increase lifetime reproductive success. For instance, parents may trade-off the survival of some offspring against their own foraging needs and use the energy gained through cannibalism to ensure continued care for the remaining current or future offspring (Rohwer, 1978; Sargent, 1992). Even

cases where parents consume all of their current progeny (total filial cannibalism [TFC]) potentially increase overall fitness by enhancing future reproduction (Sargent, 1992). While such energy-based explanations for FC dominate the literature, empirical evaluations often failed to find clear effects of energetic needs on cannibalism levels (e.g., Belles-Isles & Fitzgerald, 1991; Klug & St Mary, 2005; Lindström & Sargent, 1997).

We here pursue the alternative idea that FC serves to discard offspring that, "for example due to sickness", have reduced survival chances and thus low reproductive value. In fish with male brood care, where FC is particularly common (Manica, 2002), eggs often catch microbial infections. Common pathogens include water molds (oomycetes) of the genus *Saprolegnia*, which infect adults and eggs alike and pose a serious threat to egg viability (Hussein & Hatai, 2002;

Kitancharoen, Hatai, & Yamamoto, 1997; Knouft, Page, & Plewa, 2003; Scott & O'Bier, 1962; van West, 2006). In the absence of brood care, eggs often rapidly overgrow with water mold hyphae and die quickly (Bronstein, 1982; Brown & Clotfelter, 2012). The caring male may actively prevent infections, for example, by secreting a protective mucus (Giacomello, Marri, Marchini, Mazzoldi, & Rasotto, 2008; Knouft et al., 2003) or by creating a constant water flow via egg fanning (Côté & Gross, 1993; St Mary, Gordon, & Hale, 2004). However, when these measures fail, selective removal of sick offspring through FC might provide an efficient ultimate treatment against infection threats with the added benefit of yielding some energy via egg consumption. Although briefly discussed before (Hoelzer, 1988), this possibility has to date largely received anecdotal support (Bailey, 1952; Kraak, 1996; Winn, 1958), with the exception of a very recent study that demonstrated targeted removal of eggs carrying natural *Saprolegnia* infections in spottail darters, *Etheostoma squamiceps* (Bandoli, 2016). Our study complements this work by providing the first rigorous test for selective FC after experimental manipulation of water mold infection.

Besides dead or sick offspring, many animal fathers face the risk of caring for offspring sired by other males. A particularly high uncertainty of paternity occurs in many bird (Griffith, Owens, & Thuman, 2002) and fish species (Taborsky, 1994). In fishes, so-called sneaker males may sneak fertilizations of eggs at another male's nest, leaving the guarding male with foreign (nonkin) eggs to care for (Taborsky, 1994). Selection should favor males that recognize and cannibalize such foreign offspring and thereby not only avoid allocating costly paternal care to unrelated eggs but also use those as a cheap energy source. Recent evidence indicates that selective cannibalism of foreign eggs indeed occurs in some (Green, Mirza, & Pyle, 2008; Mehlis, Bakker, Engqvist, & Frommen, 2010; Neff, 2003a) but clearly not all fish (Bandoli, 2006; DeWoody, Fletcher, Wilkins, & Avise, 2001; Lissåker & Svensson, 2008), calling for further studies to evaluate its prevalence.

In this study, we investigated the influence of water mold infections and kinship on FC in a small marine fish with exclusive male care and common sneaking behavior (Magnhagen, 1992), the common goby (*Pomatoschistus microps*, Krøyer, Fig. 1), where recent circumstantial evidence indicated that kinship has no effect on FC (Vallon &

FIGURE 1 *Pomatoschistus microps* eggs close to hatching with embryos already clearly visible

Heubel, 2016). Nest-holding males received infected and uninfected eggs simultaneously, which were either sired by themselves ("own egg" group) or an unrelated male ("foreign egg" group). To obtain a robust assessment of cannibalism patterns, we conducted two independent replicate experiments following slightly different methodological approaches.

2 | MATERIAL AND METHODS

2.1 | Model system

Reproduction in common gobies is restricted to a single breeding season (from May to August), but individuals can complete several consecutive breeding cycles during this period (Miller, 1975). Males build nests using mussel shells (or rarely other solid objects) and sand, and competition for such nest sites can be fierce (Borg, Forsgren, & Magnhagen, 2002; Nyman, 1953). Nest-holding males try to attract females and can potentially acquire clutches from several females if the nest is large enough. The deposition of eggs by a female can take several hours (Nyman, 1953), and smaller sneaker males may try to enter the nest and sneak fertilizations of unfertilized eggs (Magnhagen, 1992; Svensson, Magnhagen, Forsgren, & Kvarnemo, 1998). The nest-holding male cares for the eggs until hatching (for about 5–12 days, depending on temperature; MV, unpublished data) while the female abandons the nest immediately after spawning. Paternal care includes fending off predators, maintaining the nest, and cleaning and ventilating the eggs (Nyman, 1953), but males also show frequent FC (Kvarnemo, Svensson, & Forsgren, 1998; Svensson et al., 1998).

2.2 | General setup

To test how FC is affected by egg infection and kinship (i.e., caring for own versus foreign eggs), we conducted two separate experiments that followed a similar general setup and mainly differed in the approach used to induce the growth of water mold on the eggs (Fig. 2). The experiments were conducted at Tvärminne Zoological Station in southern Finland in June 2013 (Experiment 1) and June and July 2014 (Experiment 2). Male and female common gobies were caught nearby, close to the shoreline at Henriksberg (59°49.75′N, 23°08.67′E) using a seine. Additional males were collected in the same bay from previously installed artificial nests (ceramic tiles) using hand nets. We measured body size of all fish as total length (TL) to the nearest mm. Males were housed individually in 35 L test aquaria and given 3 days to acclimatize to the aquarium environment while females were kept in stock tanks prior to use. To avoid interactions between neighboring males, all test aquaria were covered with black foil. Each test aquarium was equipped with a halved flowerpot (4.5 cm diameter) placed upside-down, which served as an artificial nest site and was fitted with a removable plastic sheet for females to spawn on. A flow-through system continuously supplied all aquaria with fresh seawater. Water temperature thus mirrored natural conditions and was measured daily. All fish experienced a standardized light regime (18 L:6 D), and individual males were fed with two frozen chironomid larvae twice daily.
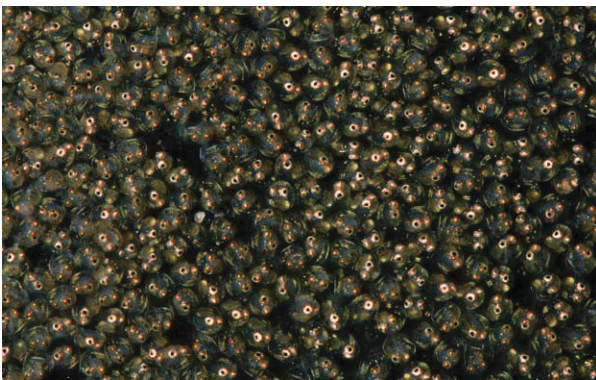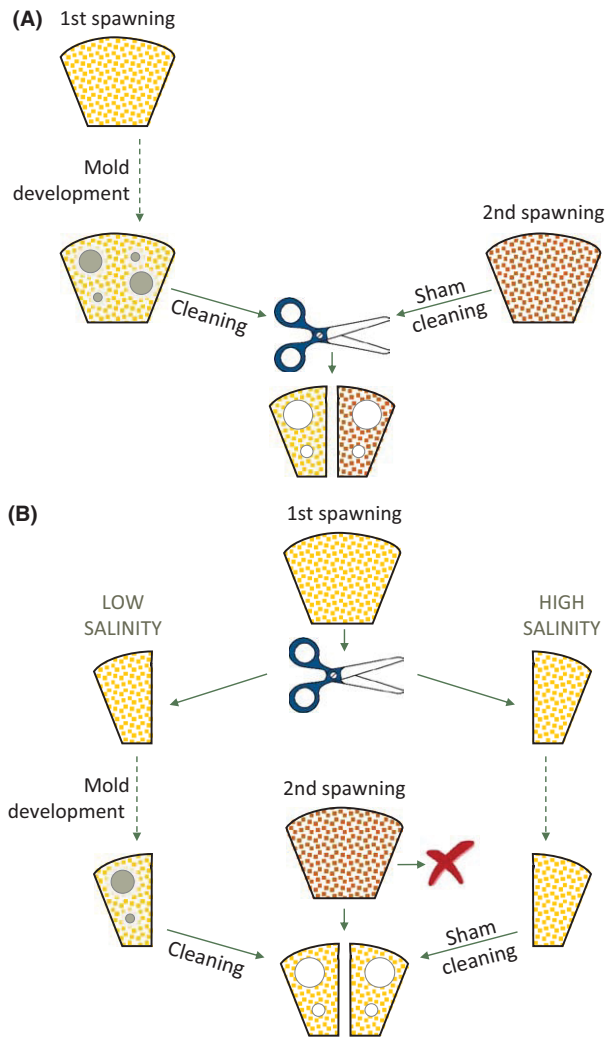
FIGURE 2 Schematic overview over experimental procedures in (A) experiment 1 and (B) experiment 2. Note that in both experiments, there was a second group of males that received foreign instead of their own eggs after the water mold treatment. See main text for details

2.3 | Treatments and procedures

2.3.1 | Experiment 1

In this experiment, each male consecutively spawned once with each of two different females. The first clutch was exposed to water mold as detailed below, and the second one maintained without water mold. The two clutches per male were halved and recombined into two clutches, each containing 50% molded old and 50% unmolded new eggs (Fig. 2A). One of these clutches was then returned to the original father ("own eggs" group), the other to an unrelated (nonkin) male ("foreign eggs" group). As a result, this experiment allowed us to simultaneously assess the effect of water mold infection and kinship on FC.

Short-term removal of egg masses is a well-established method in goby ecology with no handling effects on egg survival or parental care (Jones & Reynolds, 1999; Vallon & Heubel, 2016; Vallon et al., 2016).

However, note that the water mold treatment in this experiment confounds with egg age such that molded clutch halves contain older eggs. This confounding is conservative, however, because goby males generally value older eggs clearly higher than younger eggs (Klug & Lindström, 2008; Vallon & Heubel, 2016, see discussion). Moreover, experiment 2 (as detailed below) uses a modified experimental paradigm that excludes this confounding while yielding qualitatively similar results.

For spawning, each of initially 48 males was exposed to one female (mean ± *SE* TL: 35.7 ± 0.4 mm) for 16–18 h overnight. Males that had not received a clutch of eggs were paired with a new female up to three more times and excluded if still unsuccessful. The second spawning was initiated 3 days later with an identical approach (mean ± *SE* female TL: 36.3 ± 0.6 mm, mean ± *SE* time difference between first and second clutch: 4.0 ± 0.2 days). Twenty-six males did not spawn with two successive females and were thus excluded from further analysis.

All males that completed their two consecutive spawnings were then assigned to the "own egg" group (mean ± *SE* TL: 34.0 ± 0.6 mm, *n* = 13) or the "foreign egg" group (34.3 ± 0.9 mm, *n* = 9). All egg masses acquired by males assigned to the "foreign egg" group were now discarded—these spawnings exclusively served to initiate paternal care behavior now dedicated to their newly allocated "foreign eggs." First clutches of males in the "own egg" group were individually labeled, photographed for subsequent determination of clutch size, and placed in one of two 73 L aerated rearing tanks (up to 20 clutches per tank) without a male (Fig. 2A). To simulate nest conditions, rearing tanks were kept under low light and the plastic sheets with eggs were pinned upside-down to styrofoam plates floating at the surface. Due to the lack of paternal care, egg mold was gradually developing on all these clutches. Individual clutches were removed from the rearing tanks as soon as the corresponding male had acquired a second clutch. To reduce the risk that males rejected entire clutches just due to untypically progressed water mold infection, we removed all eggs visibly overgrown with water mold hyphae by scraping them off the plastic sheet using scissors and retaining only eggs that visually appeared healthy but were previously exposed to water mold, and thus likely still carried the infection. A similar number of eggs were removed from the second mold-free clutch as a sham treatment. Both clutches of a given male were cut in halves and recombined into two experimental clutches, each containing a molded and an unmolded half fertilized by the same sire. The new mixed clutches contained similar numbers of healthy eggs (mean ± *SE* number of eggs: 292.4 ± 32.5) and eggs exposed to water mold (314.0 ± 30.5 eggs; paired *t* test; *t* = −1.24, *df* = 21, *p* = .228). One experimental clutch each was now placed into the father's ("own egg" group) and the foreign male's ("foreign egg" group) nests, with two plastic clips holding the egg sheets in place. All males resumed paternal care on egg insertion.
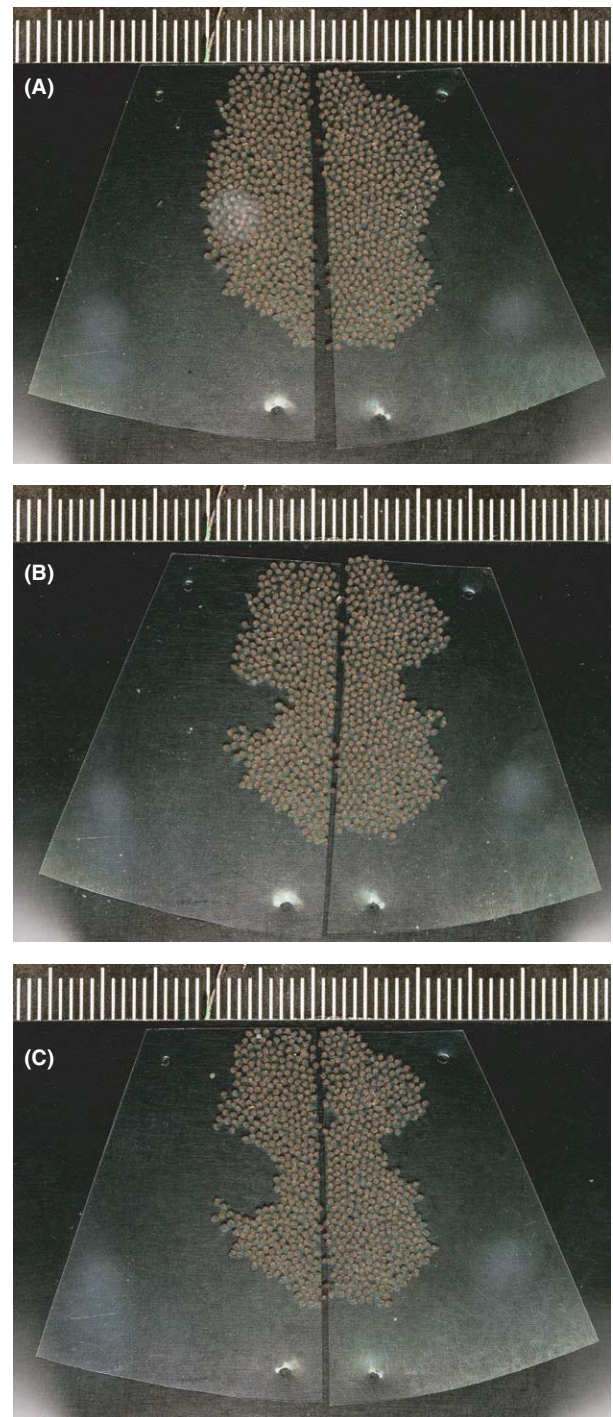
2.3.2 | Experiment 2

The overall experimental paradigm closely resembled that of experiment 1, with the exception that experiment 2 manipulated water mold infection levels independent of age within single clutches (Fig. 2B) as detailed below.

Males assigned to either the "own egg" or the "foreign egg" kinship groups (mean ± *SE* TL of males used in the main analysis; own eggs: 34.1 ± 0.5 mm, *n* = 22; foreign eggs: 35.3 ± 0.6 mm, *n* = 16; *t* test; *t* = −1.61, *df* = 36, *p* = .116) again spawned twice as outlined for experiment 1 with two similar-sized females (mean ± *SE* TL; female 1: 34.9 ± 0.3 mm; female 2: 35.1 ± 0.4 mm), now with just 2 days between spawnings (31 of initially 73 males did not spawn twice and could not be used). However, only the first clutches of "own egg" males were kept for the experiment and immediately split into two similar-sized halves that were exposed to two different salinities (mean ± *SE* number of eggs; low salinity: 403.7 ± 16.1; high salinity: 410.6 ± 15.9; Fig. 2B). As in experiment 1, own clutches of males assigned to the "foreign egg" group were only required to initiate paternal care behavior and thus discarded. Instead, the "foreign egg" males now obtained their fostered eggs from a third group of males (*n* = 16) that spawned just once, with these clutches halved and exposed to salinity treatments as outlined above for "own egg" males.

The salinity treatment draws from the well-known effects of salinity on water molds such as *Saprolegnia* (Ali, 2005; Marking, Rach, & Schreier, 1994) to establish two groups with clear differences in water mold infection risk. The low salinity treatment (mean ± *SE*: 6.36 ± 0.02 ppt) allowed water mold growth (see Experiment 1) under conditions mimicking salinity at the study site (typically between 6.2 and 6.4 ppt). The high salinity treatment (18.52 ± 0.05 ppt) mimicked conditions under which the closely related sand goby (*Pomatoschistus minutus*) showed drastically reduced water mold growth, and hence lower infection risk (Lehtonen & Kvarnemo, 2015a). Salinity differences only applied to the artificial rearing period to manipulate water mold infections. All mating, spawning, and fertilization as well as paternal care and FC took place under natural ambient salinity conditions identical to those in experiment 1. Thus, there was no direct link between salinity and fish behavior.

Salinity treatments were created by mixing 50% purified water (Milli-Q) with 50% natural Baltic Sea water and adding the appropriate amount of a sea salt mix for marine aquaria (Instant Ocean, Aquarium Systems, Sarrebourg, France). The rearing tank setup was similar to experiment 1, but we used small plastic tanks (6 L) and limited the number of clutches per tank to four to minimize the risk of infections spreading to neighboring clutches. Furthermore, we refrained from using a flow-through system for the rearing tanks to be able to maintain stable salinity levels. We instead manually exchanged approximately 50% of the water volume in each tank every second to third day. Tanks with different salinities were spatially alternated and the two differently treated clutch halves per male were always positioned in directly neighboring tanks. Average water temperature was nearly identical in both treatments (mean ± *SE*; low salinity: 14.51 ± 0.10°C; high salinity: 14.52 ± 0.10°C). We visually inspected the health status of the eggs once per day.

The experimental phase was initiated when a given male acquired its second clutch (which was exclusively needed to trigger paternal care behavior) and when we could confirm sufficient water mold growth on the low salinity half of the treated clutch halves from the first spawning (mean ± *SE* duration of artificial rearing: 3.6 ± 0.2 days).

FIGURE 3 Consecutive sample photographs of a single clutch from experiment 2, showing the clutch after (A) salinity treatment, (B) cleaning and sham cleaning, and (C) 1 day with the male. Note water mold growth on the left half in (A) and visible filial cannibalism in (C)

The two clutch halves were removed from their rearing tanks and photographed (Fig. 3A) to accurately determine whether the salinity treatment was successful. There was indeed a drastic difference in the proportion of eggs overgrown by water mold with virtually no water mold growing on the high salinity halves (Fig. 4). To maximize the difference between treatments, we excluded four males with visible
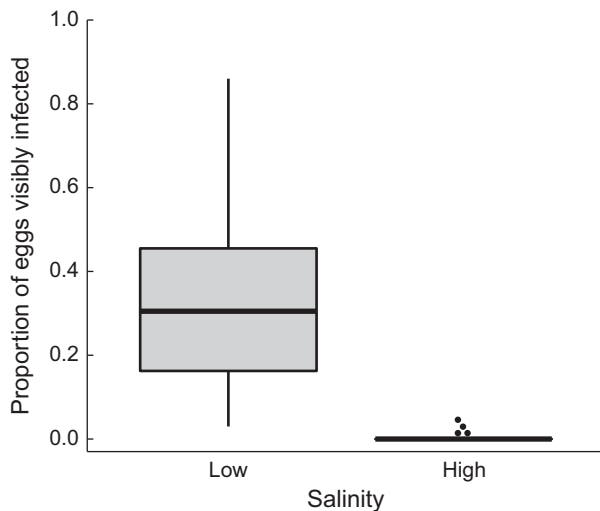
FIGURE 4 Proportion of eggs visibly infected with water mold on the differently treated clutch halves ($n = 42$ per treatment) in experiment 2

water mold growth under high salinity (see outliers in Fig. 4). Due to this clear distinction, we hereafter refer to the low salinity half as the "molded" half and to the high salinity one as the "unmolded" half.

Similar to experiment 1, we scraped off the visibly infected parts on the molded half and removed a similar pattern of eggs as a sham treatment from the unmolded half. We then took another picture of both halves combined as a baseline for FC measurements (Fig. 3B). The two halves did not differ in egg number (mean ± SE eggs; unmolded: 289.5 ± 20.5; molded: 298.5 ± 19.6; paired t test; $t = -1.35$, $df = 37$, $p = .184$). Finally, we swapped the combined clutch with the clutch the male (either "own eggs" or "foreign eggs" group) was currently caring for and discarded this second clutch.

2.4 | Data collection and statistical analysis

Data collection and analysis were performed in a similar manner in both experiments. One day after a male had received the treated clutch halves, we removed and photographed them (Fig. 3C). The male was then released into the wild. To quantify original clutch size and FC, we counted eggs manually on all consecutive images using the Cell Counter plugin (Kurt De Vos, University of Sheffield, UK) in ImageJ version 1.47v (Wayne Rasband, NIH, USA). We obtained FC measurements for 22 males in experiment 1 and 38 males in experiment 2. However, in each experiment, one male consumed the entire mixed clutch. Given that TFC is typically seen as a distinct behavior examined separately from partial clutch consumption (Manica, 2002; Sargent, 1992), we excluded these males from the main analysis.

To statistically compare cannibalism levels between molded and unmolded eggs and between "own egg" and "foreign egg" males, we fitted generalized linear-mixed models (GLMMs) with binomial error distributions using the "lme4" package version 1.1-6 (Bates, Maechler, Bolker, & Walker, 2015) in R v. 3.0.3 (R Core Team, 2014). Our response variable derived the proportion of cannibalized eggs directly

from the number eggs eaten versus those remaining untouched, and thus incorporated a measure of initial egg number (see Vallon & Heubel, 2016 and references therein). We included *Male ID* as a random factor with random intercepts and slopes over water mold treatments to reflect that each male provided paired data for cannibalism on molded and unmolded clutch halves. Fixed factors were *mold* (no or yes; i.e., unmolded or molded), *kinship* (own eggs or foreign eggs), and their interaction. In addition, we added *male length* as a covariate, which was z-transformed to improve model convergence (Korner-Nievergelt et al., 2015). Both models were reduced to the most parsimonious ones using the Bayesian information criterion (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) and ultimately contained only the fixed factors *mold* and *kinship* (retained as one of the main treatment factors) as neither their interaction nor the covariate *male length* improved model fit.

For experiment 2, we additionally tested in a separate binomial GLMM if FC was related to the proportion of infected eggs initially present on the low salinity half (corresponding data not available for experiment 1), while correcting for overdispersion in this model by including an observation-level random factor (Gelman & Hill, 2007; Korner-Nievergelt et al., 2015).

3 | RESULTS

In agreement with our main prediction, we found that in experiment 1, males cannibalized a significantly higher proportion of eggs from the molded half (mean ± SE: 56.4% ± 5.7%) than from the unmolded half (34.3% ± 6.4%; $n = 42$ observations of 21 individuals, $z = 4.92$, $p < .0001$; Fig. 5A). In contrast, average cannibalism did not significantly differ between males caring for own (40.8% ± 6.5%) and males caring for foreign eggs (51.4% ± 6.2%; $n_{own} = 12$, $n_{foreign} = 9$, $z = 0.87$, $p = .383$; Fig. 5A).

The pattern was similar in experiment 2. We found a strong positive effect on cannibalism of water mold infection (48.2% ± 4.6% of the eggs from the molded half compared to 16.6% ± 3.1% from the unmolded half; $n = 74$ observations of 37 individuals, $z = 12.70$, $p < .0001$; Fig. 5B) but none of kinship (31.5% ± 4.2% of own compared to 33.9% ± 5.5% of foreign eggs; $n_{own} = 22$, $n_{foreign} = 15$, $z = 0.38$, $p = .705$; Fig. 5B). In addition, within the molded clutch halves, FC increased with the proportion of visibly molded eggs present before the cleaning procedure ($n = 37$, $z = 2.06$, $p < .0392$; Fig. 6).

4 | DISCUSSION

Using two independent experiments with common gobies as a model system, we show that water mold infection of eggs strongly affects FC. Caring males preferentially consumed eggs that had been exposed to a water mold environment. Our results thus clearly support the hypothesis that FC, at least partly, serves the purpose of removing diseased offspring. By doing so, the cannibal can clear the nest of eggs which will likely never hatch, and thus essentially have no reproductive
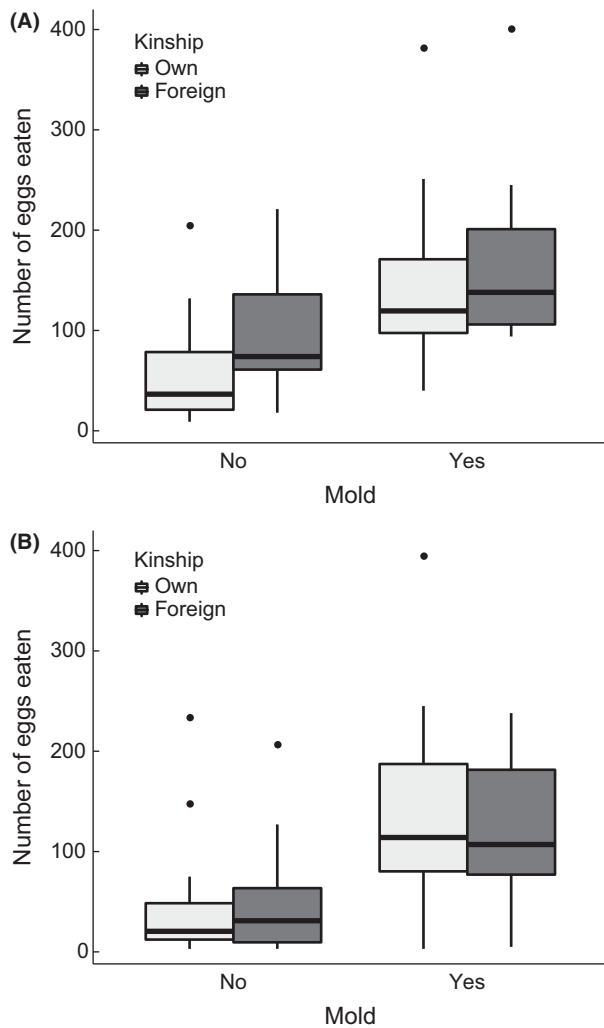
FIGURE 5 Filial cannibalism after 1 day in (A) experiment 1 and (B) experiment 2. The graphs show raw numbers of cannibalized eggs, and thus complement the proportions given in the main text and the underlying statistical analysis. See main text for sample sizes

value, while using the infected eggs as an additional food source. The energy gained by egg consumption could in turn facilitate taking care of the remaining eggs (Rohwer, 1978; Sargent, 1992). In consequence, such selective FC may be a mechanism to re-allocate paternal care to offspring of higher reproductive value (Klug, Alonzo, & Bonsall, 2012).

In the context of water mold infections, egg cannibalism likely also serves to preventing the spread of the disease. Two recent laboratory studies assessed the effect of egg density (either via natural variation or manipulation) and salinity on egg viability using artificially reared sand goby clutches (Lehtonen & Kvarnemo, 2015a,c). They found that under low but not under high salinity conditions (where water mold growth is inhibited), clutches with low egg densities had a lower prevalence of *Saprolegnia* and increased egg survival compared to clutches with high egg densities, indicating that low egg densities lead to a reduced spread of infections. Correspondingly, there is a clear evidence from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) that *Saprolegnia* infections on egg clutches spread to healthy eggs mainly by hyphal growth from adjacent infected eggs rather
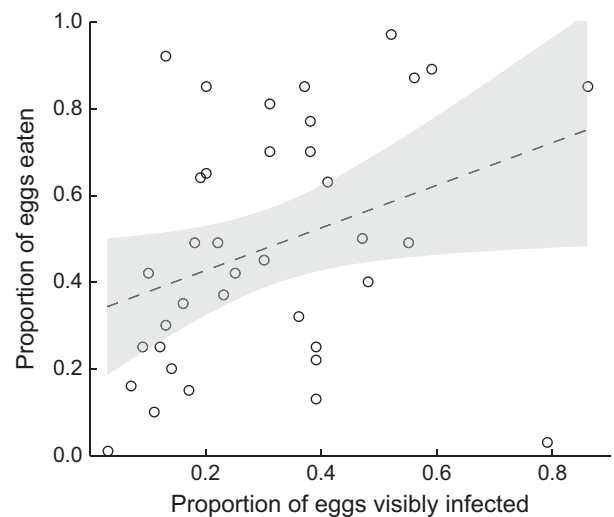
FIGURE 6 Proportion of eggs cannibalized after 1 day on the molded halves (low salinity treatment) in experiment 2 in relation to the proportion of molded eggs initially visible ($n = 37$). The gray area depicts the 95% confidence interval of the regression line

than (randomly) by zoospores (Smith, Armstrong, Springate, & Barker, 1985; Thoen, Evensen, & Skaar, 2011). This suggests that removing damaged or already dead eggs reduces the risk that surrounding eggs get infected.

Interestingly, we found in experiment 2 that male cannibalism on molded clutch halves increased with the proportion of eggs that was visibly molded after water mold exposure, even though these visibly molded eggs were experimentally removed before returning the clutch to the male (Fig. 6). This indicates that when many visibly moldy eggs had been present, there were also many potentially infected adjacent eggs, leaving more targets for selective FC. In agreement, we observed that males mostly cannibalized eggs directly neighboring the previously molded, and thus removed clutch parts (Fig. 3), while cannibalism occurred spatially more randomly on the sham-treated, unmolded clutch halves (MV, personal observation). In some cases, individual eggs that we identified as seemingly unhealthy during image analysis (e.g., because they became opaque) were missing on subsequent pictures, indicating that males specifically pick out single eggs. How fish recognize infected eggs cannot be answered with our design and provides a highly interesting research question for future studies. Recent evidence from sand gobies indicates a possible role for olfactory cues, because females avoided to lay eggs into nests that "smelled" of water mold when given the choice between nests either with or without a *Saprolegnia* odor (Lehtonen & Kvarnemo, 2015b).

In experiment 1, we prevented paternal care in one treatment for approx. 4 days to induce water mold growth on eggs while using newly laid eggs without water mold as comparison, thus additionally introducing a difference in egg age between treatments. Nevertheless, we are confident that our results are primarily caused by the difference in water mold growth. In a previous study, we found that males preferentially consume younger eggs when given the choice, presumably due to their lower reproductive value compared to more developed

eggs (Vallon & Heubel, 2016). In contrast, males cannibalized the theoretically more valuable (but infected) older eggs in the present study, indicating that water mold infections override the benefits of favoring more developed eggs. In experiment 2, the presence of egg mold was manipulated via salinity and independent of egg age. We cannot entirely rule out that higher salinity had effects on egg development beyond inhibiting water mold growth, but are confident that confounding should be mild at maximum for various reasons. First, previous evidence suggests that common goby eggs (even of marine origin) develop similarly well in 6 and 18 ppt salinities at the temperature used in our experiment (Fonds & Van Buurt, 1974), suggesting a short rearing period in manipulated salinities should not adversely affect egg development in either treatment. Second, both egg laying and paternal care for these eggs all occurred in a common environment, excluding direct physiological or behavioral parental effects on egg condition triggered by ambient water. Third, we expect the fish and eggs in our experiment to be more adapted to the low salinity conditions because the chosen treatment closely mimicked the prevailing conditions of the study population (see Material and Methods). Assuming at least some degree of local adaptation, our finding that eggs raised under these conditions were preferentially cannibalized over those exposed to a non-native, high salinity is rather opposite to the expected confounding effects. Finally, we considered manipulating water mold infections indirectly through salinity superior to direct infection in that the expressed water mold infections reflects an ecologically relevant degree of variation. Taken together, our two complementary experiments clearly highlight the importance of water mold infections for selective FC.

In contrast to water mold infections, kinship did not affect the amount of partial FC shown by males in both experiments. In addition, while we would expect nest owners to eat all and not just a fraction of the foreign eggs if they were capable to discriminate against eggs fertilized by a different father, total FC was near-absent in all our experiments. These results confirm indications from a previous study in common gobies (Vallon & Heubel, 2016), also finding no difference in FC between males caring for their own eggs and so-called surrogate males caring for foreign eggs. However, this finding was not as clear as in the present study due to methodological limitations leading to confounding between the two kinship groups. In general, the evidence for selective FC of foreign eggs is mixed (see references in the Introduction) and this behavior could well be species specific. Discriminating between own and foreign offspring may be difficult to start with. In fish, olfactory cues have been suggested to be more important than visual cues (Frommen, Brendler, & Bakker, 2007; Loiselle, 1983; Mehlis et al., 2010). We measured FC on average 4.8 days after egg deposition in our study (excluding the second clutches of experiment 1, which were only 1 day old). Possibly, paternal odor cues are only sufficiently present at later stages in development (discussed in Mehlis et al., 2010). For instance, bluegill sunfish (*Lepomis macrochirus*) are only able to recognize foreign offspring after the eggs have hatched, probably due to previously absent urinary cues (Neff, 2003b). However, for FC in common gobies, kin recognition after hatching is probably

meaningless because FC is relevant only during the egg caring period and larvae leave the nest soon after hatching and live independently (Nyman, 1953).

Males may alternatively not directly discriminate own from foreign offspring, but rather use external cues to infer the risk of paternity losses, and thus to assess the value of their brood. Males of several fish species decrease paternal effort (Neff, 2003b) or increase FC (Gray, Dill, & McKinnon, 2007; Manica, 2004) when potential sneaker males are present during spawning. Such an indirect mechanism would not have been detectable in our setup. However, corresponding studies in *Pomatoschistus* gobies did not detect an effect of sneaker presence on FC (common goby: Svensson et al., 1998; sand goby: Svensson & Kvarnemo, 2007), although nest-holder males generally react strongly to sneaker males in both species (Magnhagen, 1998; Malavasi, Lindström, & Sundström, 2001; Svensson & Kvarnemo, 2007). Interestingly, genetic data from experimental studies in sand gobies suggest that the paternity of nest-holding males remains high even after successful sneaking (Malavasi et al., 2001; Svensson & Kvarnemo, 2007). Assuming a similar pattern for common gobies, one may argue that direct or indirect mechanisms for offspring recognition never developed in these species because of just marginal costs of caring for a comparatively small fraction of foreign eggs.

To conclude, this study provides independently replicated experimental evidence that selective FC strongly responds to water mold infection but not to kinship. The common occurrence of microbial egg infections in fish on the one hand and FC on the other hand suggests that the documented link between both phenomena may not be restricted to common gobies but rather widespread at least in fish, consistent with recent findings in spottail darters (Bandoli, 2016). While FC is potentially influenced by a wide range of different (but not necessarily mutually exclusive) factors (Klug & Bonsall, 2007; Manica, 2002), our study therefore isolates removal of sick or dead offspring as one of its main drivers.

ETHICS

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.

CONFLICT OF INTERESTS

We have no competing interests.

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CHAPTER III

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 cannibalize 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.

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 & 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 & Bonsall 2007). This behaviour is surprisingly widespread despite its counterintuitive nature (e.g. Anthony 2003, Gilbert *et al.* 2005, Miller & 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 & St Mary 2005, Klug *et al.* 2006, 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 & Clotfelter 2012). Preventing infections thus likely constitutes an important part of parental care in many fish species (Bronstein 1982, Côté & 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 & 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 & 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 & 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 & Van Buurt 1974). Common gobies usually live only for one 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 & 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 x 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 ± 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.

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 airstones to provide aeration for the whole system. The low (mean ± SE salinity: 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 ± 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 one 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 ± 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 checked for the presence of eggs using a flashlight. 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 two days.
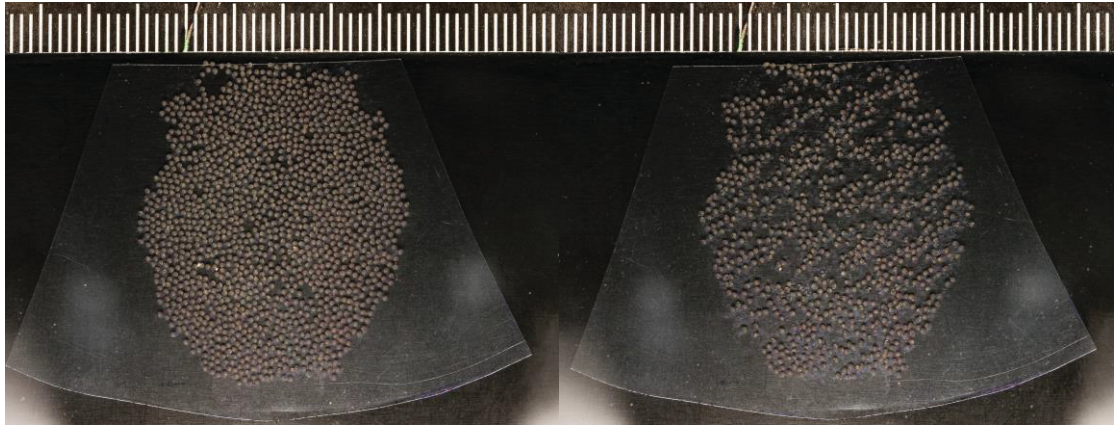
Egg density manipulation

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 ± 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. 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 ± 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 ± 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).

(a)

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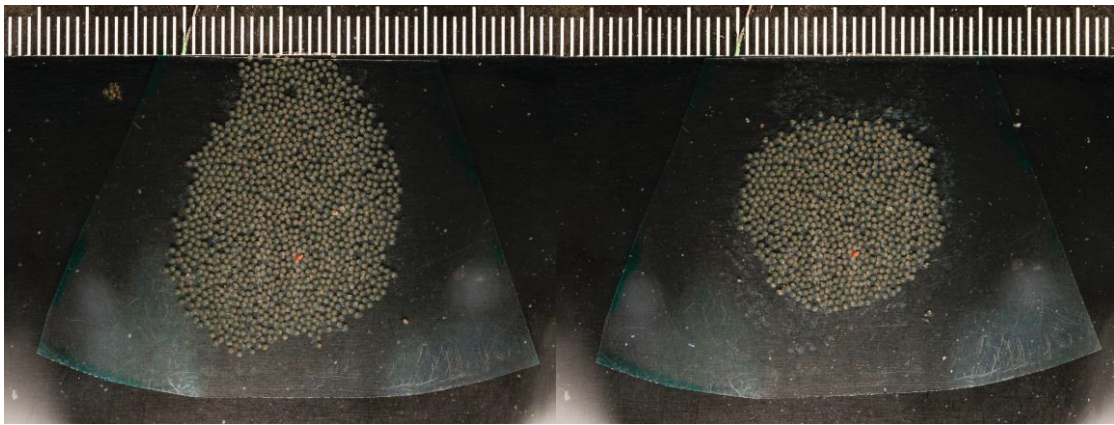
Figure 1. *Sample photographs of clutches before and after egg density manipulation for the (a) low egg density treatment and (b) high egg density treatment. See main text for details on the procedure.*

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 two 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. by turning the camera on and off), only the central 5 min of the video recording were used for analysis. We evaluated three different aspects of egg fanning behaviour using JWatcher version 1.0 (D. T. Blumstein *et al*., University of California, USA & 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 (expect one which was accidentally forgotten) were weighed once more at the end of the experiment and released to the wild one 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 versus the number of eggs remaining as response variables (Vallon *et al.* 2016a, Vallon & Heubel 2016). The random model component contained an observation-level random factor, which was added to correct for otherwise present overdispersion (Gelman & Hill 2007, Korner-Nievergelt *et al.* 2015). 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'), 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. 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).

Results

Average FC 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).
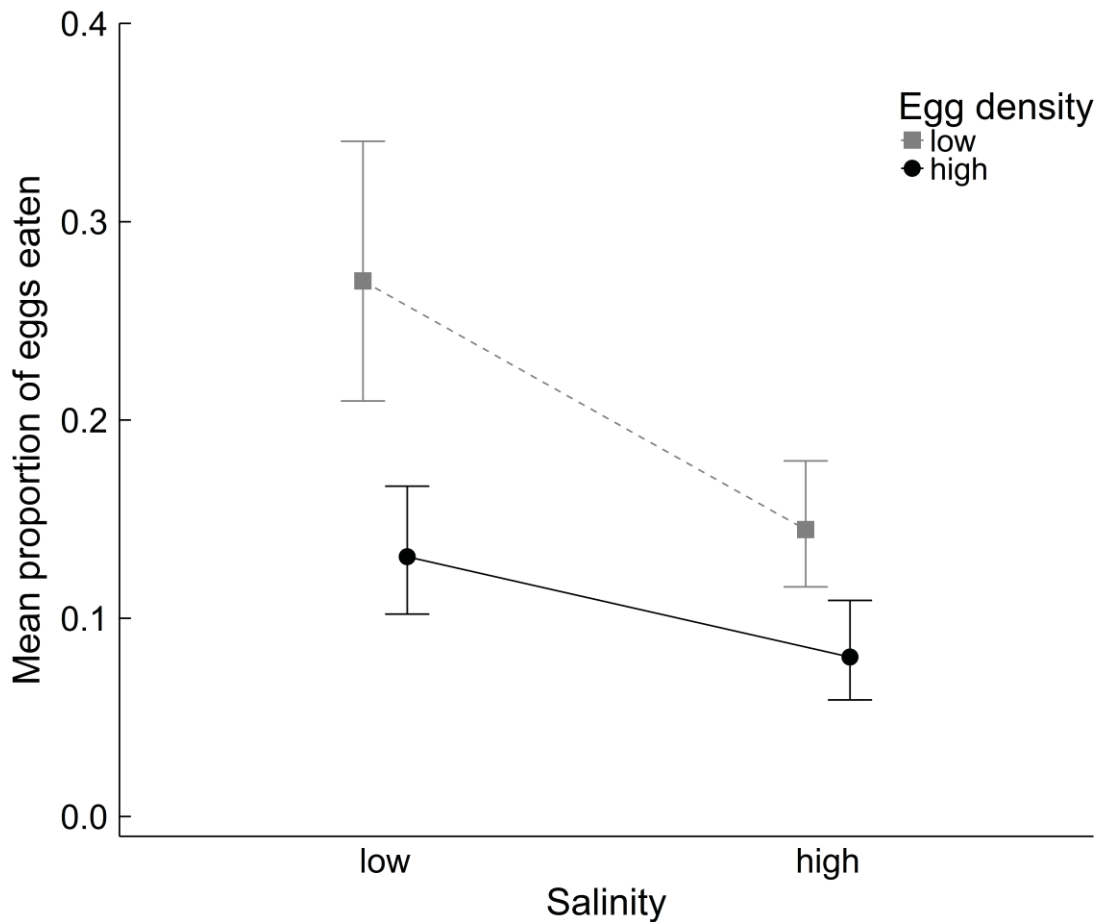

Figure 2. *Interaction plot based on model estimates for the mean proportion of eggs cannibalized after three days (excluding cases of TFC). Presented are group means and standard errors for each factor combination of* salinity *(n_{low} = 24, n_{high} = 27) and* egg density *(n_{low} = 27, n_{high}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

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 (binominal or Poisson error distribution). Bold-type P-values indicate significant effects.*

	Estimate ± 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**

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 ± 1.2 % (mean $\pm$ SE) of their body weight while low salinity males had only lost 4.0 ± 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 addition, weight loss was higher in larger individuals (*length*; Table 1).

(a)

(b)

(c)
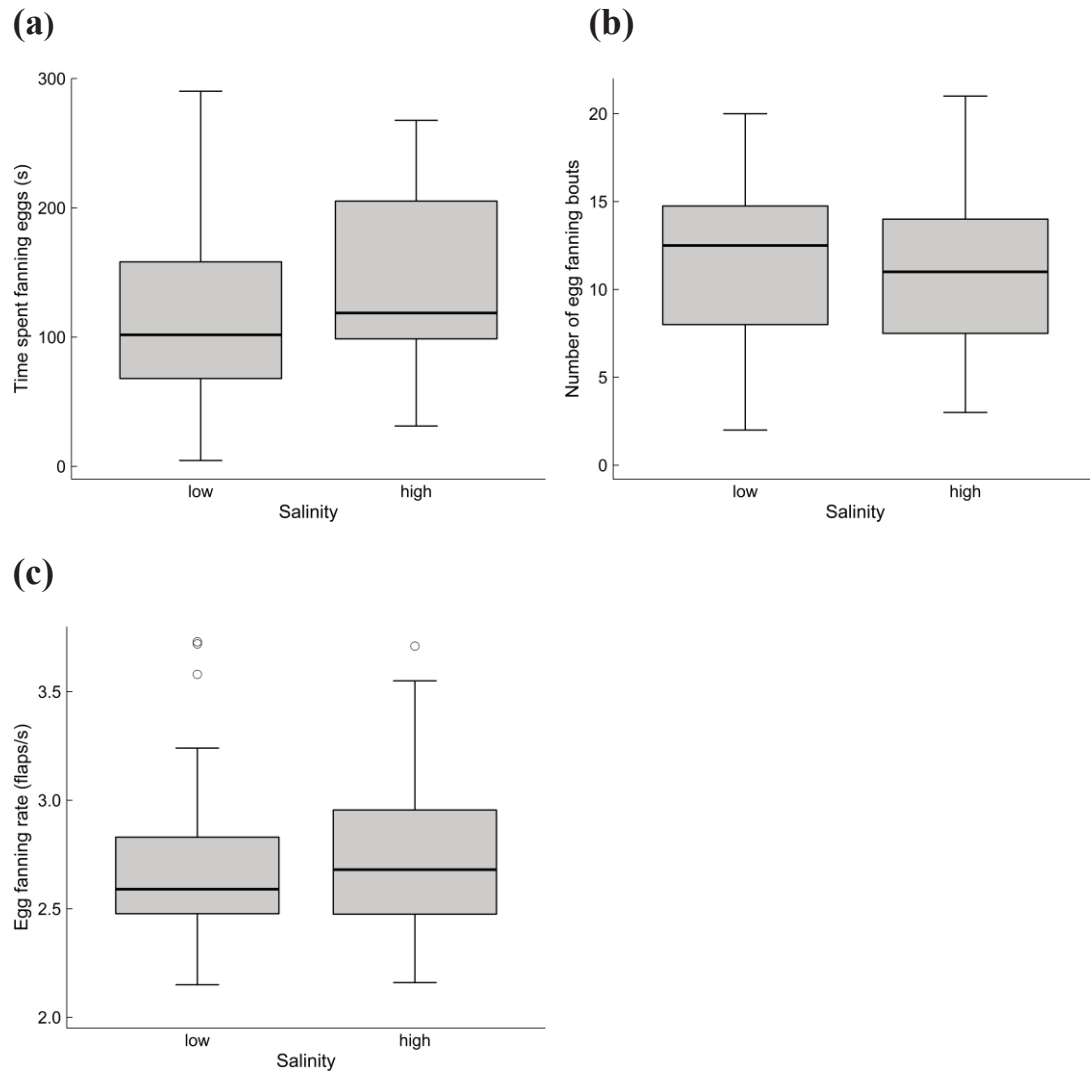
Figure 3. *Comparison of egg fanning behaviour shown by low (n = 22) and high salinity males (n = 27). Evaluated were (a) time spent egg fanning within 300 seconds, (b) number of egg fanning bouts and (c) egg fanning rate as fin flaps per second.*
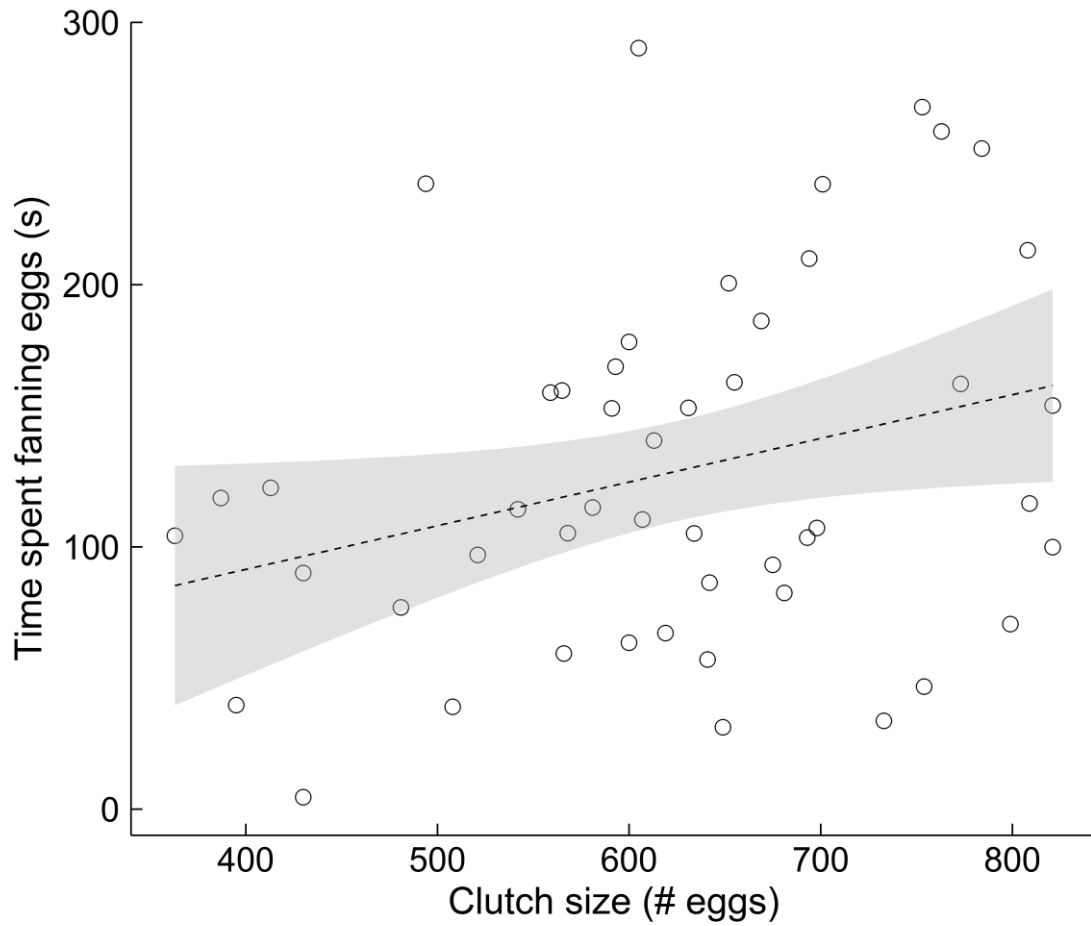
Figure 4. *Time spent fanning eggs as determined by behavioural recordings in relation to the number of eggs present after density manipulation (n = 49). The grey area depicts the 95% confidence interval of the regression line.*
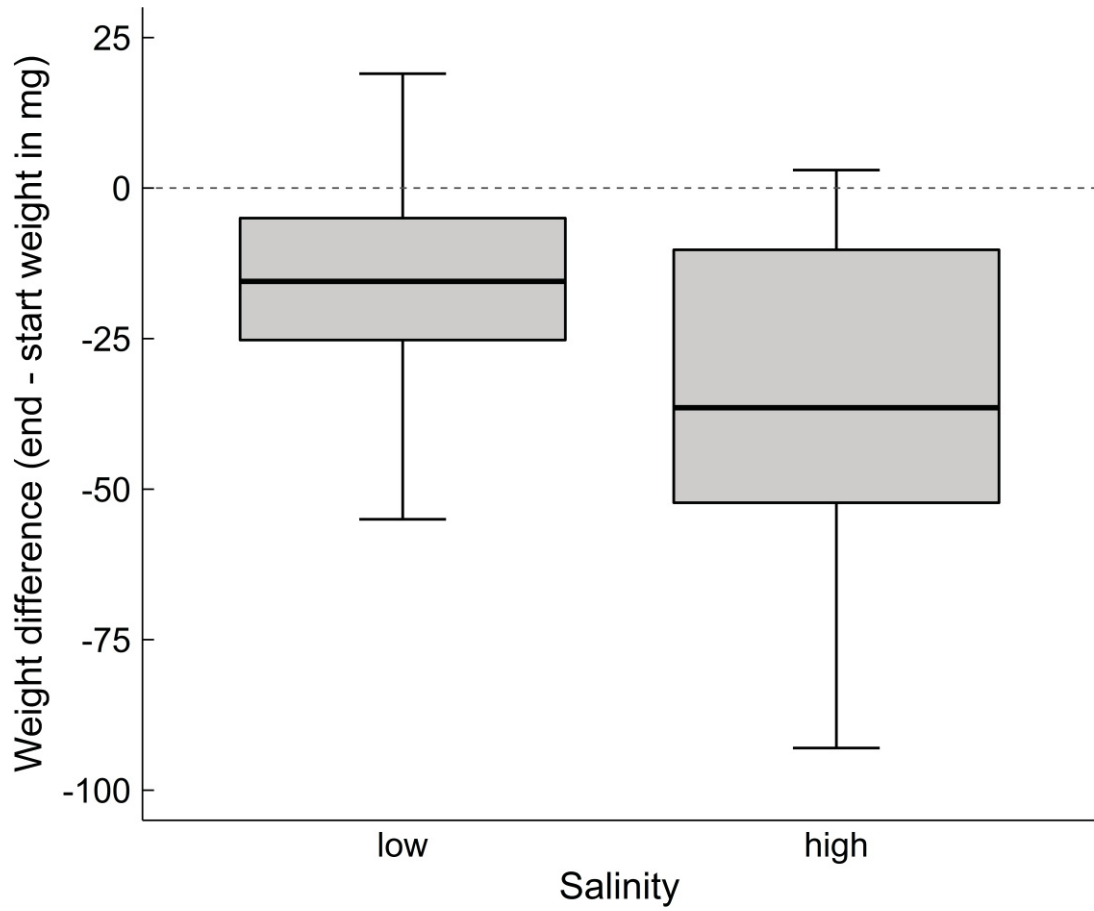
Figure 5. *Difference in weight development between males that were kept in low salinity (n = 24) versus high salinity (n = 26) conditions. Negative values indicate a weight loss over the course of the experiment.*

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 & Kvarnemo 2015b) but also in amphibians (Kiesecker & 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 clutches and argued that the possibly increased water movement between eggs could have facilitated infection by spores. However, contrary to our study, this effect was only present in high salinity 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, this was only true for total FC (a conceptually different mechanism not analysed in our study due to the low number of occurrences; see Methods), but not partial FC. The different egg densities in this 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. While 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), 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. Scraping off eggs did also not seem to affect adjacent eggs in a previous study in common gobies (Vallon *et al.* 2016a).

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, but typically only after males had already removed eggs via FC. 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.

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 instance, there is evidence from other brackish-water fish species that salinity can affect egg developmental rate (Brooks *et al.* 1997, Karås & 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 this respect (Fonds & 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 & Wootton 1985), upland bullies (*Gobiomorphus breviceps*) spent more time fanning when heavily infected with parasites (Stott & Poulin 1996). Similarly, a previous study in common gobies showed that males increase fanning rate and duration under low oxygen conditions (Jones & Reynolds 1999). This is interesting because oxygen availability is known to decrease with increasing salinity (Kinne & Kinne 1962, Fonds & 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 & 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 & Gross 1985, Sargent & Gross 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 & Choe 2002, Karino & 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 & Reynolds 1999a), 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 this study (Jones & Reynolds 1999a), 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 & 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 & 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 locomotory 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 strongly 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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DISCUSSION

Previous research on the adaptiveness of filial cannibalism has largely focussed on energy-based explanations (Manica 2002), although there are indications that FC cannot satisfy the energy requirements associated with parental care (Smith 1992). While it has been acknowledged earlier that other factors may also play a role (Klug & Bonsall 2007), corresponding empirical evidence is scarce and the theoretical framework often inconclusive. Selective filial cannibalism, i.e. selective consumption of offspring with a certain "low-quality" phenotype, seemed particularly promising to me, because of its obvious benefits to the cannibalising parent. Hence, I first assessed the general occurrence of selective FC in the common goby (chapter I).

In common gobies, as in many similar species with male brood care, males can care for clutches of several females simultaneously (Baylis 1981, Magnhagen & Vestergaard 1993). This reproductive feature has an influence on mate choice (Reynolds & Jones 1999) and operational sex ratio (Kvarnemo & Ahnesjö 1996), and often leads to significant age differences between eggs in the same nest. Age thus naturally seemed like a relevant offspring parameter that could influence the reproductive value of eggs and thus male FC.

By manipulating egg age within one batch of eggs, I could show that egg-guarding males indeed preferentially cannibalise the younger, less valuable eggs in their nest (chapter I). Firstly, this result clearly confirms the predicted general occurrence of selective FC. The ability to weed out specific offspring may be a fundamental mechanism particularly in variable environments, because it generally enables parents to alter the phenotypic composition of their current offspring after fertilization (Klug & Bonsall 2007). Secondly, this result pinpoints egg age as an important offspring phenotype where seemingly marginal differences can drive cannibalistic behaviour. While selectively removing offspring of low reproductive value could be seen as an extreme form of allocating parental care to the more valuable offspring and thus

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generally fits well with parental investment theory (Trivers 1972, Sargent & Gross 1985, Klug *et al.* 2012), it may seem surprising at first that offspring age induces such a strong response.

 However, the importance of offspring age as a determinant of offspring reproductive value has strong support from earlier studies in common gobies. Magnhagen and Vestergaard (1991) and Magnhagen and Vestergaard (1993) tested risk-taking and aggression of egg-guarding males in relation to the time the male had spent guarding a particular brood. Males were presented either with a conspecific competitor (Magnhagen & Vestergaard 1991) or with an eelpout (*Zoarces viviparus*), a potential predator (Magnhagen & Vestergaard 1991, 1993), and were subsequently chased away from their nests. The time away from the nest decreased significantly the longer the male had already spent guarding its brood in both studies. In addition, males attacking the researchers' finger, when those deliberately disturbed the nest, had on average more developed clutches than non-attacking males (Magnhagen & Vestergaard 1993). Both results strongly suggest that males use egg developmental stage as a cue to determine reproductive value of the eggs and adjust their behaviour accordingly.

Similarly, parents increase their aggression or intensity of nest defence with increasing maturation of young in a diverse array of fish (Huntingford 1976, Colgan & Gross 1977, Pressley 1981) and bird species (Møller 1984, Weatherhead 1989, Brunton 1990). Theoretical models also predict that the relative importance of offspring for the parent increases the closer the young get to maturity due to increased probability of survival (Andersson *et al.* 1980) and this effect may be particularly pronounced in species such as the common goby with only a restricted reproductive season (Sargent & Gross 1985).

After having established the general occurrence of selective FC and specifically selective FC in relation to offspring age (chapter I), I moved on to assess additional promising factors that may be connected to selective FC, namely egg infections and paternity (chapter II). By manipulating growth of microbial infections (water moulds) on common goby egg clutches and presenting egg-guarding males with eggs from two

differently treated clutches simultaneously, I could show with two independent experiments (using different methods to induce infection) that such egg infections indeed trigger selective FC (chapter II). In particular, males preferentially cannibalised eggs that had been previously exposed to water mould compared to uninfected eggs. Although egg infections were only manipulated indirectly in both experiments (either by temporarily preventing paternal care or by manipulating salinity), I am confident that the observed effect is indeed related to previous infection status of eggs and not to any of the possible confounding factors as discussed in chapter II.

Firstly, this result confirms the findings of chapter I in relation to the general occurrence of selective FC. Secondly, it confirms offspring health as modulated by infection history as an essential offspring phenotype and key trigger for selective removal by the parent. Similarly, honey bee workers selectively kill and remove developing larvae (to which they are typically closely related) infected with foul brood to prevent the spread of the disease in the hive (Rothenbuhler 1964).

Compared to offspring age, diseases may likely be an even more important driver of cannibalistic behaviour, particularly in aquatic organisms where egg infections are a ubiquitous threat that can also be directly harmful to the adult animals (Green 1999, Barber & Poulin 2002, van West 2006, Sagvik *et al.* 2008, Bandoli 2016). This is supported by the outcome of experiment 1 of chapter II, where males preferentially cannibalised the infected eggs, although those were at the same time the older, more developed ones. In addition, comparing the magnitude of the observed effects between chapter I and chapter II indicates that egg infections trigger a stronger response (while keeping in mind that the two studies were not conducted in exactly the same manner and may thus not be directly comparable): Males consumed on average 26.9 % more infected than non-infected eggs (average of both experiments), while the difference between treatments was much lower in the egg age experiment (13.9 %).

It is important to note that selective FC can generally serve different functions and that this behaviour should be considered in conjunction with the mechanisms advocated by

other hypotheses on FC. In particular, selective FC can be beneficial due to two different main processes: The first option is based on the assumption that FC is indeed generally driven by energetic needs of the parents, i.e. we assume that the energy-based hypothesis *sensu* Rohwer (1978) and Sargent (1992) is true. If this is the case, it seems reasonable to assume that a caring parent needs to consume a certain amount of eggs or young per day to satisfy its needs. Hence, by consuming preferentially offspring of low quality and thus only sacrificing the least valuable offspring, the cannibal should be able to effectively reduce the cost of FC. One potential example for this is the preferential consumption of less valuable and highly nutritious young eggs described in this thesis (chapter I).

The second option is that there are direct benefits from removing certain offspring that trigger selective FC. For instance, removal of diseased or dead offspring may inherently be advantageous to prevent spread of infections (chapter II). Option two naturally also offers the additional benefit of simultaneously providing energy via actual offspring consumption instead of simply killing or abandoning the young or eggs. Notably, there is also significant overlap possible between both options. In relation to egg developmental stage, it has also been suggested that the eggs that take longest to mature are removed to reduce the overall duration of the breeding cycle (Klug & Lindström 2008). While unlikely to be relevant in common gobies (as described in chapter II), this would represent a direct benefit independent of the energetic needs of the parent.

Likewise, selective FC by males of offspring with a low certainty of paternity may be connected to both options. If the male can in fact specifically recognise foreign offspring by olfactory or visual cues, which seems not to be the case in common gobies (chapter II) or plainfin midshipmans, *Porichthys notatus* (Bose *et al.* 2016a), but evidently in several other fish species (Loiselle 1983, Green *et al.* 2008, Mehlis *et al.* 2010), it would represent a direct benefit to remove such offspring to avoid spending unnecessary, costly care on unrelated offspring and/or to free space for potential own offspring. However, it may often be the case that paternity (or rather the lack thereof)

cannot be ascertained because it is only assessed via indirect cues such as observation of sneaker male intrusions (Neff 2003, Manica 2004, Gray *et al.* 2007), and paternity can remain relatively high even if the sneaking attempt was successful (Malavasi *et al.* 2001, Svensson & Kvarnemo 2007). In this case there might be a gradient following the uncertainty of paternity, with high uncertainty still leading to selective removal being beneficial per se, but lower uncertainty only triggering FC to potentially minimise the costs if eggs need to be sacrificed anyway.

I did not find any evidence for an effect of paternity on selective FC in common gobies (chapter II). However, the clear result regarding egg infections (chapter II) intrigued me and I aimed at investigating this further in chapter III. Following the successful manipulation of water mould growth via varying salinities in experiment 2 of chapter II, further use of salinity manipulation seemed promising to relate cannibalistic behaviour to environmental conditions. By simultaneously manipulating egg density, I aimed at addressing another well-known, but currently inconclusive hypothesis on the adaptiveness of FC (density-dependent FC; Payne *et al.* 2002, 2004, Klug *et al.* 2006), while at the same time investigating its interaction with selective FC (chapter III).

Specifically, I expected to find an interaction between salinity and egg density with FC being highest in low salinity (due to increased growth of egg pathogens) on high egg density clutches (due to easier spread of pathogens on egg clutches). However, while FC was indeed higher under low salinity conditions, I could not detect a significant interaction with egg density. Furthermore, the observed effect of egg density alone on FC was in fact contrary to my prediction since males cannibalised more on low density clutches.

Hence, I could neither directly confirm the predictions of the density-dependent FC hypothesis sensu Payne *et al.* (2004), nor the suspected interaction of egg density with selective FC. As discussed extensively in chapter III these results are indeed puzzling, particularly when considering the various earlier studies indicating that lower egg densities should in principle be beneficial in aquatic organisms (e.g. Green 1999, Klug

et al. 2006, Lehtonen & Kvarnemo 2015), and I cannot fully exclude methodological issues (egg density possibly too low or eggs too patchy; possible procedural damage on eggs during treatment).

In contrast, the increased cannibalism in low salinity generally confirmed my prediction. I could also exclude possible confounding factors by additionally assessing variation between salinity treatments in male brood care behaviour (egg fanning) and male body weight, and its potential influence on FC. While there was indeed a difference in weight loss between salinity treatments with higher losses under high salinity conditions, this difference cannot adequately explain the observed cannibalistic behaviour in relation to salinity. The results of chapter III in relation to salinity thus clearly further support and highlight the strong influence of environmental conditions and egg infections on FC already indicated in chapter II.

In this regard, it seems particularly noteworthy to consider the extensive gradient in environmental conditions, particularly in salinity, which common gobies as a whole species may experience, and also the rather extreme conditions present at the study site at the southern Finnish coast used for this thesis. Common gobies occur from the Mediterranean to the Baltic Sea (Miller 1975, 1986, Louisy 2002). Even when only considering the Baltic Sea, the variation in salinity in the shallow waters inhabited by common gobies can be immense, ranging from more than 25 ppt (close to marine conditions) at the Swedish west coast to below 2 ppt (close to fresh water) at the innermost parts (HELCOM 1996).

As indicated in chapter III, various studies on reproduction in the closely related sand goby show that average FC tends to be higher in low salinity areas compared to high salinity areas and it has been suggested that this is related to facilitated growth of egg pathogens under low salinity conditions (Lehtonen & Kvarnemo 2015). Unfortunately, most FC-related data for common gobies is derived from studies conducted in low salinity areas (i.e. Tvärminne at the eastern Baltic Sea coast; including the current studies) and a reasonable comparison with high salinity areas is thus not possible.

However, my results clearly indicate that a similar pattern as for sand gobies should be expected.

Variation in environmental conditions and in salinity in particular can generally heavily influence reproduction, e.g. via resource availability, which in turn affects competition, operational sex ratios and thus sexual selection (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996). For instance, nest resources that are typically used by sand and common gobies such as mussel shells are much scarcer in Tvärminne at the Finnish Baltic Sea coast compared to the Swedish west coast due to their limited tolerance to low salinity (Forsgren *et al.* 1996, Mück 2016), consequently leading to stronger male-male competition over nests in sand gobies in Tvärminne (Forsgren *et al.* 1996). A recent study assessed various reproductive parameters in five common goby populations across the Baltic Sea and indeed found considerable variation between populations, e.g. in mating success, brood size, egg density and egg size. However, there was only limited data available for FC. In addition, while the results indicate that egg density is generally lower in low salinity populations compared to high salinity ones, egg density was highest at intermediate locations and there was also no clear and consistent pattern observable in relation to salinity or other environmental influences for any of the other assessed parameters (Mück 2016). It thus remains unclear if and how the expected higher cannibalism levels in low salinity areas relate to general differences in reproduction and sexual selection between populations.

While the results of my thesis highlight selective removal of offspring as one of the main drivers of FC, it should be noted that selective FC as such is not necessarily mutually exclusive to other possible mechanisms affecting cannibalistic behaviour. As indicated earlier, various alternative factors have been suggested which potentially influence FC (FitzGerald 1992, Manica 2002, Klug & Bonsall 2007). Most notably, besides the already addressed energy-dependence (Rohwer 1978) and the still inconclusive relationship between FC and egg density (see chapter III), FC may also be related to the availability of potential mates (Kondoh & Okuda 2002, Deal & Wong

2016). In particular, various studies show that high mate availability leads to elevated levels of FC (Okuda & Yanagisawa 1996, Okuda *et al.* 2004, Myint *et al.* 2011, Takeyama *et al.* 2013), presumably because the cost of replacing young is reduced under such circumstances. However, the opposite has also been observed (Pampoulie *et al.* 2004, Klug *et al.* 2005) and it has been suggested that in this case parents may use FC to facilitate survival until times of higher mate availability (Deal & Wong 2016). Considering that mate availability can also be influenced by environmental conditions through the interplay of resource availability and operational sex ratio (see above), this is another factor that could affect FC in the field in combination with the directly salinity-driven effects described in this thesis.

Nevertheless, there may generally also be cases where parents cannibalise offspring accidentally. For instance, mouthbreeding fish might eat a few of their eggs by mistake while removing unfertilised eggs because they simply stick together (Mrowka 1987, FitzGerald & Whoriskey 1992). Other parents have been observed 'making the best of a bad situation' (Manica 2002): female three-spined sticklebacks participate in cannibalistic raids on conspecific nests even if they had previously laid their eggs in one. However, in this case they never initiate the attack but only join after egg consumption by other females has already started and their eggs are thus lost anyway (FitzGerald & van Havre 1987).

A more recent notion considers a fundamentally different approach to address FC based on intrinsic behavioural differences instead of extrinsic influences (Vallon *et al.* 2016b). When looking at the empirical data for FC across species not only from this thesis but also from the literature (e.g. Salfert & Moodie 1985, Nemtzov & Clark 1994, Lindström & Sargent 1997), it is apparent that there is often considerable variation in the extent of FC between individuals under very similar environmental conditions. Inter-individual behavioural differences that are consistent over time or across contexts are well established in the literature within the concept of animal personality (Gosling 2001, Sih *et al.* 2004b, Réale *et al.* 2007), which also includes so-called behavioural syndromes:

population-wide intrinsic correlations between multiple behavioural traits (Sih *et al.* 2004a). Vallon *et al.* (2016b) investigated the influence of animal personality on male FC (and other behavioural traits) using the common goby. They indeed found strong indications for an intrinsic coupling between FC and general activity, a behaviour for which consistent inter-individual differences between males were found in the same study. This indicates that individuals might not be able to adjust their cannibalistic behaviour independently of their intrinsic personality and their phenotypic plasticity regarding FC might thus be limited (Vallon *et al.* 2016b). However, more research is needed to confirm these findings and relate them to clearly environmentally-driven effects such as those found in the present thesis.

In conclusion, my thesis provides clear evidence for selective FC in relation to offspring age (chapter I) and egg infections (chapter II). In addition, I could establish the influence of environmental conditions such as salinity on FC, while the putative effects of egg density remain unclear (chapter III). The interplay of these different factors is thoroughly discussed throughout the thesis and I also highlight the role of other factors potentially influencing FC that were not directly assessed as part of the presented empirical work. Further research is thus crucial to connect the different drivers of filial cannibalism.

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