

Maternal condition influences phenotypic selection on offspring

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Summary

1. Environmentally induced maternal effects are known to affect offspring phenotype, and as a result, the dynamics and evolution of populations across a wide range of taxa.

2. In a field experiment, we manipulated maternal condition by altering food availability, a key factor influencing maternal energy allocation to offspring. We then examined how maternal condition at the time of gametogenesis affects the relationships among early life-history traits and survivorship during early development of the coral reef fish *Pomacentrus amboinensis*.

3. Maternal condition did not affect the number of embryos that hatched or the number of hatchlings surviving to a set time.

4. We found no significant difference in egg size in relation to the maternal physiological state. However, eggs spawned by supplemented mothers were provisioned with greater energy reserves (yolk-sac and oil globule size) than nonsupplemented counterparts, suggesting that provision of energy reserves rather than egg size more closely reflected the maternal environment.

5. Among offspring originating from supplemented mothers, those with larger yolk-sacs were more likely to successfully hatch and survive for longer periods after hatching. However, among offspring from nonsupplemented mothers, yolk-sac size was either inconsequential to survival or offspring with smaller yolk-sac sizes were favoured. Mothers appear to influence the physiological capacity of their progeny and in turn the efficiency of individual offspring to utilize endogenous reserves.

6. In summary, our results show that the maternal environment influences the relationship between offspring characteristics and survival and suggest that energy-driven selective mechanisms may operate to determine progeny viability.

Key-words: bet-hedging, coral reef fish, maternal effects, parent–offspring conflict, selective mortality.

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Introduction

Understanding the factors affecting variation in phenotypic patterns among individuals is of fundamental interest to evolutionary ecologists because this variation influences the dynamics and evolution of populations across a wide range of taxa (Sinervo 1990; Chambers & Leggett 1996; Mousseau & Fox 1998). Any phenotypic variation that arises in offspring as a consequence of the phenotype or environment of the parents, independent of their chro-

mosomal contribution, has been increasingly recognized as the outcome of environmentally induced parental effects (Lacey 1998). Together with the heritable component of the observed phenotypic variation (i.e. additive genetic variance; Wilson *et al.* 2005), these parental effects have been shown to strongly influence offspring development and survival and have effects that last into subsequent generations thereby determining patterns of selection response (Kirkpatrick & Lande 1989) and shaping the rate at which traits evolve (Riska 1989).

In both plants and animals, parents and particularly mothers can influence progeny phenotype in a variety of ways, including: variable provision of nutrients (LaMontagne & McCauley 2001; McCormick 2003); transmission of antibodies (Gasparini *et al.* 2002),

symbionts (Stouthamer *et al.* 1993) and hormones (Adkins-Regan, Ottiger & Park 1995; McCormick 1999; Oksanen, Koskela & Mappes 2002), as well as toxins and pathogens (Taubeneck *et al.* 1994); and through cultural conditioning as a result of parental behaviour (Bernardo 1996; Mazer & Wolfe 1998). Regardless of the way these resources are transmitted, maternal contribution is ultimately limited by the level of resources that are available to the mother for her own needs. As a result, maternal provisioning among offspring is not always equal (e.g. Mappes, Mappes & Lappalainen 1997) or maximized (e.g. Boersma 1997), causing a conflict between parents (mothers) and their offspring over resource allocation (parent–offspring conflict; Trivers 1974).

Mothers have many options in how they partition resources to their offspring. For mothers living in variable environments, past and present environmental conditions may provide an indication of the optimal investment towards reproduction. When conditions vary predictably, mothers may be expected to evolve phenotypic plasticity, whereby mothers respond to the environmental cues they experience and adjust offspring phenotype in a way that enhances offspring fitness (Mousseau & Dingle 1991). For those organisms where the maternal environment is a good predictor of the offspring environment, maternal effects may be considered adaptive (Bernardo 1996; Donohue & Schmitt 1998; Mousseau & Fox 1998) and variability in maternal allocation to their offspring may significantly influence progeny success. This is likely to be a successful strategy for organisms whose progeny does not move far from the mother, or are liberated as juveniles into the maternal environment (e.g. Bull & Baghurst 1998; Beekey, Karlson & Greenberg 2000; Dane 2002; Russell, Brown & Brown 2004). In contrast, in organisms that have a dispersive early life phase, such as wind dispersed seeds or current dispersed larvae, offspring may experience a vastly different environment from the mother, and we may not expect such a strong coupling between the maternal response and offspring environment.

When mothers face an unpredictably heterogeneous environment and cannot foresee the environment in which their progeny will develop, the adaptive value of offspring traits also become variable and unpredictable (Kaplan 1992). Under these circumstances, a trait's response to selection may evolve with a time lag or even in a direction opposite to that favoured by selection (Kirkpatrick & Lande 1989; Cowley & Atchley 1992). Accordingly, mothers may employ a 'bet-hedging' strategy, where an increase in phenotypic variance among offspring will also increase the probability that at least some individuals will survive, regardless of the changing environmental conditions (Philippi & Seger 1989). Despite the substantial theoretical work to predict how maternal investment should evolve under different conditions, the relevance of bet-hedging strategies in natural population remains controversial (Hopper 1999; Einum & Fleming 2004). Although the occurrence of different forms of bet-hedging has been shown to occur

in many animal taxa, it has yet to be demonstrated in coral reef fishes. Given that coral reef fishes have complex life cycles and are characterized by near absolute mortality of early life stages, we would expect bet-hedging to be most advantageous in this vertebrate group.

The quality of individual offspring is expressed in complex interrelationships among a suite of correlated traits. Although quality is defined by a variety of traits, studies of maternal effects on progeny quality have commonly explored the relationship between a single offspring trait, typically propagule size, and a specific aspect of the maternal environment, often food availability (Rossiter 1996). Yet maternal effects are more likely to result from the composite contribution of interacting abiotic, nutritional and other ecological characteristics of the maternal environment, and are most likely to be reflected in a number of offspring traits other than propagule size alone. While there is little doubt that propagule size is correlated with offspring fitness, potentially significant sources of maternal effects may remain undetected or misinterpreted if we assume that offspring fitness increases monotonically with egg size (the optimality propagule size theory; Smith & Fretwell 1974). In fact, maternal influence on propagule size is not able to explain variation in offspring phenotypic patterns and performance as expected from theoretical models, particularly when maternal effects are expressed in correlated traits not considered (cf. Lande & Arnold 1983).

Here, we investigated the role of maternal effects in influencing progeny characteristics from fertilization to the exhaustion of endogenous nutritional reserves in a coral reef damselfish, *Pomacentrus amboinensis*. This is the first study with marine fishes that experimentally manipulated maternal condition in the wild to quantify the consequences of maternally induced variation not only on offspring characteristics but, most importantly, on early survival. *P. amboinensis* provides a good model organism for the study of maternal investment because differences in energy allocation are often manifested in variation in a number of early life-history traits (McCormick 1999). Given that food availability is a major limiting factor influencing the growth and reproduction of individuals (Jones 1986; Kerrigan 1997; McCormick 2003), manipulation of food gave us the opportunity to alter this key resource influencing maternal energy allocation to individual offspring. By examining shifts in associations among early life-history traits and survival of individual offspring that is induced by nutritionally and non-nutritionally mediated maternal effects, this study tested the relevance of bet-hedging strategies and/or the role of phenotypic plasticity in a wild coral reef fish.

Materials and methods

EXPERIMENTAL PROCEDURE

To determine the effect of maternal condition on the quality of offspring and their survival from the egg to

the larval stage, we conducted a field experiment on 10 isolated patch reefs in the lagoon of Lizard Island (14°40'S, 145°28'E) on the Great Barrier Reef, Australia during November 2004. Experimental patch reefs were constructed on sand, 20–40 m off the edge of the main reef in 3–6 m of water. Reefs (c. 0.5 high × 1 × 2 m) were composed of a mixture of rubble and live coral, resembling patch reefs this species uses as a natural part of its habitat, and positioned 15–20 m apart. Ten breeding pairs of *Pomacentrus amboinensis* were captured from the main reef and measured (standard length, [SL]) to the nearest millimetre with callipers. Each pair was then transferred on to an experimental patch reef and randomly assigned to either a 'supplemented' or a 'nonsupplemented' feeding treatment. The diet of five pairs was supplemented with ground pilchards and barramundi pellets (Formula 87510V7 with 50% crude protein, 12% crude fat and 2.5% crude fibres) for 10 min each day (supplemented treatment), while the remaining five pairs fed on naturally available plankton (nonsupplemented treatment). Female body size (SL) for the two patch reef treatments did not differ statistically at the start of the experiment (*t*-test for independent samples, $P = 0.57$). Body size of females from the two patch reef treatments was re-measured at the end of the experiment (45 days later) to quantify the extent to which the manipulation of food altered female condition and potentially influenced maternal energy allocation to her offspring.

We monitored daily the spawning activity of the 10 pairs on the patch reefs (supplemented and nonsupplemented) and five pairs on the main adjacent reef (reef). We allowed an initial 30-day period of treatment acclimation before collecting clutches of newly fertilized eggs laid on artificial nests (plastic half-pipes). We collected one clutch per breeding pair from each of the three treatments (supplemented, nonsupplemented and reef). A total of 15 clutches were photographed with an underwater digital camera to enable the later determination of clutch size (measured as total area, mm²), and then transferred to well-aerated aquaria with flowing seawater (28.3 ± 0.03 °C). Eggs were removed from each clutch using a fresh scalpel and transferred individually to two replicate six-well tissue culture plates ($n = 12$ eggs per clutch) using a fine brush. These plates served to limit possible interaction among incubating embryos. Plates were housed in perforated plastic containers and submerged in seawater as described by Gagliano, McCormick & Meekan (in press). In total, 60 embryos from each of the three maternal conditions (supplemented, nonsupplemented and reef) were allowed to develop in isolation.

EMBRYONIC TRAITS AND SURVIVAL

To define the extent to which maternal condition influenced early life-history traits prior to and at hatching and the survival of offspring, embryos from the three treatments were individually monitored throughout

their development. Embryos were photographed under a compound microscope (at 10 × magnification) at 36 h post-fertilization (hpf) just prior to the formation of main organs and systems (McCormick & Nechaev 2002) and at 84 hpf at completion of embryonic development (2–4 h prior to hatching). Egg size (maximum egg length, mm), yolk-sac size (yolk-sac area, mm²) and oil globule size (oil globule area, mm²) were measured from the calibrated digital images using Optimas 6.5 (Media Cybernetics 1999). Treatments were randomized to minimize the effect of unavoidable delays (maximum 45 min) caused by the time required to photograph each individual embryo. Any pre-hatching mortality and the date at hatching were recorded for each embryo. Following hatching, unfed larvae were inspected every 6 h until death as a measure of post-hatching longevity based only on pre-existing reserves. This condition was chosen to represent the realistic pelagic environment in tropical waters, where food availability is notoriously limited and patchy at both temporal and spatial scales and starvation is implicated as one of the major sources of mortality in fish larvae (Kerrigan 1997).

STATISTICAL ANALYSES

To quantify the extent to which the manipulation of food altered maternal condition (measured as female body size, SL), we used a repeated-measures ANOVA with the beginning and end of experimental manipulation as the within-subject factor and treatment (supplemented and nonsupplemented) as the between-subjects factor. Differences between the supplemented and nonsupplemented feeding treatment were identified using a post-hoc Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05/k$, where k was the number of observation times ($k = 2$).

Least squares regression coefficients between clutch size and the coefficient of variation (CV) of egg size for each maternal treatment were calculated to determine whether there was evidence of bet-hedging. If bet-hedging occurred, the coefficient of variation of egg size was expected to be higher in nonsupplemented females and in larger clutches. Prior to analysis, offspring longevity measures were square root-transformed to meet the assumptions of normality and homogeneity of variance. Initial offspring traits (egg size, yolk-sac and oil globule area measured at 36 hpf), hatching success and post-hatching longevity were analysed with mixed model ANOVAs, where clutch identity (nested within maternal treatments, i.e. supplemented, nonsupplemented, reef) was defined as a random effect and maternal treatment as a fixed effect. The effect of maternal treatments on embryonic traits throughout development was analysed using a repeated-measures ANOVA with developmental time (36 and 84 hpf) as the within-subjects factor and treatments (supplemented, nonsupplemented, reef) as the between-subjects factor. Differences among treatments were identified using a post-hoc Tukey's HSD test at a corrected level of significance ($P < 0.025$).

A logistic regression model was used to determine the phenotypic traits affected by maternal condition that predicted whether embryos would successfully hatch or die before hatching. Given the high variability in the attributes of offspring within individual clutches (44–69% of the total variation), data were analysed without considering which clutch individuals were from allowing to examine how individual phenotypes affected survival. To identify the minimum number of variables that predicted hatching success within each treatment, we used the best subsets model. This involves a likelihood score criterion and the Wald test (z) to evaluate the statistical significance of each of the regression coefficients. We then used a multiple regression analysis to identify the phenotypic traits of *P. amboinensis* embryos that best predicted fitness (i.e. post-hatching longevity). Prior to the multiple regression analysis, the assumption of no collinearity of the independent variables was checked by examining the correlation among traits. By calculating partial regression coefficients of post-hatching longevity on the phenotypic traits of individual embryos, we described the effect of selection pressure on a trait when other traits were held constant. To measure the occurrence and intensity of directional phenotypic selection, we estimated directional selection gradients (β) as described in Lande & Arnold (1983). We used standardized directional selection gradients (β') to allow a direct comparison of strength of selective mortality on phenotypic traits. All statistical analyses were performed using STATISTICA 6.1 (Statsoft Inc. 2001)

Results

EFFECT OF SUPPLEMENTARY FOOD ON MOTHERS

Manipulation of food altered the condition of females from the supplemented patch reefs (treatment \times experimental time interaction, $F_{2,8} = 6.00$, $P < 0.05$). Females from supplemented patch reefs increased by 8% in body size (SL) from the start of food supplementation and were significantly larger than their nonsupplemented counterparts at the termination of the experiment (Tukey's HSD, $P < 0.01$, Fig. 1). In contrast,

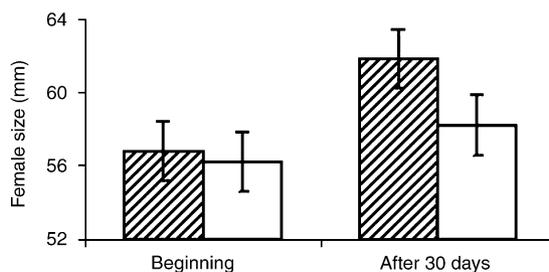


Fig. 1. Body size (SL, mm) of females from supplemented (hatched) and nonsupplemented (white) patch reefs at the beginning of the feeding experiment and after 30 days of food manipulation. Error bars represent SE.

body size (SL) of nonsupplemented females did not change during the course of the experiment (Tukey's HSD, $P = 0.17$).

EFFECT OF MATERNAL CONDITION ON OFFSPRING TRAITS

Maternal condition affected clutch size ($F_{2,12} = 4.57$, $P < 0.05$), resulting in significantly larger clutches produced by females living on the main reef compared with those produced by supplemented females (Tukey's HSD, $P < 0.05$). There was no detectable effect on egg size ($F_{2,155} = 2.44$, $P = 0.13$) and no significant relationship was found between clutch and egg size for any of the treatments (all $r^2 < 0.20$). Egg size differed substantially among clutches within maternal treatments ($F_{12,155} = 12.61$, $P < 0.001$) and size variation (measured as CV) was 1.4–1.7 times greater in clutches from nonsupplemented fish on isolated experimental reefs and those living on the main reef compared with clutches produced by supplemented mothers. However, there was no significant relationship between clutch size and CV of egg size for any of the treatments.

Maternal condition had a clear effect on the yolk sac or oil globule size of the egg ($F_{2,155} = 8.63$, $P < 0.001$ for yolk sac size; $F_{2,155} = 6.04$, $P < 0.05$ for oil globule size). Supplemented mothers produced eggs with 10% more yolk reserve than nonsupplemented mothers on either the patch reefs or the main reef (Tukey's HSD, $P < 0.001$ in both cases). Yolk-sac size also differed among clutches within maternal treatments ($F_{12,155} = 1.947$, $P < 0.05$) and supplemented mothers spawned clutches with 0.8 and 1.9 times greater range of yolk sizes compared with nonsupplemented and main reef mothers, respectively. We also found significant differences in oil globule size among all three treatments, where eggs spawned by supplemented mothers had the largest oil globules and eggs from mothers on the main reef had the smallest (Tukey's HSD, $P < 0.05$; supplemented $>$ nonsupplemented $>$ reef).

OFFSPRING QUALITY AND SURVIVAL VARIATION

Mortality occurred both before and after hatching. Different maternal feeding treatments did not affect the number of embryos that successfully hatched ($F_{2,155} = 0.60$, $P = 0.56$) or the number surviving to a set time ($F_{2,155} = 0.12$, $P = 0.89$). Despite the differences in yolk allocation among treatments, the initial amount of yolk reserve (at 36 hpf) had no direct influence on the hatching success of embryos ($z = 3.12$, $P = 0.08$, Table 1). However, initial yolk-sac size was negatively related to post-hatching longevity for embryos from the nonsupplemented treatment (Table 2), where embryos with smaller yolk-sacs survived longer after hatching. As development advanced, embryos originating from the supplemented treatment still had comparatively larger yolk reserves (treatment \times developmental time

Table 1. Hatching success of embryos based on their phenotype and maternal condition. Results are based on logistic regression model for yolk-sac and oil globule size at 36 and 84 hpf as predictor variables of hatching success ('survived to hatch' or 'died before hatching') of *P. amboinensis* larvae originating from three different maternal environments. Regression coefficients (B), their standard errors (SE) and the Wald test (z) are given with statistical significance ($P < 0.05$ in bold)

	Hatching success			
	B	SE	z	P
Natural main reef				
yolk-sac size at 36 hpf	-5.43	15.63	0.120	0.729
oil globule size at 36 hpf	-11.80	122.99	0.009	0.924
yolk-sac size at 84 hpf	0.72	14.23	0.003	0.960
oil globule size at 84 hpf	-287.56	128.36	5.018	0.025
Unfed experimental patch reef				
yolk-sac size at 36 hpf	-26.04	14.74	3.122	0.077
oil globule size at 36 hpf	143.99	96.27	2.237	0.135
yolk-sac size at 84 hpf	2.16	16.36	0.017	0.895
oil globule size at 84 hpf	-45.99	94.48	0.237	0.626
Fed experimental patch reef				
yolk-sac size at 36 hpf	12.69	11.30	1.263	0.261
oil globule size at 36 hpf	-16.36	12.35	1.753	0.185
yolk-sac size at 84 hpf	286.65	136.62	4.402	0.036
oil globule size at 84 hpf	108.99	98.00	1.237	0.266

Table 2. Post-hatching longevity of unfed larvae based on their phenotype and maternal condition. Results are based on multiple regression analysis with directional selection gradients ($\beta \pm SE$) for yolk-sac and oil globule size at 36 and 84 hpf, using post-hatching longevity of *P. amboinensis* larvae originating from three different maternal environments as a measure of fitness (i.e. survival). Standardized selection gradients (β') are in standard deviation units. R^2 -values are for the multiple regression; $P < 0.05$ in bold

	Post-hatching longevity			
	$\beta \pm SE$	$\beta' \pm SE$	R^2	P
Natural main reef				
yolk-sac size at 36 hpf	-37.34 \pm 85.67	-0.08 \pm 0.18	0.12	0.665
oil globule size at 36 hpf	120.75 \pm 670.13	0.03 \pm 0.17		0.858
yolk-sac size at 84 hpf	24.29 \pm 80.25	0.05 \pm 0.18		0.764
oil globule size at 84 hpf	-1367.53 \pm 643.10	-0.39 \pm 0.18		0.039
Unfed experimental patch reef			0.20	
yolk-sac size at 36 hpf	-155.58 \pm 71.63	-0.30 \pm 0.14		0.035
oil globule size at 36 hpf	-50.19 \pm 474.38	-0.02 \pm 0.15		0.916
yolk-sac size at 84 hpf	-87.13 \pm 83.05	-0.15 \pm 0.14		0.300
oil globule size at 84 hpf	-719.14 \pm 453.50	-0.24 \pm 0.15		0.120
Fed experimental patch reef			0.19	
yolk-sac size at 36 hpf	89.70 \pm 66.02	0.19 \pm 0.15		0.118
oil globule size at 36 hpf	-79.13 \pm 71.77	-0.18 \pm 0.17		0.276
yolk-sac size at 84 hpf	1687.48 \pm 735.54	0.09 \pm 0.15		0.027
oil globule size at 84 hpf	409.20 \pm 569.23	0.13 \pm 0.18		0.475

interaction, $F_{2,147} = 2.14$, $P = 0.12$), even after consuming 6% more yolk than embryos compared with the other two treatments in the intervening 48 h (Fig. 2a). Embryos from the supplemented treatment with large yolk-sacs just before hatching (at 84 hpf) had higher hatching success (Table 1) and survived longer after hatching (Table 2). However, in the nonsupplemented treatments, the amount of yolk reserve available to embryos just before hatching (at 84 hpf) had no relationship with hatching success (Table 1) and did not significantly influence post-hatching longevity (Table 2).

In embryos from the main reef, oil globule size rather than yolk-sac size just before hatching was related to hatching success (Table 1). Main reef embryos initially

had smaller oil globules than those originating from the patch reefs. Although these differences among treatments were maintained as development advanced (treatment \times developmental time interaction, $F_{2,147} = 1.41$, $P = 0.25$), main reef embryos reduced the size of their oil globule by 8% more than embryos from the other two treatments over the 48-h period between measurements (Fig. 2b). The size of the oil globule just prior to hatching (at 84 hpf) was important for both hatching success and post-hatching longevity for embryos originating from the main reef (Tables 1 and 2, Fig. 3). Oil globule size at both 36 and 84 hpf did not, however, show any direct relationship with embryonic success to hatch and larval longevity in the two patch reef treatments (Tables 1 and 2).

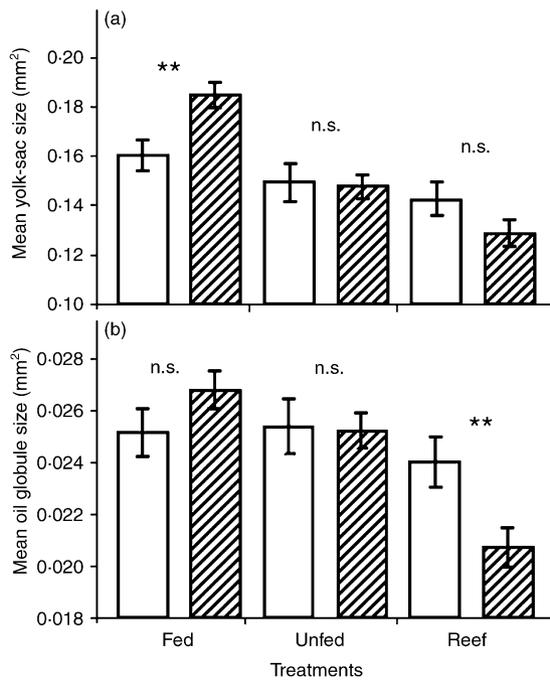


Fig. 2. Mean yolk-sac (a) and oil globule area (b) measured just prior to hatching (84 hpf) for *Pomacentrus amboinensis* embryos originating from supplemented and nonsupplemented mothers on experimental patch reefs and mothers living on a natural adjacent reef. Individuals that did not survive to hatch are represented by the white bars and those that survived to hatch are represented by the hatched bars. Error bars are SE. *n.s.*, no significant difference; **significant difference at $\alpha = 0.025$.

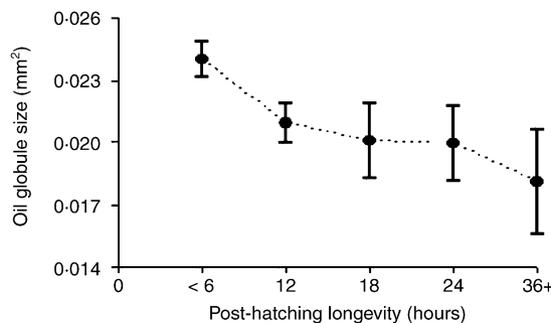


Fig. 3. Relationship between longevity of larval *P. amboinensis* originating from the natural reef and oil globule size (mm) just prior to hatching (at 84 hpf). Error bars represent SE.

Discussion

By manipulating the maternal environment of *P. amboinensis*, we were able to demonstrate that maternal condition at the time of gametogenesis influences traits that predict larval survival. Maternal energy allocation to offspring, measured as yolk-sac and oil globule size, induced shifts in the intensity of selective mortality throughout the early development of this species.

Smith and Fretwell's optimal offspring size theory suggests that maternal reproductive investment is traded-off between egg size and egg number with an increase in one requiring a corresponding decrease in the other.

Although we found evidence that the nutritional condition of mothers did significantly affect clutch size, there was no evidence for a linear trade-off between egg size and clutch size. Surprisingly, maternal condition had no effect on the number of offspring that successfully completed the embryonic phase, nor did it influence the number of individuals that survived to a given time after hatching. Regardless of their physiological condition, *P. amboinensis* mothers produced a wide-variety of egg sizes, suggesting that diversified bet-hedging is likely to be an evolved response to the unpredictability of the offspring environment. Accordingly, the present findings extend the range of vertebrate taxa in which the relevance of bet-hedging strategies has been demonstrated in natural populations.

None the less, maternal nutritional state did significantly affect offspring quality by causing substantial changes in individual egg composition (i.e. yolk sac and oil globule size) and thus, the energetic value of embryos and hatchlings. We found that supplemented females enhanced the quality of their eggs by provisioning them with significantly larger energy reserves. Both yolk-sac and oil globule sizes were larger in eggs from nutritionally supplemented mothers suggesting that the trade-off may be more accurately reflected by the relationship between egg quality and clutch size rather than egg size and clutch size. This finding underscores two important observations that have traditionally been assumed for many different taxa. First, egg size may not always be a good proxy for egg quality, and secondly, egg size may not accurately reflect maternal investment in reproduction.

Provision of energy reserves in the yolk sac and oil globule more closely reflected the differences in the maternal environment and influenced larval survival. Supplementary feeding enhanced body condition of females, which led to the production of offspring with the largest yolk-sacs, a finding consistent with previous studies (e.g. Kerrigan 1997; McCormick 2003). To our knowledge, the present study is the first to demonstrate that increased yolk provisioning is translated into a survival advantage during the embryonic period and after hatching in a tropical species. Given that the yolk reservoir of free and protein bound amino acids is the substrate for energy production and synthesis for tissue growth during the egg stage (Finn *et al.* 1996), it is not surprising that a larger amount of yolk during embryogenesis is associated with higher hatching success. At hatching and thereafter, the main metabolic fuel for species with discrete oil droplets in the yolk (e.g. *P. amboinensis*) is derived from neutral lipids enclosed in the oil globule (Rønnestad *et al.* 1998). However, yolk constituents are essential for sustaining maintenance and tissue growth when the developing fish have just hatched and are learning to feed effectively (Heming & Buddington 1988). Larger amounts of yolk reserves may therefore lead to hatchlings that survive longer before reaching irreversible starvation, as suggested by numerous studies on temperate fish (e.g. Blaxter & Hempel 1966; Chambers, Leggett & Brown 1989).

Depending on the offspring environment, benefits associated with a larger yolk-sac may not always be detectable (Gagliano *et al.*, in press). By examining the relationship between yolk-sac size and survival of *P. amboinensis* eggs developing at three different temperatures, Gagliano *et al.* (in press) showed that yolk-sac size was only important for embryonic success to hatching at the highest temperature and had no ramification on post-hatching longevity of larvae. The link between yolk-sac size and selective larval survival may also only be detectable when maternal investment in individual offspring within a clutch is highly variable. We found that there was high variability in the attributes of larvae within individual clutches, regardless of the food resources available to *P. amboinensis* mothers. However, this differential maternal investment to eggs within a clutch was particularly marked in the supplemented treatment, where strong phenotypic selection on yolk-sac size was detected. Our finding suggests that supplemented mothers may have sufficient resources to be able to allocate the same amount of energy in each egg (which would be in the best interest of individual offspring, thereby reducing within-clutch yolk-size variability), but instead they hedge their bets by provisioning some offspring with more yolk reserves while investing less energy in others, which maximizes maternal investment under unpredictable environmental conditions.

In low food conditions, represented by both the non-supplemented treatment and the main reef, mothers may be limited in the amount of energy available to fuel gametogenesis (Jones & McCormick 2002) and this may result in a reduction of maternal investment in offspring quality (e.g. yolk sac). Although under these circumstances selection for a larger yolk-sac size is expected (Kerrigan 1997), we found that yolk-sac size had no effect on embryonic survival. For post-hatching longevity, yolk-sac size was also either inconsequential (main reef) or offspring with smaller yolk-sac sizes were actually found to be favoured (nonsupplemented treatment). The reasons why a larger yolk sac may be an undesirable trait in offspring originating from the nonsupplemented treatment are unclear. One possible explanation for differences in the selective value of yolk-sac size may be differences in the conversion efficiency of yolk reserves. von Westernhagen (1988) provides examples in marine fish of how metabolic or osmotic disturbance in embryos can prevent the proper use of the energy stored in the yolk. Important embryonic physiological functions such as metabolic activity, development and osmoregulation are governed by maternally derived hormones (Lam 1994). Because offspring rely entirely on maternally derived hormones until the development of their own endocrine system after hatching (Lam 1994; Sampath-Kumar *et al.* 1997), we suggest that the initial level of hormones such as testosterone that nonsupplemented females transferred into the egg yolk may have affected the efficiency of yolk use (McCormick 1999). Given that testosterone levels in breeding females are closely associated with,

and triggered by, behavioural interactions with conspecifics (McCormick 1998), the social isolation of nonsupplemented females on patch reefs may cause a reduction in the natural levels of this hormone (McCormick 1998). Low hormonal levels have been previously reported to inhibit embryonic physiological functions in fish (e.g. sturgeon larvae, Blaxter 1969) and other vertebrates (e.g. birds, Jacobs & Wingfield 2000; and humans, Parker *et al.* 1989). We suggest that eggs from the nonsupplemented treatment may be less efficient than those with a different hormonal balance in transforming yolk into body tissue, thereby retarding growth and reducing survivorship.

Survival benefits associated to the size of the oil globule were observed in offspring from the main reef exclusively, and the direction of the relationship between this trait and offspring hatching success and post-hatching longevity was opposite to that typically predicted. We found that a smaller oil globule just prior to hatching confers higher hatching success (i.e. number of eggs successfully hatched) and greater post-hatching longevity (Table 2; Fig. 2). These results are unexpected, given that the oil globule provides the main substrate in the aerobic energy from hatching and thereafter (Norton, Macfarlane & Mohr 2001), and consequently a positive relationship between oil globule size and post-hatching longevity rather than hatching success would intuitively be predicted. Size of the oil globule was positively correlated with larval survival in capelin *Mallotus villosus* (Chambers *et al.* 1989) and in walleye *Stizostedion vitreum* (Moodie *et al.* 1989). Larvae from walleye eggs with the smallest oil globule were both smaller and slower-growing compared with those larvae hatched from eggs with larger oil globules. Recently, Berkeley, Chapman & Sogard (2004) also showed that oil globule size strongly affects larval growth and survival in black rockfish *Sebastes melanops*, suggesting that enhanced growth rates of larvae with larger oil globules at hatching provide clear benefits in allowing larvae to quickly grow through a window of vulnerability from predation and other environmental challenges. Although it is generally accepted that fast growth can provide many survival advantages (Stearns 1992), these benefits vary and are not always detectable (cf. Meekan & Fortier 1996; Metcalfe & Monaghan 2003) because fast growth clearly comes at an energetic (metabolic) cost (Rombough 1994). Given that energy expenditure associated with physiological rates, such as growth, is closely tied to metabolism (Savage *et al.* 2004), we suggest that slow-growing larvae with low metabolic rates may be favoured by energy-driven selective mechanisms in stressed (e.g. food limited) environments. The rate at which energy stored in the oil globule are used up could explain how embryos with smaller oil globules, and presumably slower growth rates (Moodie *et al.* 1989; Berkeley *et al.* 2004) survived for a significantly longer time after hatching. Obviously further work is required to clarify this interesting result, but the result does highlight that a large oil globule does not always purvey a survival advantage.

In the present study, we showed that offspring viability arising from maternal investment in progeny quality is not always reflected in offspring size. Instead, it may be directly affected by the quantity, and probably the quality, of endogenous reserves available to individual propagules. We did not examine the susceptibility of individual offspring to other directional selection agents such as predation. Consequently, we cannot establish how the relationship between early life-history traits such as egg and larval size, and behavioural processes such as predator avoidance may interact to influence differential survival among offspring (but see Fuiman *et al.* 2005), and subsequently how maternal investment may be related to performance of their offspring (but see Mappes *et al.* 1997). This study emphasizes the complexity of selective processes during the embryonic and larval phase and highlights the limitations in our current understanding of larval development and energetics.

In conclusion, our findings suggest that maternal condition influences offspring physiological capacity and in turn their efficiency to utilize endogenous reserves. This suggests that energy-driven mechanisms may operate to determine offspring viability, particularly in stressful maternal environments (i.e. low food levels and high breeding density). Although there is little doubt that an offspring's phenotype integrates information derived from the maternal environment, offspring themselves are directly affected by local environmental conditions, which can shift the optimal value of a trait or a combination of traits (Gagliano *et al.*, in press). The ramifications of maternal effects on offspring performance in relation to the offspring environment clearly merit further investigation.

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