



Indirect effects of heterospecific interactions on progeny size through maternal stress

Mark I. McCormick

M. I. McCormick (mark.mccormick@jcu.edu.au), School of Marine and Tropical Biology, James Cook Univ., Townsville, Queensland 4811, Australia.

Maternal effects are increasingly being recognized as an important pre-natal source of life history variation in the next generation. The present study uses a field experiment to explore the influence of heterospecific interactions on the reproductive output and offspring characteristics of a common Indo-Pacific damselfish, *Pomacentrus amboinensis*. On the Great Barrier Reef pairs of breeding *P. amboinensis* were placed on isolated patch reefs and to half of the pairs resource competitors (other planktivorous damselfishes), and predators of eggs and juveniles were added. Females inhabiting patches with heterospecifics had more aggressive interactions and higher levels of the stress hormone, cortisol. Neither the number of clutches nor number of eggs produced differed among treatments. The size of larvae at hatching was found to be reduced as a result of the stress associated with increased interactions with heterospecific and the transfer of cortisol to offspring. This stress-associated mechanism appears to be an important and directional source of life history variability, but the individual nature of the maternal response is likely to result in a conclusion of a diversified bet hedging reproductive strategy when viewed at the local population level. These findings highlight the complex determinants of individual success and the important role of parental well-being in the population dynamics of the next generation.

Heterospecific interactions govern the organization of communities through the dominant processes of competition, predation and facilitation. Competition has been hypothesized to be important in driving divergence by natural selection, whilst predation is attributed the role of removing individuals that are inferior competitors and exhibit low performance. The direct effects of these ecological processes can be seen in the spatial distribution of individuals and their behavioural regimes (Sih et al. 1985). However, the indirect effects are less readily observed. These can occur through the mere presence of a heterospecific influencing the interactions of the focal species, or through the heterospecific's influence on food web connections (Menge 1995, Werner and Peacor 2006). Such indirect effects may influence growth (Werner and Peacor 2006), phenotypic expression (Benard 2006), mating opportunities (Sih and Krupa 1996), the reproductive output of local populations (Wootton 1994) and performance of offspring (Allen et al. 2008); all of which influence fitness and survival.

Behavioural interactions can influence individual success through their impact on the physiology and health of breeding individuals. When species compete for resources it can have negative effects on all involved regardless of dominance rank (Verhulst and Salomons 2004, Poisbleau et al. 2005). Interactions with heterospecifics take time away from fitness associated activities, such as foraging, mating or offspring defence. Increased interaction regimes

may therefore influence energy directed to storage and available for reproductive efforts (Alvarez et al. 2006). Enhanced interaction regimes may also influence the stress physiology of those individuals who interact (Sloman and Armstrong 2002), as well as those in the vicinity of the interaction (Oliveira et al. 2001, Summers et al. 2005), leading to elevated levels of parental corticosteroids and catecholamines (Schreck et al. 1997, DeVries 2002, McCormick 2006). Interaction regimes will thus have an impact on individual and population fitness by altering the energy devoted to reproduction and the relative allocation to the quantity versus the quality of offspring. While both parents may be subject to stressful and energy-demanding interactions, it is generally the condition of the mother that has greatest influence on the number and attributes of the offspring (Price 1998, Green and McCormick 2005, Trippel et al. 2005).

Maternal effects, defined as contributions of the mother other than her genotype (Bernardo 1996a), have been increasingly recognized as an important means whereby progenitors can optimize or adapt the state of their offspring to best suit the environment into which they are liberated (Mousseau and Fox 1998, Marshall and Uller 2007). Maternal health or body condition can influence progeny size and survival through maternal contributions to nutrition. Offspring survival can also be enhanced by non-genetic contributions to immunology, metabolism and endocrinology. Life history theory suggests that the phenotypic state of

the mother, and her perception of the environment into which she will liberate her offspring, determine her relative allocation to the quantity and quality of offspring (Smith and Fretwell 1974, Roff 1992, Stearns 1992, Bernardo 1996a). While many studies have supported these hypotheses (Mousseau and Dingle 1991, Galloway 1995, Agrawal et al. 1999, Agrawal 2002, Allen et al. 2008), a number of studies have found that mothers may fail to endow their progeny with characteristics that will necessarily benefit them for their future environment (Bernardo 1996a, 1996b, Kofman 2002, McCormick 2006). Our understanding of the mechanistic links between the maternal environment, reproductive physiology and progeny characteristics is currently poor, which has made the discrimination between hypotheses difficult.

Fishes are an ideal group for the study of life history adaptability because they exhibit a high degree of plasticity in many traits that are important to success, such as offspring size. Coral reefs are of particular interest since they are characterized by high species diversity and high densities of species that have similar ecological requirements leading to high levels of behavioural interaction. Interaction frequencies can be high accounting for a substantial proportion of activity budgets (McCormick 2006), particularly in species that maintain territories (McCormick 2006, unpubl.). A recent study has shown that intraspecific interactions act with a density dependent influence on levels of maternal stress, and that this directly influences offspring characteristics through a hormonal mechanism (McCormick 2006). While we now have some understanding of the influence of conspecific interactions on reproductive output, it is currently unknown how heterospecific interactions impact maternal reproductive output and offspring characteristics, and the relative importance of heterospecific versus conspecific interactions. Knowing this is as fundamental as understanding the relative importance of intra- versus inter-specific competition.

The present study is the first to explore the influence of heterospecific interactions on the reproductive output and progeny characteristics of a common Indo-Pacific damselfish, *Pomacentrus amboinensis*. A field experiment is undertaken which manipulates heterospecific interactions experienced by spawning pairs and examines the impact of interaction frequency on clutch size, clutch mortality and larval characteristics. Theory predicts that if females anticipate the juvenile environment to be poor for growth and survival she should shift towards larger and fewer offspring to improve her fitness (Roff 1992, Marshall and Uller 2007). In previous studies on a range of taxa, poor environments have included the perception by the mother of low resource abundance, high competition or high predation (Brockelman 1975, Sibly and Calow 1983, Clutton-Brock 1991, Roff 1992). In the present study heterospecific interactions are enhanced by the addition of resource competitors and egg and juvenile predators. Maternal measures of ovarian cortisol are correlated to egg output and larval characteristics to advance a mechanism whereby heterospecific interactions may influence larval attributes.

Methods

Study species and location

This project was undertaken at Lizard Island (14°41'S, 145°27'E) on the northern Great Barrier Reef (GBR), Australia between 25 November 2002 and 3 February 2003. On the GBR the ambon damselfish, *Pomacentrus amboinensis*, inhabits a broad range of habitats involving a mixture of rubble and live coral. They are commonest at the base of the slope of shallow reefs, where they live in loose groups involving a male and up to seven females, which interact in a size-based dominance hierarchy. The species is a protogynous hermaphrodite, with males guarding a nest site that is visited by females during the breeding season (October to February on the northern GBR). Males often make a nest site under a piece of coral rubble or upturned clam shell, but will readily accept an artificial spawning surface, such as a plastic half pipe used in this and other studies (McCormick 1998, 2003, 2006). Females spawn with the male before dawn (McCormick and Smith 2004) and males guard the eggs for 4.5 d (at 28°C) until they hatch about 20 min after sunset. There is a strong relationship between the levels of cortisol in the ovary of breeding females and the levels of cortisol within newly spawned eggs (McCormick 1998). Males take the primary role in nest site defence, although when he leaves the nest to court other females, the dominant female of the social group may take over this role until he returns (McCormick unpubl.). During the breeding season the male guards the nest against any fish that may attempt to eat eggs from his nest, regardless of size; these include other *P. amboinensis* (male, female or juvenile), other damselfishes (e.g. *P. nagasakiensis*) and some wrasses (particularly *Thalassoma lunare*) (McCormick and Meekan 2007).

Natural densities of interacting fishes

To establish the densities of fishes that breeding pairs of *P. amboinensis* may potentially interact with, the densities of all fish species were counted by a scuba diver within a cylindrical sampling unit of 1 m radius on the contiguous reef edge. Because the nest guarding activity of *P. amboinensis* males influences the distribution patterns of some fish species (McCormick and Meekan 2007), fish densities were quantified inside and outside male nesting territories to obtain a good estimate of the sorts of densities of fish breeding females could potentially interact with on the continuous reef. Half the counts (18) were centered on a nest guarded by a male *P. amboinensis*. The remainder (18) were undertaken in an adjacent section of reef that did not contain the territory of a breeding male, and which was far enough away to not be influenced by the fishes within the nest-centered count (3–4 m apart). Eighteen nest and non-nest sites were surveyed. Multivariate analysis of variance (MANOVA) was used to test for a difference in the fish assemblage between inside and outside male *P. amboinensis* territories. Fish were placed into seven categories which covered the main species that interact with that *P. amboinensis* at this locality (Fig. 1 legend). Canonical

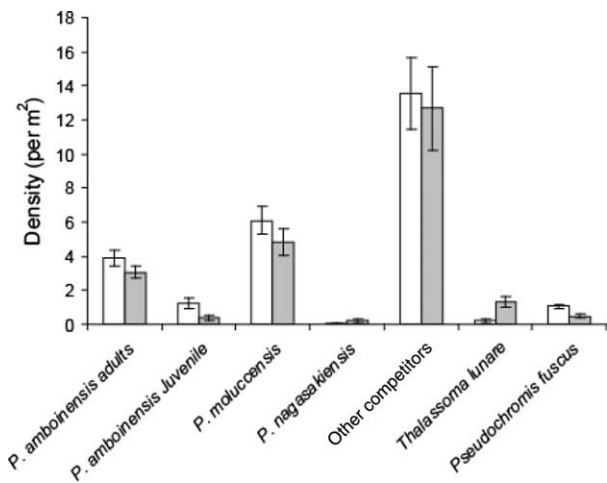


Figure 1. Densities of interacting fish near (clear) and away (shaded) from *Pomacentrus amboinensis* nest sites. Densities are from visual counts 1 m radius from a defended nest site, or from a random non-defended area nearby.

discriminant analysis (CDA) was used to explore the nature of the significant difference found with MANOVA.

Experimental design

In synopsis, in the present field experiment breeding pairs were established on patch reefs and exposed to one of two treatments: no heterospecifics, or the presence of heterospecifics. Interaction regimes of the parents were recorded together with their reproductive output and characteristics of the larvae produced. These results are compared to the progeny of breeders from the reef edge, where fish are exposed to a wide variety of con- and hetero-specific interactions. The present experiment was conducted at the same time, using a similar protocol, as another experiment that looked at the effects of female density on maternal reproductive output (McCormick 2006). This enables a further, unique comparison of the heterospecific effects to the effect of a varying density of conspecifics, conducted in the discussion.

Males and female *Pomacentrus amboinensis* were collected with hand-nets from the reef edge of the Lizard Island lagoon. They were randomly placed in reproductive pairs onto 19 lagoonal patch reefs (2 × 2 m), composed of mostly coral rubble with some live coral, and isolated by at least 15 m of sand in 3 to 4 m water depth. Patch reefs are part of the natural habitat range of this species. Reefs were cleared of all fishes prior to the release of the breeding pair. A plastic half pipe (30 cm long, 18 cm ϕ) was placed on each reef and this was adopted quickly by the male as a breeding site. Ten reefs had additional fishes that were known egg predators or resource competitors placed onto them. These were 1 to 2 dottedback, *Pseudochromis fuscus* (family Pseudochromidae), and two moon-wrasse, *Thalassoma lunare* (family Labridae), both of which are common opportunistic predators of the eggs and juveniles of *P. amboinensis* (Emslie and Jones 2001). Although moon-wrasse are a resident predator on the main reef edge of many tropical lagoons, they have a more transient association with isolated reefs. Therefore, to keep moonwrasse on

the isolated reefs, and prevent them moving between reefs of different treatments, they were individually enclosed in 3 l clear plastic containers with mesh bases. Wrasse within the containers were replaced every ~5 d to avoid detrimental stress to the captives. A pilot study showed that *P. amboinensis* had no behavioural response to the presence of empty plastic containers. Two species of resource competitors were also added to the patch reefs: 1 to 2 adult *Pomacentrus nagasakiensis*, and 1 to 5 *Pomacentrus moluccensis*. These species are planktivores (like *P. amboinensis*) and compete for nesting sites. On the remaining nine isolated reefs breeding pairs were maintained without competitors or predators of eggs or juveniles. Male *P. amboinensis* used in the experiment ranged in size between 63 to 78.3 mm standard length (SL), whilst females ranged between 57.1 to 65 mm SL. There was no difference in the size of males or females between treatments (males: $F_{1,17} = 1.87$, $p = 0.190$; females: $F_{1,16} = 0.139$, $p = 0.714$).

In addition, the natural nests of 11 males on the shallow edge of the main reef adjacent to the patch reefs were exchanged with the standardized nesting surfaces to facilitate efficient monitoring of egg clutches. The behavioural regime of females on the isolated patch reefs, and the attributes of the larvae they produced (detailed below), were compared to those of dominant females within social groups from the main population on the main reef edge. Dominant females were chosen as targets for study since other data suggests that the social status of the females influences their levels of ovarian cortisol (McCormick unpubl.).

Due to the complexity of undertaking these manipulative experiments in the field the numbers of replicates are unbalanced and differ between comparisons of measured variables. Reasons for these differences include females being lost from the patch reef after breeding but prior to capture, the loss (through predation) of a male prior to capture, or the lack of breeding by a reproductive pair. Replicate numbers used in each comparison are therefore given in the text.

Parental behaviour

Behavioural budgets were determined for all females on isolated patch reefs (seven per treatment) and dominant females in social groups on the nearby main reef edge (11 females). Behavioral observations were made between 10:30 and 14:30 hours over a 5-day period, with observations of replicate fish within each treatment spread approximately evenly over the census period (7, 7 and 11 replicate female were assessed for the control, heterospecific and main reef treatments respectively; 7, 7 and 10 replicates for males). Behaviour occurrence was recorded in categories every 15 s over a 10 min focal sampling period. Behaviours were categorized into: display (showing side to target and erecting dorsal and anal fins); circling (swimming close, stopping frequently and looking at the target fish); chasing; biting; undirected swimming; feeding and courting. Target species of interactions were also recorded. Total aggressive interactions were quantified as the sum of the first four behavioural categories. Mean aggression was compared

among treatments using ANOVA, and residual analysis was used to check the assumptions of normality and homogeneity of variance. A Tukey's (HSD) means comparison was used to determine the nature of the significant differences found by ANOVA.

Clutch size and larval morphology

Egg clutches were photographed in situ on the morning of spawning, with a transparent 1 cm square grid positioned on top for a scale. Clutch size and the density of eggs were estimated from the digital images using an image analysis program (Optimas 6.5, Media Cybernetics 1999) and this enabled the calculation of the total number of eggs produced. Clutches were brought into the laboratory and placed into aerated aquaria just prior to natural hatching. Immediately upon hatching larvae were collected with a small net and preserved in 2.5% glutaraldehyde in seawater for 2 h at room temperature, rinsed in seawater, then transferred to fresh seawater and refrigerated for morphometric analyses. To maximize the opportunity for the treatments to affect the provisioning of eggs by fish on patch reefs, characteristics of larvae spawned were quantified from clutches that had been laid at least three weeks after establishment of the treatments. In all but two instances the clutches analysed were the second clutch spawned by the experimental pair, with the exceptions being their first clutch. Because male *P. amboinensis* will spawn with many females when available (see McCormick and Smith 2004; hence the use of isolated patch reefs for the experimental manipulation) the identity of the females that laid eggs on nest from the main reef, and their spawning history, were unknown.

Standard length (SL) and yolk sac area of 30 larvae from each nest were measured from calibrated digital images with the aid of image analysis software (Optimas). Yolk sac area provided a measure of the endogenous reserves available for subsequent development. Mean larval morphology per breeding female was compared among treatments with ANOVA, after examining the assumptions of normality and homogeneity of variance with residual analysis. Tukey's (HSD) means comparisons were used to determine the nature of significant differences found by ANOVA. The number of replicate pairs for which egg clutches were collected was seven for the control and nine for the heterospecific treatment. Larval attributes were compared to attributes of larvae from seven clutches of eggs collected from pairs of *P. amboinensis* nesting on the main reef edge.

Cortisol assays

Females from the patch reefs were collected upon the successful hatching (and larval sampling) of their second clutch of eggs, five to six days after females had laid their second clutch. At the end of the experiment, dominant females associated with the monitored nests on the main reef edge were collected for comparison to females on experimental patch reefs. Females were euthanized using a clove oil-ethanol-seawater solution overdose, weighed and measured (SL with calipers) and their ovaries were removed. A piece of ovary tissue (≈ 0.1 g wet wt) was freeze-dried and

homogenized in 200 μ l 0.05 M phosphate buffer containing 0.1% gelatine and 0.01% Thimerosal (Sigma). One ml of ethyl acetate was added, vortexed and centrifuged. Duplicate 100 μ l aliquots were evaporated in assay tubes. Cortisol in ovary extracts was assayed according to the protocol of Pankhurst and Carragher (1992). The radioactive tracer used was (1,2,6,7- 3 H) cortisol (TRK407; Amersham, UK) while the antiserum was developed against cortisol-3-carboxymethyloxime-bovine serum albumin in rabbits (UCBA907/R1Y; Accurate Chemical and Scientific Corporation, NY). This antiserum is reported to have cross reactivities to corticosterone (1.8%) and aldosterone ($< 0.02\%$). Interassay variability for cortisol was 8.3% CV, $n = 6$.

Results

Natural densities of interacting fishes

Mean densities of fish that may interact with female *P. amboinensis* differed slightly between inside and outside the immediate area of male nesting territories (Pillai's trace = 0.399, DF = 7, 28, $p = 0.03$; Fig. 1). CDA suggested that this significance was due to higher densities of juvenile *P. amboinensis* and *Pseudochromis fuscus* inside male territories, and higher numbers of *Thalassoma lunare* in non-territory areas. Densities of total heterospecifics around nest sites range from 1.9 to 46.8 m^{-3} .

Parental behaviour

The presence of heterospecifics on isolated patch reefs significantly increased the percentage of time females devoted to aggressive interactions ($F_{2,46} = 28.362$, $p < 0.0001$; Tukey's HSD, $p = 0.0002$; Fig. 2). Interaction levels for females on isolated reefs with heterospecifics were

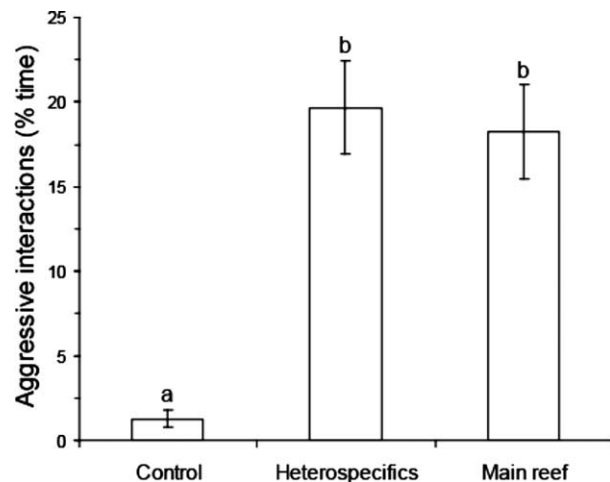


Figure 2. Comparison of aggressive behaviour of female *Pomacentrus amboinensis* in breeding pairs on isolated patch reefs when exposed or not to heterospecific fishes. Mean behaviour of dominant females (\pm SE) from the main reef are also given. Behaviour was determined from 10min focal animal samples with behaviour categorized every 15 s. $n = 9$ (no heterospecifics; controls), 10 (with heterospecifics), 21 (main reef).

similar to those found for dominant females in social groups on the adjacent contiguous reef (Tukey's HSD, $p = 0.984$), with both spending $\sim 18\%$ of their time undertaking displays, chases and bites. The levels of interactions provoked by the addition of heterospecifics to the reefs were well within the range of time spent on aggressive interactions on the main reef, which found one female devoting 85% of her time to such behaviours (Fig. 2). Male behaviour exhibited a similar, but non-significant ($F_{2,22} = 2.955$, $p = 0.07$), trend in the levels of aggression among treatments to that of the females, however males displayed much lower levels of aggression ranging from 5.3% (control) to 15.4% (main reef) of the total time budget on the main reef.

Egg production

There was no relationship between female size and the number of eggs produced over a 30d monitoring period ($r = -0.08$, $n = 16$, $p > 0.05$). There was no difference in the production of eggs over a 30d period between the control females and females from reefs stocked with heterospecifics ($F_{1,14} = 0.225$, $p = 0.642$; overall mean 16 884 eggs in 30 d). No clutches were lost to egg predation from either patch reef treatment during the course of the study.

Larval morphology

Females on control reefs produced larger larvae than either females that interacted with heterospecifics on patch reefs or on the main reef ($F_{2,20} = 42.90$, $p < 0.0001$; Fig. 3). There was no difference in the size of larvae from the heterospecific and main reef treatments (Tukey's HSD, $p > 0.05$). The range in the size of larvae from main reef spawns was 2.12 to 2.71 mm. There was no difference in the size of the larval yolk sac among the three treatments ($F_{2,20} = 0.104$, $p = 0.902$).

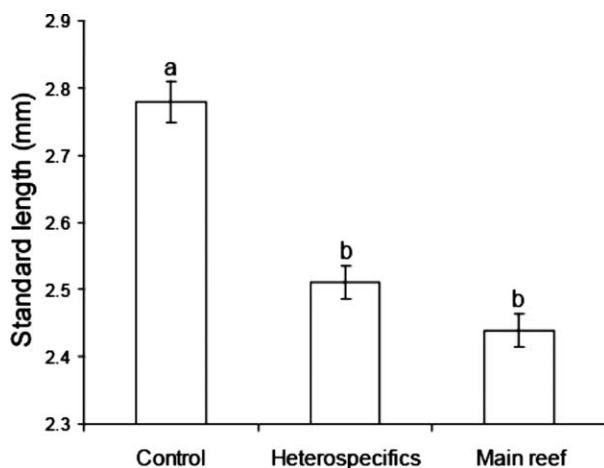


Figure 3. Standard length of larvae at hatching from female *Pomacentrus amboinensis* in breeding pairs on isolated patch reefs when exposed or not to heterospecific fishes. Mean length of larvae (\pm SE) produced by females from the main reef is also given.

Cortisol levels

Ovarian cortisol levels of the females differed among treatments. Females on reefs with heterospecifics had almost double the cortisol of females on patch reefs without heterospecifics, or females from the main reef ($F_{2,35} = 4.900$, $p = 0.013$; Fig. 4). Ovarian cortisol levels within females collected from the main reef ranged between 17 and 172 ng g^{-1} .

Discussion

The intergenerational transmission of non-genetic effects are increasingly being found to be important to the phenotypic expression of offspring (Dufty et al. 2002), and a variety of covarying traits such as development rate (Eising et al. 2003, Ostrand et al. 2004), growth (Janczak et al. 2005), personality traits (Kofman 2002), social dominance (Janczak et al. 2005), spatial awareness (Zielinski et al. 1992), reproductive maturation (Clark et al. 1993), reproductive periodicity and lifetime fecundity (Clark and Galef 1998). The present study is the first to show a link between maternal interactions with heterospecifics and offspring phenotype for an organism with a complex lifecycle. The finding highlights the complex determinants of individual success and the likelihood of the important role of parental well-being in population dynamics of the next generation.

Enhanced interaction regimes were found to increase levels of physiological stress in mothers, as indicated by elevated levels of cortisol. Individuals that have high interaction rates, such as highly interacting individuals within dominance hierarchies, have been found to show elevated catecholamines and corticosteroids in a wide range of taxa, including mammals (Abbott et al. 2003), birds (Poisbleau et al. 2005), lizards (Greenberg et al. 1984) and fishes (Fox et al. 1997). For breeding individuals a variably strong relationship between maternal stress levels and egg corticosteroid levels (McCormick 1998, Takahashi et al.

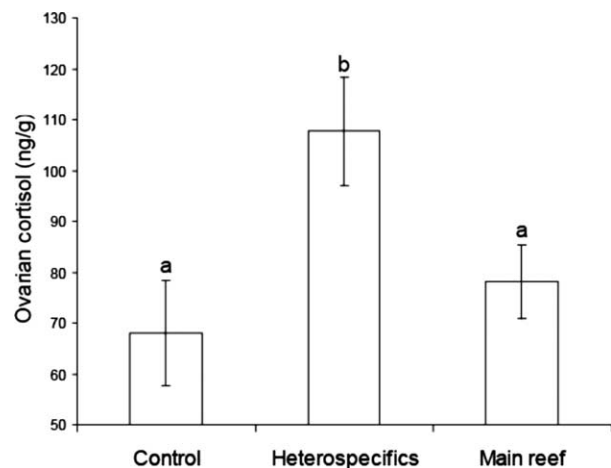


Figure 4. Ovarian cortisol levels of female *Pomacentrus amboinensis* in breeding pairs on isolated patch reefs when exposed or not to heterospecific fishes. Mean cortisol levels of females (\pm SE) from the main reef are also given.

1998, Schreck et al. 2001) results in offspring that are exposed to higher concentrations of stress hormones compared to embryos produced by similar females in less stressful environments. Cortisol has been shown in field and laboratory experiments to directly influence offspring size (Campbell et al. 1992, Contreras-Sanchez et al. 1998, McCormick 1998, 1999, 2006, Corner et al. 2007). Fish embryos exposed to higher levels of cortisol have higher developmental rhythms, less endogenous energy to devote to growth and so are smaller at hatching than embryos exposed to lower cortisol levels (McCormick and Nechaev 2002). The result can be a higher frequency of abnormalities (Morgan et al. 1999, Eriksen et al. 2006, Gagliano and McCormick unpubl.) and an impact on performance potential, given the strong link between larval size and swimming ability (Fisher et al. 2000).

The smaller offspring that are a product of elevated stress levels in their mothers may also have higher mortality. A recent study by Gagliano and McCormick (unpubl.) manipulated cortisol within the eggs of *P. amboinensis* and found that embryos exposed to higher levels of cortisol resulted in offspring with greater otolith fluctuating asymmetry (FA); which is commonly used as an indicator of developmental stability in bilaterally symmetrical organisms (Palmer 1996). Evidence suggested that fish with higher asymmetry were also selectively lost during the larval phase (Gagliano et al. 2008). Furthermore, Lemberget and McCormick (2008) related the monthly recruitment of a Caribbean lizardfish from the larval phase to otolith FA and found that FA accounted between 70 to 97% of the variability in fish recruitment (in wet and dry seasons respectively). Months with low recruitment had a high proportion of asymmetrical fish, suggesting that asymmetry is indicative of a higher probability of larval mortality. An increasing number of studies are underscoring the importance of maternal effects on the initial condition of offspring and the long-term influence of these prenatal effects on individual success and population dynamics.

If the production of smaller offspring does represent a negative impact on the offspring, then why would mothers resort to reducing offspring quality rather than altering the number of offspring produced? In the present organism, and possibly many others that have shown a similar relationship between stress and offspring size (Contreras-Sanchez et al. 1998), the answer may lie in the importance of cortisol as a physiologically active hormone. The transmission of maternal cortisol occurs during gametogenesis and is unlikely to be a trait that can be selected on, but rather an unfortunate consequence of the physiological machinery that all vertebrates share (i.e. the hypothalamo-pituitary-adrenocortical axis). From the maternal side cortisol is a glucocorticoid involved in energy mobilization, osmoregulation, and the reallocation of energy away from growth and reproduction (Pankhurst and Van Der Kraak 1997). From the developing offspring's perspective, cortisol plays many important roles during vertebrate development and interacts with many developmental hormones, such as thyroxine, tri-iodothyronine and growth hormone (particularly during metamorphosis) (Mathiyalagan et al. 1996, Kim and Brown 1997, Schreck et al. 2001). Thus, the diverse functionality of corticosteroids negates targeted selection on the mechanisms underlying the impacted

offspring traits (e.g. size, growth), particularly since many of these traits will only be selected against under some environmental conditions (e.g. low food availability and/or high predator pressure). Findings of a recent study that serially sampled a cohort of *P. amboinensis* in the wild suggested that the direction and intensity of selection pressures on key life history traits, such as growth, can change quite rapidly during larval and juvenile life stages (Gagliano et al. 2007). If selection pressures on offspring are variable then diversified bet hedging may be the best reproduction strategy for parents to adopt to optimize offspring survival.

Other information suggests that *P. amboinensis* uses to a diversified bet hedging allocation strategy across local populations (Gagliano and McCormick 2007). However, in this species there is no evidence that stressed females anticipate the juvenile environment and produce fewer and larger offspring (McCormick 1999). This may be because fish of similar size have different responses to stress and consequently differing levels of cortisol, which in *P. amboinensis* influence offspring developmental rhythms and size. The production of larger and fewer offspring have been found for low growth environments in comparisons among field populations of many different taxa (Berven 1982, Orton and Sibly 1990, Tamate and Maekawa 2000, Johnson and Leggett 2002, Gregersen et al. 2006), and it may be that the balance of mechanisms determining offspring characteristics in these species is different from the damselfish in the current study.

The present study emphasizes the transgenerational impact of heterospecific interactions on reproductive females, however, it may be expected that interactions with conspecifics may be of greater influence because they use exactly the same resources and competition is expected to be more intense (Forrester et al. 2006). In a companion study to the present research, McCormick (2006) manipulated the number of interactions that breeding female *P. amboinensis* had with conspecifics. He found that there was a density dependent response, with higher densities of conspecifics leading to higher levels of maternal cortisol and smaller larvae. When data from the present experiment is added to the data on conspecifics effects (Fig. 5), the present data fits well into the relationship between maternal cortisol levels and offspring size, with an overall relationship that accounts for 92% of the variability among treatments. The present combination of heterospecific species on the patch reefs elevated the cortisol in the breeding female to the same extent as when 6 conspecific females were present, and yielded a similar reduction in larval size. Since both the number of interacting heterospecifics and conspecifics are well within naturally occurring levels, this field study suggests that both types of behavioural interactions are important influences on maternal stress levels and aid to shape the characteristics of the next generation.

Not surprisingly, the pattern found on the main reef does not fit the trend found for fish on the experimental patch reefs, with the average female showing high levels of aggressive interactions, producing small larvae but having low levels of ovarian cortisol. This is due to the polygamous nature of the species mating system where one male will attract as many breeding females as possible to spawn on his nest site (McCormick and Smith 2004). Many of the larvae

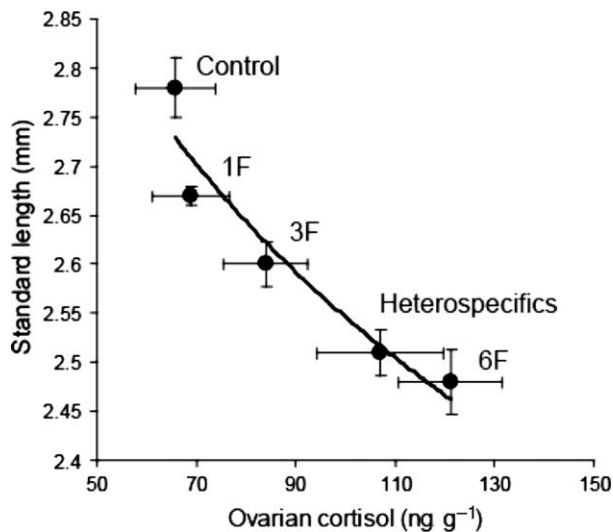


Figure 5. Comparison of conspecific versus heterospecific influence on larval size. Relationship between maternal ovarian cortisol levels and the standard length of larvae at hatching. Data are treatments means with standard errors (numbers from left to right; females: $n = 10, 10, 10, 9, 6$; larvae: $n = 7, 6, 6, 9, 5$). Treatments correspond to the addition of 0 (control), 1, 3, or 6 extra female (F) *P. amboinensis* onto patch reefs containing a breeding pair (after McCormick 2006), or the addition of a group of heterospecifics. The semi-logged relationship is represented as: $SL = -0.4364 \ln(\text{Cortisol}) + 4.556$, $r^2 = 0.92$, $p < 0.05$.

that were collected during the study from the nest sites on the main reef were therefore unlikely to have originated from the dominant female collected at the end of the study. Contributors to the nests on the main reef include subordinate females that have varying levels of cortisol, which are higher than dominant females (McCormick unpubl.). This contribution of subordinates will therefore obscure the relationship between behaviour, cortisol and larval length on natural nests. It is for this exact reason that experiments were conducted on patch reefs, where the social system could be controlled in an attempt to explore the link between maternal and progeny characteristics.

The present study has shown that behavioural interactions are an important source of naturally occurring stress that may have carryover effects to the next generation. Maternal stress may have lasting effects that are amplified (or attenuated) through ontogeny (Walker et al. 2007, McCormick and Gagliano in press). Broad scale changes in habitat health, such as coral bleaching, may influence the progeny quality of local populations through this means. However, given the small (among individual) scale on which the mechanism operates it is likely that the maternal environment-offspring link through cortisol will functionally be a mechanism underlying the moderate levels of variability in larval characteristics that naturally occur at the site level (McCormick 1999), which has been described before as diversified bet hedging (Gagliano and McCormick 2007). Manipulations in the laboratory and field of prenatal cortisol in *P. amboinensis* have found that modifying cortisol levels within naturally occurring limits can explain all of the variability in offspring size that is found in the wild (McCormick 1999, 2006). Whether this mechanism

of maternal influence on progeny is of similar importance in other fishes or vertebrates is currently unclear.

A large body of evidence in vertebrates indicates that maternal stress during gamete production and release affects the hormonal and behavioural development of offspring (Braastad 1998, Schreck et al. 2001, Kofman 2002, Harper 2005). Currently the details of these effects have only been documented through experiments on human models (such as rats and monkeys). Given the similarity of the endocrine systems of vertebrates it is likely that fishes will also exhibit complex responses to maternal stress, including enhanced responsiveness to stressful situations, increased mortality, reduced growth, deviant morphology and depressed immune function (Braastad 1998, Eriksen et al. 2006). Behaviour may also be affected by prenatal stress, causing increased anxiety, reduced stress-coping, impaired spatial learning and cognitive deficits (Braastad 1998, Welberg and Seckl 2001, Kofman 2002). It is unclear how many of these influences will be relevant to particular species, however, the Pandora's box of maternal effects has been opened and what has been revealed to date suggests that maternal influences are likely to be found to have diverse, profound and lasting impacts on population dynamics. Further work is required for most non-mammalian vertebrates (and invertebrates generally), particularly those with complex life cycles, before predictions can be made about the response of populations and communities to environmental change.

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References

- Abbott, D. H. et al. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. – *Horm. Behav.* 43: 67–82.
- Agrawal, A. A. 2002. Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. – *Ecology* 83: 3408–3415.
- Agrawal, A. A. et al. 1999. Transgenerational induction of defences in animals and plants. – *Nature* 401: 60–63.
- Allen, R. M. et al. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. – *Am. Nat.* 171: 225–237.
- Alvarez, M. D. et al. 2006. Digestive capacity, growth and social stress in newly-metamorphosed Japanese flounder (*Paralichthys olivaceus*). – *Environ. Biol. Fish.* 77: 133–140.
- Benard, M. F. 2006. Survival tradeoffs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). – *Ecology* 87: 340–346.
- Bernardo, J. 1996a. Maternal effects in animal ecology. – *Am. Zool.* 36: 83–105.
- Bernardo, J. 1996b. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. – *Am. Zool.* 36: 216–236.
- Berven, K. A. 1982. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. – *Evolution* 36: 962–983.

- Braastad, B. O. 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. – *Appl. Anim. Behav. Sci.* 61: 159–180.
- Brockelman, W. Y. 1975. Competition, the fitness of offspring, and optimal clutch size. – *Am. Nat.* 109: 677–699.
- Campbell, P. M. et al. 1992. Stress reduces the quality of gametes produced by rainbow trout. – *Biol. Reprod.* 47: 1140–1150.
- Clark, M. M. and Galef, B. G. 1998. Perinatal influences on the reproductive behaviour of adult rodents. – In: Mousseau, T. A. and Fox, C. W. (eds), *Maternal effects as adaptations*. Oxford Univ. Press, pp. 261–271.
- Clark, M. M. et al. 1993. Hormonally mediated inheritance of acquired characteristics in Mongolian gerbils. – *Nature* 364: 712.
- Clutton-Brock, T. H. 1991. *The evolution of parental care*. – Princeton Univ. Press.
- Contreras-Sanchez, W. M. et al. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). – *Biol. Reprod.* 58: 439–447.
- Corner, R. A. et al. 2007. The effect of mid-pregnancy shearing and litter size on lamb birth weight and postnatal plasma cortisol response. – *Small Ruminant Res.* 73: 115–121.
- DeVries, A. C. 2002. Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. – *Horm. Behav.* 41: 405–413.
- Dufty, A. M. et al. 2002. Hormones, developmental plasticity and adaptation. – *Trends Ecol. Evol.* 17: 190–196.
- Eising, C. M. et al. 2003. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. – *Gen. Comp. Endocrinol.* 132: 241–247.
- Emslie, M. J. and Jones, G. P. 2001. Patterns of embryo mortality in a demersally spawning coral reef fish and the role of predatory fishes. – *Environ. Biol. Fish.* 60: 363–373.
- Eriksen, M. S. et al. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. – *J. Fish Biol.* 69: 114–129.
- Fisher, R. et al. 2000. The development of swimming abilities in reef fish larvae. – *Mar. Ecol. Prog. Ser.* 202: 163–173.
- Forrester, G. E. et al. 2006. Assessing the magnitude of intra- and interspecific competition in two coral reef fishes. – *Oecologia* 148: 632–640.
- Fox, H. et al. 1997. Stress and dominance in a social fish. – *J. Neurosci.* 17: 6463–6469.
- Gagliano, M. and McCormick, M. I. 2007. Maternal condition influences phenotypic selection on offspring. – *J. Anim. Ecol.* 76: 174–182.
- Gagliano, M. et al. 2007. Survival against the odds: ontogenetic changes in selective pressure mediate growth–mortality trade-offs. – *Proc. R. Soc. Lond. B* 274: 1575–1582.
- Gagliano, M. et al. 2008. Dispersal without errors: symmetrical ears tune into the right frequency for survival. – *Proc. R. Soc. Lond. B* 275: 527–534.
- Galloway, L. F. 1995. Response to natural environmental heterogeneity: maternal effects and selection on life history characters and plasticities in *Mimulus guttatus*. – *Evolution* 49: 1095–1107.
- Green, B. S. and McCormick, M. I. 2005. Maternal and paternal influences determine size, growth and performance in a tropical reef fish larvae. – *Mar. Ecol. Prog. Ser.* 289: 263–272.
- Greenberg, N. et al. 1984. Social status, gonadal state, and the adrenal stress response in the lizard, *Anolis carolinensis*. – *Horm. Behav.* 18: 1–11.
- Gregersen, F. et al. 2006. Egg size differentiation among sympatric demes of brown trout: possible effects of density-dependent interactions among fry. – *Ecol. Freshwater Fish.* 15: 237–246.
- Harper, L. V. 2005. Epigenetic inheritance and the intergenerational transfer of experience. – *Psychol. Bull.* 131: 340–360.
- Janczak, A. M. et al. 2005. Behavioural effects of embryonic exposure to corticosterone in chickens. – *Appl. Anim. Behav. Sci.* 96: 69–82.
- Johnson, T. A. and Leggett, W. C. 2002. Maternal and environmental gradients in the egg size of an iteroparous fish. – *Ecology* 83: 1777–1791.
- Kim, B. G. and Brown, C. I. 1997. Interaction of cortisol and thyroid hormone in the larval development of Pacific threadfin. – *Am. Zool.* 37: 470–481.
- Kofman, O. 2002. The role of prenatal stress in the etiology of developmental behavioural disorders. – *Neurosci. Biobehav. Rev.* 26: 457–470.
- Lemberget, T. and McCormick, M. I. 2008. Replenishment success linked to fluctuating asymmetry in larval fish. – *Oecologia* doi: 10.1007/s00442-008-1212-x.
- Marshall, D. J. and Uller, T. 2007. When is a maternal effect adaptive? – *Oikos* 116: 1957–1963.
- Mathiyalagan, A. et al. 1996. Effects of cortisol on growth and development in tilapia larvae, *Oreochromis mossambicus*. – *Fish. Physiol. Biochem.* 15: 453–458.
- McCormick, M. I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. – *Ecology* 79: 1873–1883.
- McCormick, M. I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. – *Oecologia* 118: 412–422.
- McCormick, M. I. 2003. Consumption of coral propagules after mass spawning enhances larval quality of a damselfish through maternal effects. – *Oecologia* 136: 37–45.
- McCormick, M. I. 2006. Mothers matter: crowded reefs lead to stressed mothers and smaller offspring in marine fish. – *Ecology* 87: 1104–1109.
- McCormick, M. I. and Nechaev, I. V. 2002. Influence of cortisol on developmental rhythms during embryogenesis in a tropical damselfish. – *J. Exp. Zool.* 293: 456–466.
- McCormick, M. I. and Smith, S. A. 2004. Efficacy of passive integrated transponder tags to determine spawning site visitations by a tropical fish. – *Coral Reefs* 23: 570–577.
- McCormick, M. I. and Meekan, M. G. 2007. Social facilitation of selective mortality. – *Ecology* 88: 1562–1570.
- McCormick, M. I. and Gagliano, M. Carry-over effects—the importance of a good start. – *Proc. 11th Int. Coral Reef Symp.*, Session number 10, in press.
- Menge, B. A. 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. – *Ecol. Monogr.* 65: 21–74.
- Morgan, M. J. et al. 1999. The effect of stress on reproduction in Atlantic cod. – *J. Fish Biol.* 54: 477–488.
- Mousseau, T. A. and Dingle, H. 1991. Maternal effects in insect life histories. – *Annu. Rev. Entomol.* 36: 511–534.
- Mousseau, T. A. and Fox, C. W. 1998. The adaptive significance of maternal effects. – *Trends Ecol. Evol.* 13: 403–407.
- Oliveira, R. F. et al. 2001. Watching fights raises fish hormone levels. – *Nature* 409: 475.
- Orton, R. A. and Sibly, R. M. 1990. Egg size and growth rate in *Theodoxus fluviatilis* (L.). – *Funct. Ecol.* 4: 91–94.
- Ostrand, K. G. et al. 2004. Effects of stress on largemouth bass reproduction. – *N. Am. J. Fish. Manage.* 24: 1038–1045.
- Palmer, A. R. 1996. Waltzing with asymmetry. – *Bioscience* 46: 518–532.
- Pankhurst, N. W. and Carragher, J. F. 1992. Oocyte maturation and changes in plasma steroid levels in snapper *Pagrus* (= *Chrysophrys*) *auratus* (Sparidae) following treatment with human chorionic gonadotropin. – *Aquaculture* 101: 337–347.
- Pankhurst, N. W. and Van Der Kraak, G. 1997. Effects of stress on reproduction and growth of fish. – In: Iwana, G. K. et al.

- (eds), Fish stress and health in aquaculture. Cambridge Univ. Press. Vol. 62, pp. 73–93.
- Poisbleau, M. et al. 2005. Linear social dominance hierarchy and corticosterone responses in male mallards and pintails. – *Horm. Behav.* 47: 485–492.
- Price, T. 1998. Maternal and paternal effects in birds: effects on offspring fitness. – In: Mousseau, T. A. and Fox, C. W. (eds) *Maternal effects as adaptations*. Oxford Univ. Press, pp. 202–226.
- Roff, D. A. 1992. The evolution of life histories. – Chapman and Hall.
- Schreck, C. B. et al. 1997. Behavioral responses to stress. – In: Iwama, G. K. et al. (eds), *Fish stress and health in aquaculture*. Cambridge Univ. Press, pp. 145–170.
- Schreck, C. B. et al. 2001. Effects of stress on fish reproduction, gamete quality and progeny. – *Aquaculture* 197: 3–24.
- Sibly, R. and Calow, P. 1983. An integrated approach to life-cycle evolution using selective landscapes. – *J. Theor. Biol.* 102: 527–547.
- Sih, A. and Krupa, J. J. 1996. Direct and indirect effects of multiple enemies on water strider mating dynamics. – *Oecologia* 105: 179–188.
- Sih, A. et al. 1985. Predation, competition, and prey communities: a review of field experiments. – *Annu. Rev. Ecol. Syst.* 16: 269–311.
- Sloman, K. A. and Armstrong, J. D. 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena. – *J. Fish Biol.* 61: 1–23.
- Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. – *Am. Nat.* 108: 499–506.
- Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.
- Summers, C. H. et al. 2005. Dynamics and mechanics of social rank reversal. – *J. Comp. Physiol. A* 191: 241–252.
- Takahashi, L. K. et al. 1998. Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies. – *Psychoneuroendocrinology* 23: 571–581.
- Tamate, T. and Maekawa, K. 2000. Interpopulation variation in reproductive traits of female masu salmon, *Oncorhynchus masou*. – *Oikos* 90: 209–218.
- Trippel, E. A. et al. 2005. Maternal and paternal influences on early life history traits and processes of Baltic cod *Gadus morhua*. – *Mar. Ecol. Prog. Ser.* 303: 259–267.
- Verhulst, S. and Salomons, H. M. 2004. Why fight? Socially dominant jackdaws, *Corvus monedula*, have low fitness. – *Anim. Behav.* 68: 777–783.
- Walker, S. P. W. et al. 2007. Rapid larval growth promotes sex change and growth acceleration in a protogynous hermaphrodite, *Paraperis snyderi* Jordan & Starks 1905. – *J. Fish Biol.* 71: 1347–1357.
- Welberg, L. A. M. and Seckl, J. R. 2001. Prenatal stress, glucocorticoids and the programming of the brain. – *J. Neuroendocrinol.* 13: 113–128.
- Werner, E. E. and Peacor, S. D. 2006. Lethal and nonlethal predator effects on an herbivore guild mediated by system productivity. – *Ecology* 87: 347–361.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. – *Annu. Rev. Ecol. Syst.* 25: 443–466.
- Zielinski, W. J. et al. 1992. The effect of intrauterine position on the survival, reproduction and home range size of female house mice (*Mus musculus*). – *Behav. Ecol. Sociobiol.* 30: 185–191.