



Original Article

Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*

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Caring for offspring is energetically expensive, and when the costs of care are sufficiently high, the use of cost-reducing strategies can be favored. Such strategies may include the avoidance of nest construction through nest take-over and the restoration of depleted energy reserves through offspring cannibalism. Despite extensive theoretical and empirical work on parental care, neither the actual energetic costs of care nor the putative benefits of cost-reducing strategies have been systematically measured. Using plainfin midshipman fish, *Porichthys notatus*, we assessed how energy reserves of caring parents varied with duration of care, offspring cannibalism, and nest take-overs. We show that liver glycogen and lipid contents declined by 58% and 18.7%, respectively, that liver investment (measured via a hepatosomatic index) declined by 32.6%, and that muscle protein content declined by 8.8%. Other measures of body condition and energy reserves, such as hepatic glucose and adenosine triphosphate, remained stable over the extraordinarily long care period (3 months). Experimentally starved fish showed depletions of energy stores similar to caring fish. Fish that took over nests or that cannibalized eggs both had higher glycogen reserves than fish that did not adopt these strategies. These findings show that even when parental care is energetically costly, starvation may not be the dominant driving factor behind parent–offspring cannibalism.

Key words: cannibalism, glycogen, lipids, nest take-overs, paternal care, toadfish.

INTRODUCTION

Parental care can be costly when the energy and resources put into care trade-off against alternative investments, such as those made into somatic growth, which can diminish future reproductive success (Williams 1966). Although parental care enhances offspring fitness, caring parents may have less energy for growth and bodily maintenance and suffer reduced fecundity, mating opportunities, and/or survival (Alonso-Alvarez and Velando 2012). Investments made into current offspring may diminish resources available for future offspring, and parents should balance the benefits of providing care against the associated costs (Trivers 1972; Gross 2005; Smiseth et al. 2012). Although the costs of parental care have been well studied theoretically, few empirical studies have investigated the direct costs of care on parental energy reserves (Alonso-Alvarez and Velando 2012).

It has been long argued that parents can offset some of the high costs of care through offspring cannibalism, even when the offspring consumed are genetically related to the cannibal (i.e., filial cannibalism, Rowher 1978; Sargent et al. 1995). Offspring

cannibalism by caring-giving individuals is a taxonomically widespread phenomenon (Polis 1981; Soulsby 2013) and can be categorized into distinct types including filial versus nonfilial or partial-brood versus whole-brood cannibalism. Each of these types of cannibalism differ in terms of the selection pressures that drive them, how they influence the investment of parental resources, and the adaptive benefits that they confer (Smith and Reay 1991). Offspring cannibalism, both filial and nonfilial, is known to occur in birds (Stanback and Koenig 1992), mammals (Elwood 1992), and insects (Bartlett 1987; Thomas and Manica 2003) and is thought to occur for a variety of reasons including food shortage, mating competition, selective termination of low-quality young, and low certainty of parentage (Polis 1981; Smith and Reay 1991; Manica 2002). All types of parent–offspring cannibalism have also been observed among teleost fishes (Smith and Reay 1991; FitzGerald and Whoriskey 1992). As in other taxa, a large number of fish studies have focused on offspring cannibalism in the context of parental care, in which cannibalism of a portion of the brood may serve as a means to replenish dwindling energy reserves that would otherwise compromise the quality or quantity of care. According to this “energy-based hypothesis,” the recouped energy may then be allocated towards future reproductive efforts or toward the care for any remaining unconsumed offspring (Klug and Bonsall 2007).

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Such energy-driven cannibalism is clearly profitable when targeting non-kin offspring, but it can also be adaptive even when targeting kin offspring if the future benefits of the energetic gains outweigh the immediate loss of progeny (Rowher 1978). Although cannibalism may serve to recoup energy, nest take-overs may provide a means to preserve energy, by bypassing the costs associated with nest construction. However, in previous studies, the lack of comprehensive energy reserve measures of offspring cannibalism or nest take-overs has not permitted an unequivocal testing of the energy-based hypothesis.

To date, most studies have tested the energy-based hypothesis using coarse measures of body condition or manipulations of food availability, and results from these studies have provided mixed support for the hypothesis (Manica 2002). For example, negative correlations between body condition and cannibalism have been uncovered in the bluegill sunfish, *Lepomis macrochirus* (Neff 2003), the freshwater goby, *Rhinogobius* sp. (Okuda et al. 2004), and the assassin bug, *Rhinocoris tristis* (Thomas and Manica 2003). In female earwigs, *Anisolabis maritima*, filial cannibalism appears as a last resort to sustain a female through the nesting period (Miller and Zink 2012). Increased food availability via supplemental feeding (mitigating the energy depletion during care) has reduced but not abolished cannibalism in the Cortez damselfish, *Stegastes rectifraenum* (Hoelzer 1992), sphinx blenny, *Aidablennius sphynx* (Kraak 1996), common goby, *Pomatoschistus microps* (Kvarnemo et al. 1998), and scissortail sergeant, *Abudefduf sexfasciatus*, (Manica 2004). However, such feeding studies have had no effect on cannibalism in the three-spined stickleback, *Gasterosteus aculeatus* (Belles-Isles and FitzGerald 1991), fantail darter, *Etheostoma flabellare* (Lindström and Sargent 1997), or beaugregory damselfish, *Stegastes leucostictus* (Payne et al. 2002). No link between energy depletion and observations of filial cannibalism could be made for orangutans, *Pongo abelii* (Dellatore et al. 2009) and house finches, *Carpodacus mexicanus* (Gilbert et al. 2005). Additionally, in stark contrast to the predictions of the energy-based hypothesis, Klug and St. Mary (2005) showed that male flagfish, *Jordanella floridae*, placed on an enhanced diet cannibalized more than fish placed on a restricted diet. In many systems, the importance of cannibalism as a means for restoring energy remains unclear, possibly because studies are often conducted in artificial environments and have assumed the energetic costs of care but not obtained any direct measures of energy stores.

Adequately testing the energy-based hypothesis requires several steps. First, the energetic costs associated with care should be demonstrated. Second, the available energy reserves of individuals that engage in cannibalism should be measured and compared with those that do not cannibalize. Measures of energy reserves should be taken soon after cannibalism occurs, but before nutrients from the meal can be absorbed and stored. In this study, we implemented these 2 steps and investigated the energetic costs of parental care and how these costs are linked to cannibalism and nest take-overs using a wild fish, the plainfin midshipman, *Porichthys notatus*. This species is well suited for testing the energy-based hypothesis. Males dig and maintain nests under rocks, providing sole parental care in the form of embryo fanning and brood defense (Arora 1948) for up to 3 continuous months (Cogliati et al. 2013). Guarding males do not leave their nests during the care period, and must therefore rely on food items found within their nests (Sisneros et al. 2009; Bose et al. 2014; Cogliati et al. 2015). We tested 3 distinct predictions pertaining to the energy-based hypothesis. First, if parental care is costly then declines in energy reserves should reflect the length of time a parent provides care. Second, individuals that

take over a nest should have higher energy reserves than those that have invested energy into building their own nest and caring for offspring. Third, if a dwindling energy reserve triggers cannibalism, then cannibals should have lower energy reserves than noncannibals.

METHODS

Field sample collections

In 2013, we located and marked 122 plainfin midshipman nests along Crescent Beach, British Columbia, Canada (49°02'N, 122°52'W). A plainfin midshipman nest is a small, excavated cavity beneath an intertidal rock that contains a guarding male and a monolayer of eggs, which are deposited on the nest ceiling. We found and sampled these nests during the early, mid, and late parts of the breeding season (23–26 May; 22–26 June; 19–24 July). Nests were photographed using an Olympus TG-820 digital camera. Males were measured (total and standard lengths to the nearest 0.1 cm and total mass to the nearest 0.1 g) and uniquely marked with nontoxic injectable elastomer (Northwest Marine Technology, Inc., Shaw Island, WA) before the rocks were carefully repositioned. On the following day, we checked if the male from the previous day still remained in the nest, or whether a new unmarked male was present, and the nest was rephotographed. Nest photographs were later used for offspring quantification in the software ImageJ (v1.45, <http://rsbweb.nih.gov/ij/>). New untagged males that had replaced the original males were classified as nest “take-over” males, whereas original males were categorized as “remaining resident” males. We examined the stomach contents of a subset of fish from each sampling period, to determine whether take-over males or remaining resident males had recently cannibalized. Fish were sacrificed with an overdose of benzocaine. During dissection, a caudal vein blood sample was taken from each fish and frozen in liquid nitrogen. All organs were weighed (to the nearest 0.01 g) and both liver and muscle samples were frozen in liquid nitrogen to later assess energy stores.

Of the 122 males sampled over the season, 50 were randomly targeted for detailed energetic analyses. Twenty fish were selected from the May sampling period so that 10 of these had embryos in their digestive tracts, hereafter called cannibals, and 10 had empty digestive tracts, hereafter called noncannibals. Another 20 males were selected from the June sampling period (10 cannibals and 10 noncannibals). Only 10 fish were selected from the July sampling period (all of these were noncannibals because we did not find evidence of cannibalism in any fish sampled during July, see Bose et al. 2014). Of the 40 fish analyzed from the May and June sampling periods, 11 were take-over males.

Starvation sample collections

Wild caring males eat little over the breeding season (Cogliati et al. 2015). Thus, we also held 12 males under controlled food deprivation for 82 days to serve as a reference against which to compare changes in body condition and energy reserves due to caring. Midshipman males can care for 90 days or longer (Cogliati et al. 2013). The males for this experiment were collected from Ladysmith Inlet, British Columbia, Canada (49°1'N, 123°50'W) in early May 2013. They were measured and weighed (as above) and housed individually in 300-L outdoor aerated tanks supplied with filtered 12 °C seawater, lined with a sand substrate, and provided with shelter. Fish were monitored daily and experienced no mortality. After 82 days in

these tanks, all 12 fish were remeasured, euthanized, and dissected. All organs were weighed (to the nearest 0.01 g) and blood plasma, liver, and muscle samples were preserved as previously described.

Measures of body condition and energy reserves

To measure body condition, we employed 2 commonly used indices: 1) a relative condition factor (RCF), based on residuals from a regression of \ln body mass against \ln standard length (Blackwell et al. 2000) and 2) a hepatosomatic index (HSI), based on the residuals of \ln liver mass regressed against \ln eviscerated body mass. A gonadosomatic index (GSI) was also calculated for each fish using residuals of \ln gonad mass against \ln eviscerated body mass, as well as a gut investment index using the residuals of \ln empty gut mass against \ln eviscerated body mass.

Frozen liver samples were measured for water, glycogen, free glucose, adenosine triphosphate (ATP), total lipids, and total protein contents. Frozen muscle samples were analyzed for water and total protein contents. Frozen plasma samples were analyzed for ammonia content. A full description of the lab protocols used can be found in the [Supplemental Materials](#).

Total lipids and glycogen per whole liver were also determined for each fish by multiplying total liver dry weight (g) by either mass-specific lipid (mg/g dry weight) or glycogen content ($\mu\text{mol/g}$ dry weight). These reserves were then converted into a condition index using residuals similar to RCF and HSI as described above.

Egg digestion study

We used the presence or absence of embryos within the digestive tract as an indicator of whether offspring cannibalism had recently occurred. However, this indicator offers only minimal information regarding the rate of cannibalism that occurs in the field or the rate at which nutrients from a meal are accessed. To gain more insight into this, we estimated gastrointestinal evacuation rates by conducting an egg-feeding study between 17 May and 14 June 2013. Thirty males were collected from Ladysmith Inlet, held in aerated tanks of seawater, and fasted for 42 h. Each male was then fed 5 eggs. Six randomly selected fish were sacrificed and dissected at one of 5 time points: 6-, 12-, 24-, 36-, or 48-h postfeeding. Fish body size did not differ between time point groups ($F_{(4,24)} = 0.11$, $P = 0.98$). To track the progression of digestion, a digestion index was created using a 4-level scale. Fully intact undigested eggs were given a score of 1; mild digestion (loss of spherical shape but yolk still present) was given a 2; major digestion (loss of shape and yolk) was given a 3; and passed from the track entirely was given a 4. Gut content mass (g) was also recorded for each fish by weighing the full gut mass and subtracting the empty gut mass once the contents had been removed (see [Cogliati et al. 2015](#) for details).

Data and statistical analysis

All analyses were performed in R version 3.1.0 (R Core Team 2014). We compared overall body condition (RCF) and body size (based on standard length in cm) between those males that were no longer present in their nests on the second day of sampling with those males that remained in their nests using exact Wilcoxon rank sum tests (“exact RankTests” package, [Hothorn and Hornik 2015](#)). We also tested whether males that took over the ownership of a nest were larger than the males that they replaced with a 1-sample t -test using difference scores in their standard lengths.

We tracked how measures of body condition and energy reserves changed across the breeding season in wild fish, and also compared these measures with those from the lab-held fish that were

experimentally starved for 82 days. Only remaining resident males that were noncannibals were considered for these analyses. For each measure of body condition and energy reserves that we quantified, we ran a linear model (LM) that included sampling time (i.e., May, June, July, and “starved”) as a predictor. All models also included a parameter for fish size (standard length, cm) unless the analysis was conducted on an index that already accounted for body size. Both plasma ammonia concentration and liver glucose content were \ln -transformed to meet parametric assumptions.

We then compared cannibals with noncannibals. We used a Wilcoxon rank sum test to test whether the nests of cannibal males experienced a larger reduction in relative brood size than the nests of noncannibal males. This analysis only considered nests sampled in May and June, the 2 months in which cannibalism was observed. We compared energy reserves between cannibals and noncannibals, and between take-over males and remaining resident males. We ran LM that included sampling time (i.e., May, June, July, and “starved”) and either cannibal status (i.e., cannibal or noncannibal) or take-over status (i.e., take-over male or remaining resident male) as predictor variables. Fish size (standard length, cm) was also included in all models, except for the aforementioned indices. Nest take-overs and cannibalism were only detected in the first 2 sampling periods of the breeding season, and so only fish sampled from these first 2 periods could be included in these analyses. All take-over males were excluded from the comparison of cannibals to noncannibals. All fish sampled during the first 2 periods were included in the comparison of take-over males to remaining resident males. Muscle water and protein contents were measured from noncannibal males only. Liver glucose content was \ln -transformed to meet parametric assumptions for both the cannibalism and take-over comparisons, and liver protein content was \ln -transformed for the take-over comparison only. A \ln -transformation could not resolve the heteroskedasticity in total glycogen per liver for the take-over comparison, and so a generalized least squares (GLS) regression was used in this case to accommodate the uneven variance (“nlme” package, [Pinheiro et al. 2014](#)). Lastly, each model also included an interaction term between sampling period and either cannibal status or take-over status, removing the term if it was nonsignificant.

Finally, a cumulative link mixed model (CLMM) for ordinal data (“ordinal” package, [Christensen 2014](#)) was used to correlate the digestion index scores with elapsed time in the egg digestion study. Change in gut content mass with time was analyzed with a LM including elapsed time (hours) and fish size (body mass, g) as predictors.

Ethical Note

Plainfin midshipman fish are neither threatened nor endangered ([Collette et al. 2010](#)). All animals were collected and handled quickly in accordance with the Canadian Department of Fisheries and Oceans protocols/rules/guidelines (Scientific license XR 14 2013). All procedures were approved by the McMaster University Animal Research Ethics Board, DFO’s Animal Care Committee (AUP number 13-003), and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

RESULTS

Body size and condition do not predict loss or retention of nest ownership

Throughout the breeding season, we observed numerous occurrences of changes in nest ownership (see [Bose et al. 2014](#)). Such events indicate the take-over of an occupied nest, where the

previous resident was ejected, or the take-over of an empty nest, where the previous resident had already abandoned. Early in the season (May), 32% of nests experienced a change in nest ownership between the 2 consecutive sampling days, 7% in the midseason (June), and 0% in the late season (July). Additionally, the proportion of nests found to be empty on the second sampling day were 4%, 5%, and 2% for the early, mid, and late seasons, respectively.

No significant difference could be detected in the overall body condition (RCF) of males that were no longer in their nests on the second sampling day and the males that remained in their nests (Wilcoxon rank sum, May: $W = 223$, $N = 46$, $P = 0.71$; June: $W = 207$, $N = 56$, $P = 0.93$; July: $W = 26$, $N = 48$, $P = 0.92$). Furthermore, no differences in body size could be detected between these 2 groups of males (May: $W = 299$, $N = 46$, $P = 0.18$; June: $W = 250.5$, $N = 56$, $P = 0.39$; July: $W = 23.5$, $N = 48$, $P = 1$).

Energy reserves decline across the breeding season

Both body condition indices and energy reserve measures indicated deteriorating condition across the season (Figure 1, Supplementary Tables 1 and 2). Significant declines in liver investment ($P = 0.02$, Figure 1c), weight-specific liver glycogen content ($P = 0.003$, Figure 1e), total glycogen per whole liver ($P = 0.007$, Figure 1f), total lipids per whole liver ($P = 0.003$, Figure 1d), and muscle protein content ($P = 0.016$, Figure 1j) were observed over the season. The males sampled at the end of the breeding season also had higher liver water ($P = 0.02$, Figure 1g), liver protein ($P = 0.005$, Figure 1h), and muscle water ($P < 0.0001$, Figure 1i) contents relative to males sampled early in the season, and they also tended to have smaller gastrointestinal tracts for their body size ($P = 0.07$, Supplementary Table 2). There were no changes in plasma ammonia levels across the season (Supplementary Table 2). We found that liver glycogen and lipid contents declined by 58% and 18.7%, respectively, that HSI declined by 32.6%, and that muscle protein content declined by 8.8% over the season.

Starved fish have significantly reduced energy reserves

After 82 days, the food-deprived reference males had lost $16.8 \pm 0.6\%$ (mean \pm standard error [SE]) of their initial body mass. They were also in lower body condition and had smaller hepatic energy reserves when compared with wild fish sampled early in the breeding season (Figure 1, Supplementary Table 3). Specifically, relative to early season fish, these starved fish had lower RCF ($P = 0.038$, Figure 1a), smaller livers ($P = 0.001$, Figure 1c) and digestive tracts ($P = 0.036$), lower weight-specific liver glycogen content ($P = 0.004$, Figure 1e), total glycogen per whole liver ($P = 0.009$, Figure 1f), total lipids per whole liver ($P < 0.001$, Figure 1d), and muscle protein content ($P = 0.019$, Figure 1j, Supplementary Table 3). The starved fish also had significantly higher water content in their livers ($P = 0.023$, Figure 1g) and muscles ($P < 0.0001$, Figure 1i), and also higher gonadal investment ($P < 0.001$, Figure 1b) relative to early season fish. Except for higher liver-free glucose, few differences could be detected between the starved fish and late season (July) fish from the wild that had presumably been caring for 3 months (Supplementary Table 4).

Nest take-over males have greater energy reserves

Take-over males were not significantly different in body size (standard length) than the males that they replaced (t -test, $t = -1.5$,

degrees of freedom = 17, $P = 0.15$). However, take-over males had higher weight-specific liver glycogen content and total glycogen per whole liver than males that had remained on their nests, but these measures were significantly different only later in the season (glycogen content LM, interaction effect, est. \pm SE = 154.9 ± 70.3 , $t_{(35)} = 2.2$, $P = 0.03$, Figure 2a; total glycogen GLS, interaction effect, est. \pm SE = 0.72 ± 0.32 , $t_{(35)} = 2.2$, $P = 0.03$). Take-over males also had higher gonadal investment compared with males that remained the resident of their nests (LM, est. \pm SE = 0.61 ± 0.16 , $t_{(36)} = 3.79$, $P < 0.001$, Figure 3b). All other measures of body condition and hepatic energy reserves were nonsignificant (all $P > 0.11$).

Cannibals do not have lower energy reserves than noncannibals

Cannibalistic males were found to have an average of 17.8 ± 3.5 (mean \pm SE) eggs in their guts. Additionally, the nests of cannibal males suffered a greater reduction in brood size between the 2 sampling days than the nests of noncannibal males (proportion of brood disappearing overnight, mean \pm SE = $23.1 \pm 1.2\%$ for cannibals vs. $6.3 \pm 0.3\%$ for noncannibals; Wilcoxon rank sum, $W = 48$, $N = 40$, $P = 0.002$).

Cannibals appeared to have higher HSIs (LM, est. \pm SE = 0.16 ± 0.09 , $t_{(25)} = 1.79$, $P = 0.09$) compared with noncannibals though this difference did not reach significance (Figure 3a). However, cannibals had higher weight-specific liver glycogen content (LM, est. \pm SE = 112.4 ± 33.7 , $t_{(25)} = 3.33$, $P = 0.003$; Figure 3b) and total glycogen per whole liver (LM, est. \pm SE = 0.634 ± 0.266 , $t_{(24)} = 2.38$, $P = 0.03$). All other measures of body condition and hepatic energy reserves were nonsignificant (all $P > 0.17$).

Gastric evacuation rates

Extent of egg digestion was strongly related to time elapsed since feeding in the egg digestion study (CLMM, $z = 4.6$, $N = 138$, $P < 0.001$, Figure 4). Gut content mass also declined significantly with time elapsed since feeding (LM, est. \pm SE = -0.02 ± 0.006 , $t_{(26)} = -2.3$, $P = 0.03$).

DISCUSSION

We predicted that if parental care was energetically costly, then the longer an individual provides care the more their energy reserves should deteriorate. We corroborated this prediction in the plainfin midshipman, as parental condition as well as hepatic and somatic energy stores declined with time in the breeding season, a proxy for duration of care provided. We also predicted that if care is costly, then late-coming males that avoid nest building by taking over nests from previous nest owners should be in better condition than those males that have spent more time caring and building a nest. Indeed, we found that take-over males represent a subset of the population that is in better condition than males that have cared for and maintained a nest and offspring for a longer period of time. Finally, we also predicted that if cannibalism was used as a means for caregivers to replenish dwindling energy reserves, as the energy-based hypothesis suggests, then cannibals should have lower energy reserves than noncannibals. We did not find any evidence to support this hypothesis as cannibals had similar energy reserves to noncannibals.

Males that lost their nest are similar to those that retained it

Any male found to be absent from his nest on the second day of sampling could not be collected for dissection. As such, we were

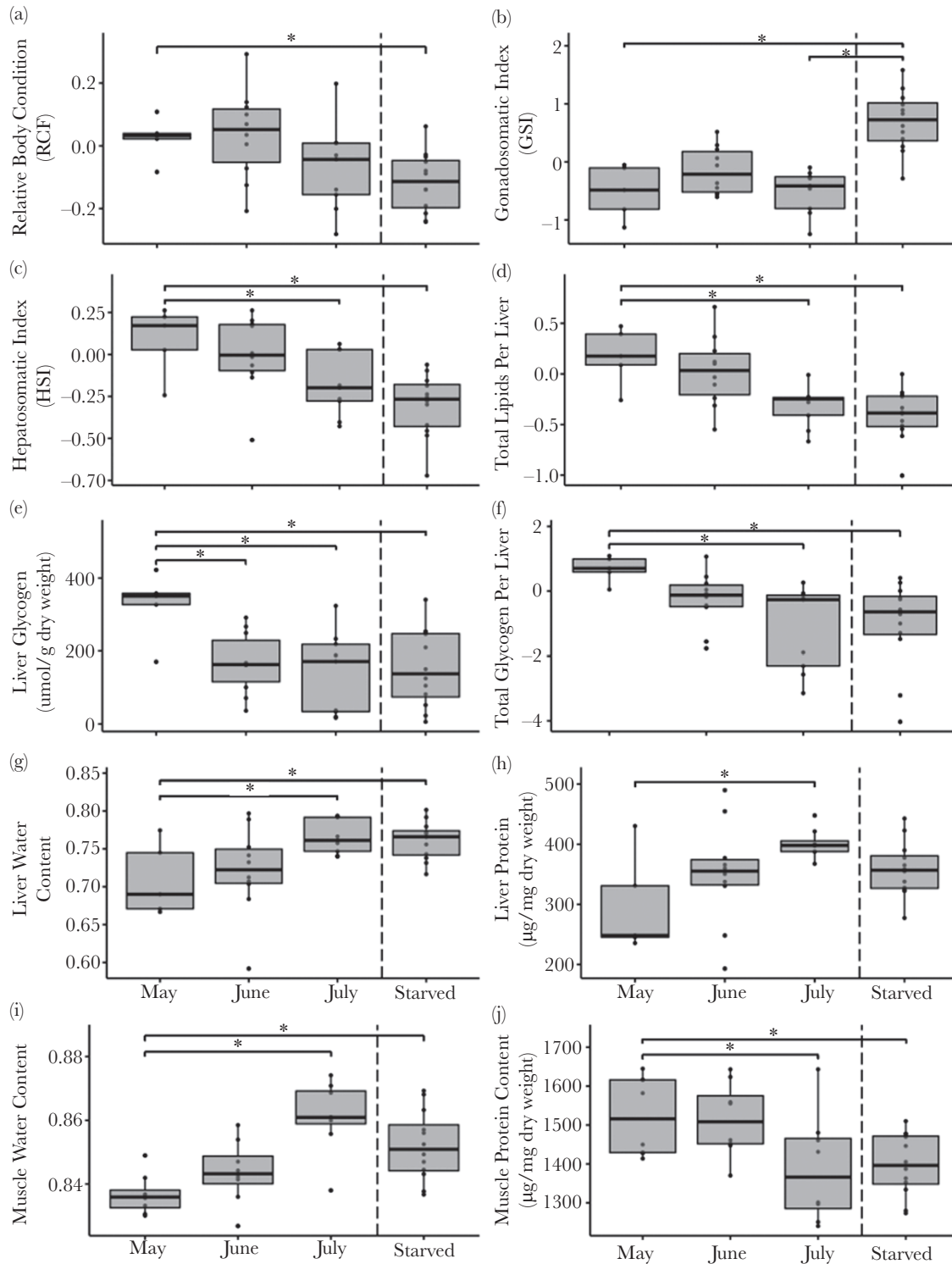


Figure 1

Seasonal variation in (a) relative body condition (RCF), (b) gonadosomatic index (GSI), (c) hepatosomatic index (HSI), (d) total lipids per whole liver, (e) weight-specific liver glycogen content, (f) total glycogen per whole liver, (g) liver water content, (h) weight-specific liver protein content, (i) muscle water content, and (j) weight-specific muscle protein content from nesting and forced starved male midshipman fish. The dashed line separates the fish sampled over the breeding season from the fish held under food deprivation. Brackets with * indicate a significant difference between 2 groups at $P < 0.05$.

unable to directly compare energy reserves between these absent males and those that remained in their nests. However, we could compare relative condition factor (RCF) and skeletal body length, 2

measures that did not require dissection, between these 2 groups of males and did not detect any significant difference. Previous studies have suggested that larger and stronger males may be more likely

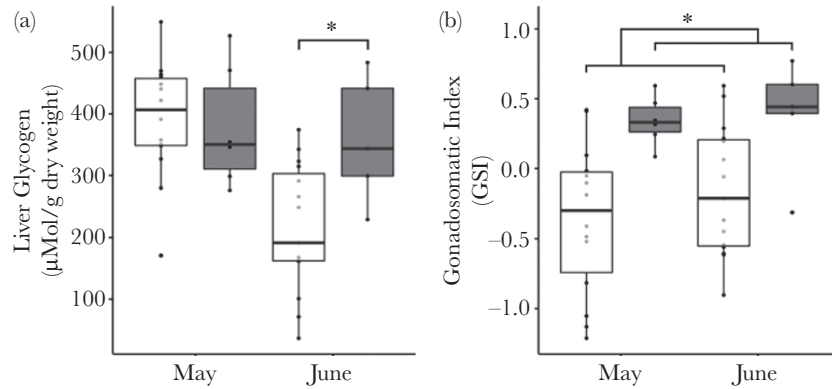


Figure 2

Variation in weight-specific liver glycogen content (in $\mu\text{mol/g}$ dry weight) and gonadosomatic index (GSI) between male midshipman fish that took over a nest (nest take-over male shown in dark gray boxes) and males that remained in their nest (remaining resident male, white boxes). Brackets with * indicate a significant interaction (a) or a main effect (b) at $P < 0.05$.

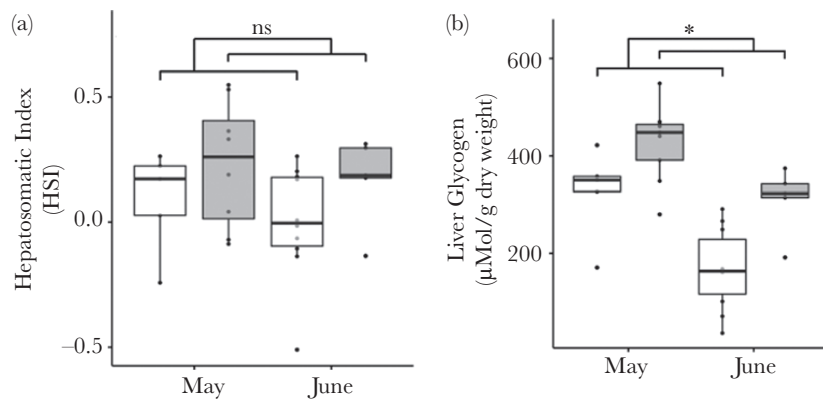


Figure 3

Variation in a body condition index (HSI) and weight-specific liver glycogen content (in $\mu\text{mol/g}$ dry weight) between midshipman fish found to have recently cannibalized (light gray boxes) and fish with empty guts (noncannibals, white boxes). Brackets with * indicate a significant main effect at $P < 0.05$.

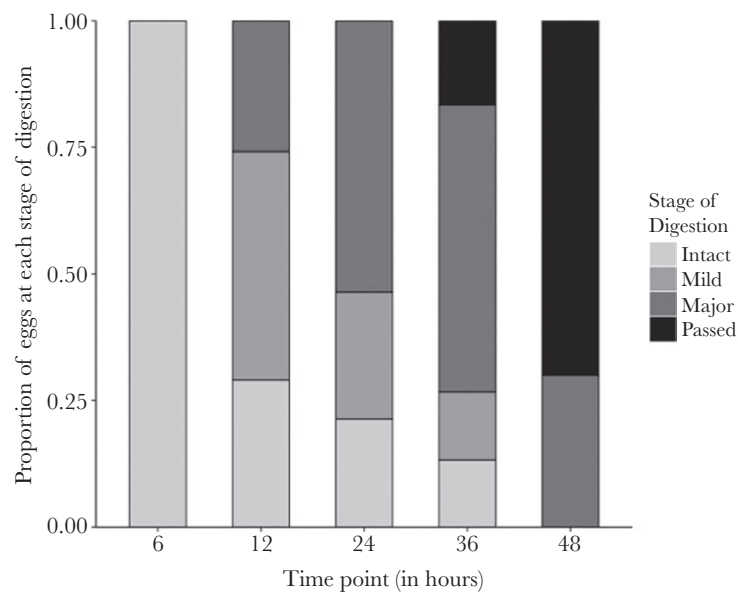


Figure 4

Progression of egg digestion (in hours) postfeeding in plainfin midshipman guarder males.

to abandon a nest because their higher resource holding potentials enhance their probability of obtaining an alternate site (sand goby, *Pomatoschistus minutus*, Lindström and Pampoulie 2005). However, we found no evidence to suggest that either body condition or body size influenced the likelihood of a male retaining his nest. Thus, it seems unlikely that our disturbance introduced a systematic bias in the males that remained in their nests to be collected on day 2.

Parental care period is associated with declining parental energy reserves

Many animals undergo fasting during one or more parts of their life history and may rely on endogenous fuels for extended periods of time. For example, in many bird species, the defense of young is most crucial during egg incubation, and parents may forgo foraging to remain on the nest during this stage (Mrosovsky and Sherry 1980; Clutton-Brock 1991). This also appears to be true of male plainfin midshipman, as their diet during the breeding season is comprised of the few limited food items found in their nests, suggesting that they remain confined to their nests throughout the care period (Cogliati et al. 2015). Animals undergo 3 well-defined phases of starvation with predictable depletions of available endogenous fuel stores (Bar 2014). Phases I and II rely first on glycogen and then primarily lipid stores. By phase III, animals have crossed a critical body lipid threshold, and have switched to protein catabolism (Bar 2014). Liver glycogen decreased significantly between the early and mid-breeding season and then remained relatively stable throughout the remainder of the season. Liver lipid reserves declined slowly and steadily over the season, revealing a significant decrease only by the late season. Muscle protein decreased significantly between the mid- and late-breeding season. This suggests that hepatic glycogen, hepatic lipids, and somatic proteins are mobilized over the course of starvation in the midshipman fish, but specifically implicates glycogen as an important fuel source during the initial stages of starvation. Water content of the liver and muscle tissues also increased over the season, consistent with a general trend for starving animals to replace lost mass with water (McCue 2010). Taken together, we find it likely that the midshipman males in our study were in phase II of starvation after 2 months of parental care and were in phase III of starvation after 3 months.

Several previous studies have also demonstrated results generally consistent with ours. For example, in three-spined sticklebacks, *G. aculeatus*, males lose the majority of their liver glycogen and lipid reserves and also a considerable amount from their somatic muscle over the course of their approximately 3-month breeding season (Chellappa et al. 1989). *Gasterosteus aculeatus* also displays a similar pattern of fuel use, with liver glycogen being preferentially mobilized before liver lipids. Male grass goby, *Zosterisessor ophiocephalus*, also experience a large decrease in HSI and somatic body lipids between the beginning and end of their breeding season (Malavasi et al. 2004). An increase in liver protein concentration has also been documented in starving Atlantic cod, *Gadus morhua* (Black and Love 1986).

We also found that the relative mass of gastrointestinal tissues declined with duration of both parental care and the food deprivation treatment, suggesting that midshipman males can adaptively reduce investment into unused tissues. Gastrointestinal tissues have high rates of protein synthesis, fast cellular turnover, and are metabolically expensive to maintain, and a broad range of animal taxa are known to reduce digestive tract size during periods of fasting (Piersma and Lindström 1997; Zaldúa and Naya 2014).

Starved males were in a similar physiological state as the late-season males, with similar declines in energy reserves. The only

notable difference was that starved males had larger gonads. We interpret the large GSI in starved fish as a result of these males not having an opportunity to spawn repeatedly over the season. Starved males also had lower liver ATP and higher liver glucose levels than did wild males, suggesting an increasing mismatch between ATP demand and supply as food deprivation is prolonged.

Interestingly, a recent diet analysis by Cogliati et al. (2015) revealed that food abundance within the guts of guarder males generally does not change throughout the season and that food availability within the nest, mostly in the form of small invertebrates, may actually increase over the season. Therefore, any decrease in body condition experienced by caregivers is unlikely to be the result of a decline in food availability as the season progresses. Overall, the similarities between laboratory starved males and those providing care in the wild further support the idea that parental care is energy demanding and restricts foraging beyond the nest.

Take-over males are in better condition than resident males that remain on nests

Males can be motivated to take-over nests when appropriate nesting sites are limited (e.g., Bessert et al. 2007) and when nest ownership provides higher reproductive output than cuckoldry strategies alone (e.g., Gomagano and Kohda 2008; Cogliati et al. 2013). Here, we show that take-over males are in better condition than guarder males that have constructed nests and likely spent time caring for offspring. Midseason take-over males had higher levels of liver glycogen (Figure 2a). Here, liver glycogen can be used as an indicator of recent energetic strain as we showed it to be the first hepatic fuel source to display a measurable decline in response to care duration in the plainfin midshipman. Any decrease in other fuels, such as lipids or proteins, should first be preceded by a decrease in glycogen, but not vice versa.

These late-coming take-over males are likely to be in better condition as a result of 2 non-mutually exclusive factors. First, take-over males are likely to have invested fewer resources overall into nest construction or parental care. Indeed, take-over males also had higher GSI (Figure 2b) suggesting that they had invested less into recent spawning. Second, take-over males may represent a sample of the guarder male population with sufficient energy to be able to out-compete already established nest owners. Interestingly however, take-over males were not larger than the males that they replaced, as has been found in other taxa (e.g., Lindström and Pampoulie 2005; Peixoto and Benson 2011). In the future, to explicitly test the importance of body condition and size on nest tenure in this species, resource contests between already caring and newly arriving males would be valuable.

Energy reserves in cannibals are not lower than in noncannibals

There are several lines of evidence to suggest that cannibalistic guarder males consume eggs from the nest directly under their care. First, all nests experienced a reduction in brood size between the 2 sampling days. This reduction is likely due to a combination of natural mortality, predation, cannibalism, and nest disturbance. However, cannibal fish were associated with nests that experienced a much larger decrease in brood size, consistent with the hypothesis that guarder males cannibalize from the nests under their care as opposed to consuming the eggs of neighbors. Second, Cogliati et al. (2015) made use of genetic paternity testing to show that some eggs consumed by males are in fact related to the cannibal. Cannibalism in this system therefore appears to be a

mixture of both filial and nonfilial cannibalism. Third, recent video footage of guarder males within their nests shows the fish periodically engaging in cannibalism of offspring from the roof of their nest (Bose APH, personal observations). The captured cannibalistic behaviors are associated with a characteristic arching of the back, positioning of the nares close to the offspring, and then a forceful suction or expulsion of water from the mouth powerful enough at times to dislodge offspring from the rock surface.

The energy-based hypothesis predicts that parents in poor condition should be the most likely to cannibalize, yet our results do not support this contention; similar to take-over males, cannibals possessed higher levels of liver glycogen suggesting that they had, overall, experienced lower recent energetic strain. Offspring found within the guts of cannibal males were most likely consumed within the 24-h window prior to fish dissection, based on our gastric evacuation rate data. The majority (~90%) of offspring found within the guts of wild cannibals showed little sign of digestion (i.e., classified as either 1, intact, or 2, with mild loss of shape). We therefore assert that 1) it is unlikely for the recently consumed offspring to have contributed to the elevated liver glycogen detected in cannibals and 2) that the energy reserves of the cannibals are likely representative of their condition when they began consuming the offspring. Thus, despite the significant costs of parental care it appears unlikely that low-energy reserves drive offspring cannibalism in the midshipman fish system. Furthermore, egg cannibalism among midshipman males is most frequent early in the breeding season when energy reserves are still high (Bose et al. 2014). Interestingly, our results are consistent with several previous studies. Both Klug and St. Mary (2005) and Klug et al. (2006), respectively, recorded filial cannibalism correlating positively with either the amount of supplemental food provided to caregiving parents (flagfish, *J. floridae*) or the initial body condition of cannibal parents (sand goby, *P. minutus*). They suggest that if a decline in body condition is also associated with diminished expected future reproduction, then this should reduce cannibalism and promote investment into current offspring.

Alternate selective forces that may drive offspring cannibalism in the plainfin midshipman system are paternal uncertainty and mating competition. Cuckoldry and competition among males is likely to influence paternity, or paternity certainty, thereby decreasing the reproductive value of the offspring at hand and the optimal level of investment that a caregiver should provide (Klug et al. 2012). Future studies will investigate these possible factors. Cannibalism could also be an incidental component of nest/brood cleaning or the selective termination of unhealthy offspring. However, the high prevalence and intensity of cannibalism especially in the early season (see Bose et al. 2014) suggests that incidental offspring consumption associated with cleaning is not the primary explanation of this behavior. Furthermore, offspring found in the guts of cannibals appeared healthy. Finally, cannibalism in this system may simply represent an investment in future reproduction. Cannibalism is most common in the early season, when males have invested relatively little into the offspring, and time remains for the males to attract new females. The extent to which body condition affects reproductive success (e.g., nest tenure, offspring survival, female mate choice) still remains to be evaluated.

CONCLUSIONS AND IMPLICATIONS

Few studies on reproductive costs in fish have directly quantified on-board energy reserves over an extended offspring-care period or to the level of detail of this study. Our results show that there is a considerable decline in parental energy reserves associated with

progression through the care season. We show that such behavioral strategies such as nest take-overs are associated with an energetic advantage. Lastly, we refute the energy-based hypothesis, at least in this batrachoidid species by demonstrating that low-energy reserves do not drive offspring cannibalism. Thus, it is apparent that energetic need is not a ubiquitous driving factor for offspring cannibalism. Furthermore, we expect that if other species also possess comparable starvation-tolerance and similar systems of mating and parental care to the plainfin midshipman, then they too should be similarly unaffected by declining energy reserves.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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REFERENCES

- Alonso-Alvarez C, Velando A. 2012. Benefits and costs of parental care. In: Royle NJ, Smiseth PT, Kölliker M, editors. *Evolution of parental care*. Oxford: Oxford University Press. p. 40–54.
- Arora HL. 1948. Observations on the habits and early life history of the batrachoid fish, *Porichthys notatus* Girard. *Copeia*. 1948:89–93.
- Bar N. 2014. Physiological and hormonal changes during prolonged starvation in fish. *Can J Fish Aquat Sci*. 71:1447–1458.
- Bartlett J. 1987. Filial cannibalism in burying beetles. *Behav Ecol Sociobiol*. 21:179–183.
- Belles-Isles JC, FitzGerald GJ. 1991. Filial cannibalism in sticklebacks: a reproductive management strategy? *Ethol Ecol Evol*. 3:49–62.
- Bessert ML, Brozek J, Ortí G. 2007. Impact of nest substrate limitations on patterns of illegitimacy in the fathead minnow, *Pimephales promelas* (Cypriniformes: Cyprinidae). *J Hered*. 98:716–722.
- Black D, Love RM. 1986. The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *J Comp Phys B*. 156:469–479.
- Blackwell BG, Brown ML, Willis DW. 2000. Relative weight (Wr) status and current use in fisheries assessment and management. *Rev Fisher Sci*. 8:1–44.

- Bose APH, Cogliati KM, Howe HS, Balshine S. 2014. Factors influencing cannibalism in the plainfin midshipman fish. *Anim Behav*. 96:159–166.
- Chellappa S, Huntingford FA, Strang RHC, Thomson RY. 1989. Annual variation in energy reserves in male three-spined stickleback, *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae). *J Fish Biol*. 35:275–286.
- Christensen RHB. 2014. ordinal—regression models for ordinal data. R package version 2014.11-14. Available from: <https://cran.r-project.org/web/packages/ordinal/ordinal.pdf>.
- Clutton-Brock TH. 1991. The evolution of parental care. Princeton (NJ): Princeton University Press.
- Cogliati KM, Danukarjanto C, Pereira AC, Lau MJ, Hassan A, Mistakidis AF, Bolker BM, Neff BD, Balshine S. 2015. Diet and cannibalism in plainfin midshipman *Porichthys notatus*. *J Fish Biol*. 86:1396–1415.
- Cogliati KM, Neff BD, Balshine S. 2013. High degree of paternity loss in a species with alternative reproductive tactics. *Behav Ecol Sociobiol*. 67:399–408.
- Collette B, Acero A, Betancur R, Cotto A, Rojas P. 2010. *Porichthys notatus*. The IUCN Red List of Threatened Species. Version 2014.3. Available from: www.iucnredlist.org (Accessed 27 March 2015).
- Dellatore DF, Waitt CD, Foitova I. 2009. Two cases of mother-infant cannibalism in orangutans. *Primates*. 50:277–281.
- Elwood R. 1992. Pup-cannibalism in rodents. In: Elgar MA, Crespi BJ, editors. *Cannibalism: ecology and evolution among diverse taxa*. Oxford: Oxford University Press. p. 299–322.
- FitzGerald GJ, Whoriskey FG. 1992. Empirical studies of cannibalism in fish. In: Elgar MA, Crespi BJ, editors. *Cannibalism: ecology and evolution among diverse taxa*. Oxford: Oxford University Press. p. 239–251.
- Gilbert WM, Nolan PM, Stoehr AM, Hill GE. 2005. Filial cannibalism at a house finch nest. *Wilson Bull*. 117:413–415.
- Gomagano D, Kohda M. 2008. Partial filial cannibalism enhances initial body condition and size in paternal care fish with strong male-male competition. *Ann Zool Fenn*. 45:55–65.
- Gross MR. 2005. The evolution of parental care. *Q Rev Biol*. 80:37–45.
- Hoelzer GA. 1992. The ecology and evolution of partial-clutch cannibalism by paternal Cortez damselfish. *Oikos*. 65:113–120.
- Hothorn T, Hornik K. 2015. exactRankTests: exact distributions for rank and permutation tests. R package version 0.8-28. Available from: <https://cran.r-project.org/web/packages/exactRankTests/exactRankTests.pdf>.
- Klug H, Alonzo SH, Bonsall MB. 2012. Theoretical foundations of parental care. In: Royle NJ, Smiseth PT, Kölliker M, editors. *The evolution of parental care*. Oxford: Oxford University Press. p. 21–39.
- Klug H, Bonsall MB. 2007. When to care for, abandon, or eat your offspring: the evolution of parental care and filial cannibalism. *Am Nat*. 170:886–901.
- Klug H, Lindström K, St. Mary CM. 2006. Parents benefit from eating offspring: density-dependent egg survivorship compensates for filial cannibalism. *Evolution*. 60:2087–2095.
- Klug H, St. Mary CM. 2005. Reproductive fitness consequences of filial cannibalism in the flagfish, *Jordanella floridae*. *Anim Behav*. 70:685–691.
- Kraak SB. 1996. Female preference and filial cannibalism in *Aidablennius sphyinx* (Teleostei, Blenniidae); a combined field and laboratory study. *Behav Processes*. 36:85–97.
- Kvarnemo C, Svensson O, Forsgren E. 1998. Parental behaviour in relation to food availability in the common goby. *Anim Behav*. 56:1285–1290.
- Lindström K, Pampoulie C. 2005. Effects of resource holding potential and resource value on tenure at nest sites in sand gobies. *Behav Ecol*. 16:70–74.
- Lindström K, Sargent RC. 1997. Food access, brood size and filial cannibalism in the fantail darter, *Etheostoma flabellare*. *Behav Ecol Sociobiol*. 40:107–110.
- Malavasi S, Fiorin R, Franco A, Torricelli P. 2004. Somatic energy storage and reproductive investment in the grass goby *Zosterisessor ophiocephalus*. *J Mar Biol Assoc UK*. 84:455–459.
- Manica A. 2002. Filial cannibalism in teleost fish. *Biol Rev Camb Philos Soc*. 77:261–277.
- Manica A. 2004. Parental fish change their cannibalistic behaviour in response to the cost-to-benefit ratio of parental care. *Anim Behav*. 67:1015–1021.
- McCue MD. 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp Biochem Physiol A Mol Integr Physiol*. 156:1–18.
- Miller JS, Zink AG. 2012. Parental care trade-offs and the role of filial cannibalism in the maritime earwig, *Anisolabis maritima*. *Anim Behav*. 83:1387–1394.
- Mrosovsky N, Sherry DF. 1980. Animal anorexias. *Science*. 207:837–842.
- Neff BD. 2003. Paternity and condition affect cannibalistic behavior in nest-tending bluegill sunfish. *Behav Ecol Sociobiol*. 54:377–384.
- Okuda N, Ito S, Iwao H. 2004. Mate availability and somatic condition affect filial cannibalism in a paternal brooding goby. *Behaviour*. 141:279–296.
- Payne AG, Smith C, Campbell AC. 2002. Filial cannibalism improves survival and development of beaugregory damselfish embryos. *Proc Biol Sci*. 269:2095–2102.
- Piersma T, Lindström A. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol Evol*. 12:134–138.
- Piexoto PEC, Benson WW. 2011. Fat and body mass predict residency status in two tropical satyrine butterflies. *Ethology*. 117:722–730.
- Pinheiro J, Bates D, DebRoy S, Sarkar D. 2014. The R Development Core Team. nlme: linear and nonlinear mixed effects models. R package version 3.1–117.
- Polis GA. 1981. The evolution and dynamics of intraspecific predation. *Annu Rev Ecol Syst*. 12:225–251.
- R Core Team. 2014. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>.
- Rowher S. 1978. Parent cannibalism of offspring and egg raiding as a courtship strategy. *Am Nat*. 112:429–440.
- Sargent RC, Crowley PH, Huang C, Lauer M, Neergaard D, Schmoetzer L. 1995. A dynamic program for male parental care in fishes: brood cycling and filial cannibalism. *Behaviour*. 132:1059–1078.
- Sisneros JA, Alderks PW, Leon K, Sniffen B. 2009. Morphometric changes associated with the reproductive cycle and behaviour of the intertidal-nesting, male plainfin midshipman *Porichthys notatus*. *J Fish Biol*. 74:18–36.
- Smiseth PT, Kölliker M, Royle NJ. 2012. What is parental care? In: Royle NJ, Smiseth PT, Kölliker M, editors. *The evolution of parental care*. Oxford: Oxford University Press. p. 1–14.
- Smith C, Reay P. 1991. Cannibalism in teleost fish. *Rev Fish Biol Fisher*. 1:41–64.
- Soulsby D. 2013. *Animal cannibalism: the dark side of evolution*. Sheffield (UK): 5m Publishing.
- Stanback MT, Koenig WD. 1992. Cannibalism in birds. In: Elgar MA, Crespi BJ, editors. *Cannibalism: ecology and evolution among diverse taxa*. Oxford: Oxford University Press. p. 277–298.
- Thomas LK, Manica A. 2003. Filial cannibalism in an assassin bug. *Anim Behav*. 66:205–210.
- Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. *Sexual selection and the descent of man*. Chicago (IL): Aldine Publishing Company. p. 136–179.
- Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's Principle. *Am Nat*. 100:687–690.
- Zaldúa N, Naya DE. 2014. Digestive flexibility during fasting in fish: a review. *Comp Biochem Physiol A Mol Integr Physiol*. 169:7–14.