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BEHAVIORALLY INDUCED MATERNAL STRESS IN A FISH INFLUENCES PROGENY QUALITY BY A HORMONAL MECHANISM

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Abstract. The survival and quality of progeny can be strongly influenced by nongenetic effects derived from the physiological condition of the mother during gametogenesis. The influence of maternal condition on the size and quality of larvae at dispersal was examined for the tropical damselfish, *Pomacentrus amboinensis*, through a series of field studies during 1994. In this species, males guard a demersal nest of eggs contributed to by nearby females. The largest and most dominant female stays near the nest and contributes most to the egg clutches, limiting egg contributions from subordinate females. Maternal effects dramatically influenced the size of larval progeny at hatching, four days after laying. Much of the variability in progeny size was explained by levels of the stress-associated steroid hormone, cortisol, in the female. A field experiment manipulating maternal levels of cortisol found that cortisol levels strongly influenced the morphology and yolk size of larval progeny at hatching. Variation in the density of egg predators and competitors together explained 38% of the observed variance in maternal cortisol levels. These competitors and predatory fish appear to elevate maternal cortisol levels and consequently influence larval morphology through a stress-related response. This study suggests that the behavioral interaction regime of a fish population can determine larval quality and potentially govern a female's contribution to the next generation.

Key words: coral reef fishes; cortisol; hormonal mechanism; maternal effects; Pomacentrus amboinensis; *progeny quality; social stress; stress, behaviorally induced; variable progeny development.*

INTRODUCTION

Density-dependent processes, such as competition, can act to emphasize phenotypic differences among individuals in the breeding population (Jones 1987, Massot et al. 1992, Booth 1995, Ostfeld and Canham 1995) and determine reproductive output (Jones 1984, Booth 1995, Ostfeld and Canham 1995, Kerrigan 1996). Differences in the reproductive condition of females can influence phenotypic patterns across generations and dynamics of a population in both ecological and evolutionary time for a broad range of taxa (Sinervo 1990, Mousseau and Dingle 1991, Rossitter 1994, Bernardo 1996*a*, *b*, Chambers and Leggett 1996, Reznick et al. 1996; Kerrigan, *in press*). Unfortunately, we know little about the mechanisms by which behavioral interactions influence maternal reproductive fitness, and whether there are downstream effects to offspring performance.

Nongenetic maternal contributions to an offspring's phenotype can be manifested in a diversity of ways and have lasting effects. These maternal effects often influence offspring survival by the differential provisioning of propagules (Roach and Wulff 1987, Levitan 1993, Kerrigan 1996), which alone suggests their potentially significant impact on population processes. Moreover, small changes in early development can permanently and dramatically alter developmental trajectories (Bernardo 1996*a*). Evolutionary models that incorporate traits influenced by maternal effects have found that these traits can act in an unpredictable manner. Specifically, traits influenced by maternal effects may evolve in a direction opposite to that favored by selection and will cause time lags in a population's response to selection on those traits (Kirkpatrick and Lande 1989, Cowley and Atchley 1992). Such studies emphasize that determining the consequences of variability in maternal investment, and the mechanisms responsible for producing and maintaining such variation, are central to understanding the evolution of life history strategies.

The physiological condition of the mother at the time of gametogenesis determines the provisioning of a propagule. There is a relationship for many taxa between maternal characteristics (e.g., size or condition) and the propagule size and number, although this can be complicated by environmental variability (reviewed by Bernardo 1996*b*). Recent research on higher vertebrates has revealed that direct maternal effects go well beyond simply nutritional contributions but also involve maternal manipulation of the endocrinology of the embryo that may lead to enhanced growth and survival in later life stages, and even competitive success in adult life (Schwabl 1993, Stamps 1994, Migliaccio et al. 1996). For instance, the reproductive behavior of rodents is greatly affected by the levels of exposure to androgens that they experienced prenatally (Clark and Galef 1995).

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In mammals and teleost fishes the maternal endocrine system and that of the offspring can be closely aligned (Schreck et al. 1991, Hwang et al. 1992). Events that affect the hormonal regime of the breeding females may have downstream effects on subsequent offspring. Studies of salmonid fishes have shown that embryos cannot produce the required developmental hormones until well after hatching and that the necessary hormones are aliquoted into the nutritive yolk sac during oogenesis in quantities that reflect female plasma levels (Schreck et al. 1991, Hwang et al. 1992, Mylonas et al. 1994). Laboratory experiments on domesticated salmonids have shown that chronic stress elevates maternal levels of the corticosteroid, cortisol, and results in lower survival of offspring from stressed females (Campbell et al. 1992, 1994). Although elevated cortisol levels are an almost universal response to stress in vertebrates (Pickering 1993, Saltzman et al. 1994, Astheimer et al. 1995, Coddington and Cree 1995), the details of the secondary effects of stress, elevated cortisol levels and the interaction of cortisol with developmental hormones are poorly understood for most taxa and appear to be species specific. Most of the studies of stress in fishes have been on salmonids that are often reared and domesticated, then exposed to chronic levels of stress for unnaturally long periods of time. Stress levels are seldom related to those of fishes in wild populations. How the responses to chronic stress relate to the events that occur in natural populations is largely unknown.

In this study, I examine for the first time the role of maternal effects in influencing larval phenotype in a tropical fish population and identify a mechanism by which the mother may influence progeny characteristics. Specifically, I examine the hypothesis that intra- and interspecific interactions influence the investment by breeding females in their progeny. Tropical demersal fishes are a good model organism for examining the effects of maternal investment. These fish are similar to many other ectothermal vertebrates, in that differences in levels of maternal investment are often evident as differences in the amount of yolk invested by a female during vitellogenesis (Chambers and Leggett 1996; B. A. Kerrigan and M. I. McCormick, *unpublished manuscript*). This variability in provisioning can lead directly to variation in ovum size and hatchling size. For example, laboratory studies showed that maternal effects accounted for 71% of the variability in egg size in the capelin, *Malotus villosus* (Chambers and Leggett 1996). The high mortality inherent in early life stages means that these small differences in larval characteristics can have a major impact on the number of progeny surviving to maturity (Houde 1987, Leggett and Deblois 1994).

Coral reef fish are of particular interest since they are characterized by high species diversity and high population densities (Sale 1980, 1991). Interactions with conspecific and interspecific competitors, especially during the breeding season, can account for substantial proportions of daily activity budgets and energy expenditure (e.g., Thompson and Jones 1983; M. I. McCormick, *unpublished data*). As with many social animals in high-density populations, the number and intensity of interactions, and an individual's response to them, influences how much energy can be directed to reproduction and the physiological status of the breeding individuals (Knapp and Kovach 1991, Pankhurst and Barnett 1993, Sikkel 1993). The exposure of fish to stressors, such as the competition inherent in high density populations, leads to an endocrinological response that may be passed to the next generation during reproduction (Campbell et al. 1994).

In the present study I demonstrate the importance of maternal effects in influencing offspring phenotype by manipulating the maternal environment and looking for a nongenetic basis for the response in the offspring. The fish chosen for the study, the ambon damselfish *Pomacentrus amboinensis,* is typical of most damselfishes being a protogynous hermaphrodite with males guarding demersal nests during a summer breeding season. Associated with each nesting male are between one and six females in various states of reproductive condition. The largest and most dominant female (here known as the ''primary female'') contributes most of the eggs in the nest and seldom strays >1 m from the nest site (M. I. McCormick, *unpublished data*). Her proximity to the nest, and her investment in the progeny suggests that she plays an active role in determining access to the nest site. During the breeding season, nest sites become centers of activity, with a myriad of egg predators waiting for the opportunity for a high energy meal and conspecific females competing with the primary female to breed in the nest site. I hypothesize that maternal levels of the stress hormone, cortisol, may govern the quality of her larval offspring. To address this hypothesis, I examine levels of the hormone in breeding female damselfish exposed to a range of densities of competing con- and contra-specifics and egg predators on a coral reef. I also examine whether the highly variable levels of cortisol found in female ovaries are transferred to the larval yolk. Cortisol levels in eggs produced by these females are then related to features of the larvae which are important in determining survival in the early life stages (larval size, yolk sac size, and eye development). A field experiment is conducted that manipulates maternal levels of the stress hormone to determine whether it directly influences offspring phenotype. Lastly, I investigate what factors may be driving the variable levels of the stress hormone seen in the field population of breeding females. This study demonstrates, for the first time, that developmental characteristics of newly hatched larvae are influenced by parental interactions prior to spawning. Field experiments reveal that a hormonal mechanism is involved that is partly triggered by the behavioral interaction regime of the breeding females. These

findings illustrate the potential importance of maternal effects in influencing the variability of phenotypes in the reef population.

METHODS

Natural variability in stress hormone levels of eggs and larval quality

During October to December 1994 I monitored the spawning output of reproductive pairs of the damselfish *Pomacentrus amboinensis* around the Lizard Island fringing reef on the northern Great Barrier Reef, Australia (14°41′ S, 145°27′ E). At each of five locations (1–7 km apart) I replaced the nests of 10 breeding males (2–10 m apart) with an artificial nesting surface, which consisted of an upturned terracotta roof tile. These tiles presented a uniform concave nesting surface that were of similar dimension and defensibility as natural nests (upturned clam shells).

I collected part of each egg clutch spawned on these tiles in situ within 4 h of spawning (at dawn; Meekan 1992) and preserved them in liquid nitrogen for later hormonal analysis. Egg clutches spawned on the tiles subsequent to sampling could be readily identified from the sampled clutches by their stage of development (as indicated by pigmentation) and location on the tile. Egg clutches that had been partly sampled just after spawning were carefully brought into the laboratory at the end of a 4-d incubation period (at 28°C field and hatching-tank temperature), a few hours prior to hatching. These were placed in well aerated aquaria with a strong unidirectional water flow and larvae hatched within 30 min after sunset. There was no variation in the time to hatching over the course of the study. Newly hatched larvae were preserved in 2.5% glutaraldehyde buffered in seawater for 2 h at room temperature, rinsed with seawater and stored in seawater at 4°C until measurement. Larvae were measured using an image analysis system linked to a binocular microscope. Standard length (SL), head depth (through the eye), eye diameter (maximum), and yolk sac area of 25–50 larvae from each clutch were measured. Yolk sac area provided a measure of yolk reserves available for subsequent development.

Primary females associated with the monitored nests were collected at the end of the monitoring period (December 1994) to: examine the natural levels of variability in cortisol among primary females for comparison to experimentally manipulated levels; to determine the relationship between levels of cortisol in the ovaries and newly laid eggs; and to determine whether there was a relationship between cortisol levels and the densities of interacting fish species. To boost sample size I also measured ovarian cortisol levels of 100 primary females collected during the 1993 reproductive season. For each of the primary females I recorded standard length, mass, and ovary mass, and ovaries were stored in liquid nitrogen prior to hormonal assays. Egg clutches spawned no more than 5 d prior to the collection of monitored females were used to examine the relationships between female and egg cortisol. This reduced the confounding influence of temporal fluctuations in female ovarian cortisol levels.

Hormonal stress mechanism

Endocrine systems are a complex of interacting hormones, wherein altering the levels of one often flows to alterations in the levels of other hormones or metabolites. This is particularly the case with cortisol, where elevation of cortisol in response to stress is coincident with elevations in lactate, glucose, and reductions in reproductive hormones (i.e., steroids such as testosterone) in the blood plasma (Campbell et al. 1994, Carragher and Rees 1994). To determine whether the experimental supplementation of cortisol was affecting changes in the steroids that govern reproductive behavior and fecundity, the sex steroid testosterone was measured in experimental females. Testosterone and estradiol, which are both present in ovaries, react to stress in the same way, with plasma testosterone levels often being more sensitive than estradiol (Foo and Lam 1993, Campbell et al. 1994).

Female *Pomacentrus amboinensis* were too small (6 to 9 g) to enable the humane extraction of blood, so hormonal assays were undertaken on samples of ovary tissue. I determined cortisol and testosterone concentrations using standard radioimmunoassay (RIA) techniques (a modified version of the protocol of Pankhurst and Carragher [1992]). Ovary tissue (~0.1 g wet mass) was freeze-dried and homogenized in 200 μL 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 μL aliquots were evaporated in assay tubes. Extraction efficiencies, determined by the recovery of ^{3}H-steroid added to ovary homogenate and incubated for 18 h, were 87.6% (cortisol) and 96% (testosterone). Cortisol was assayed using (1,2,6,7-^{3}H) cortisol (Amersham, UK). The antibody used was an antiserum raised in rabbits against a cortisol-3-(0-carboxymethyl) oxime-bovine serum albumin conjugate (BioClinical Services, Cardiff). This cortisol antiserum had the following cross reactivities: 11-deoxycortisol 25%, cortisone 8.5%, corticosterone 4.5%, 17α-hydroxyprogesterone, deoxycorticosterone 1.4%, and progesterone 0.06%. Interassay variability (CV) for cortisol was 11.2%, $n = 3$. Testosterone was assayed using (1,2,6,7-^{3}H) testosterone (Amersham, UK). The testosterone antiserum was raised in rabbits against a testosterone-3(-0-carboxymethyl) oxime-bovine serum albumin conjugate (BioClinical Services, Cardiff). This testosterone antiserum had the following cross reactivities: 5α-dihydrostestosterone 16%; 5α-androstane-3α, 17β-diol 5.8%; 5α-androstane-3β, 17β-diol 3.7%; androstenedione 2.1%; and cortisol <0.01%. Since ovary and egg samples were not purified using column chromatog-

raphy prior to RIA it is possible that the testosterone levels measured may include small contributions from other androgens that also cross-react with the assay antibody. Testosterone levels were determined in a single assay. For the egg samples, a known number (200–500 eggs per clutch) were homogenized and assayed as above. Cortisol levels in eggs were measured in a single assay, with an extraction efficiency of 84.1%.

Experimental test of stress hormone impact on progeny quality

To test whether maternal cortisol levels directly influenced larval morphometrics, I ran a 6-wk field experiment. Breeding pairs of *Pomacentrus amboinensis* were transplanted to 18 isolated 2 × 2 m coral patch reefs (one pair per reef) on a sandflat 30 m from the edge of the backreef and 40 m apart. I cleared all patch reefs of fish prior to the transplantation of breeding pairs so that reefs did not harbor inter- or intraspecific competitors that may have confounded results. Patch reefs are part of the natural habitat range of this species. Females were all of a similar size. After a 2-wk acclimation period all translocated fish had started a regular 2- to 4-d spawning cycle. I elevated cortisol levels in 10 females by abdominal injection at two different concentrations (50 and 25 μg/g body mass). Cortisol was dissolved in cocoa butter to make a viscous mixture to extend and moderate the release of cortisol into the blood system (Pankhurst et al. 1986, Foo and Lam 1993). Using this method, cortisol is elevated for up to 10 d after injection (M. I. McCormick, *unpublished data*). I injected females on the morning that they had spawned a clutch of eggs so that the treatments would potentially influence the development of the next batch of eggs spawned 3 to 5 d postinjection. Two controls were run concurrently with the experimental manipulations: (1) an ''injection control'' in which transplanted females were only injected with the vehicle for the hormone (cocoa butter). This treatment controlled for the combined effects of the disturbance associated with injection and the addition of cocoa butter and (2) a ''transplantation control'' consisting of pairs of fish that had been transplanted to patch reefs but were otherwise unmanipulated. Capture and injection was done underwater to minimize handling effects. I allocated between four and five randomly chosen breeding pairs to each of the four treatments. I monitored spawning daily and eggs were brought into the laboratory and hatched for larval collections in the same way as for the monitored breeding pairs. Egg and larval samples were collected from all pairs within 5 d postinjection. To determine the extent to which the experimental treatments had influenced ovarian cortisol levels, I reinjected each of the experimental females the same cortisol treatment or the injection control 12 d after the successful sampling of eggs. Females were then collected 3 d after injection, killed by cold shock, and their ovaries were assayed. Fish were not collected immediately upon their first postinjection spawning since the number of days after injection to spawning varied among individual females.

Influence of the behavioral interaction regime on female stress levels

To quantify the potential of an individual primary female to have stress-causing interactions, which may elevate cortisol levels (Pickering 1993), I made visual counts of all interacting species of fish within a 5-m radius of the monitored nest sites. These counts were made during the egg collection period (described in *Methods*: *Natural variability*) and prior to collection of the primary females. Conspecifics were placed into one of four categories identifiable by size, coloration, and behavior: juveniles; primary females; secondary females, not closely associated with a nesting male; and males. The relationship between cortisol concentrations in the ovary of the primary females and the densities of species and species groups were then examined using linear regression procedures. Subsets of the independent variables entering the model that best predicted the level of female cortisol were determined using stepwise, forward, and backward elimination procedures (SAS 1987).

Statistics

I tested for differences in mean cortisol concentrations in ovaries of females collected from five locations and among the four experimental treatments using separate analyses of variance (GLM; SAS 1987). Type III sums of squares were used due to unequal sample sizes. Assumptions of homogeneity of variance and normality were examined by residual analysis, and data were transformed if required. Tukey's (HSD) tests were used to compare means found different using analysis of variance. Multivariate analysis of variance (MANOVA; Tabachnick and Fidell 1989) was used to test the hypothesis of no difference in larval morphology at hatching among the two cortisol supplementation treatments and two control treatments. Canonical discriminant analysis (CDA) was used to identify and display the nature of the significant differences among treatments found by MANOVA. CDA identifies a number of trends in the data set (canonical variates) that maximally discriminate among the identified groups (in this case, treatments) and sequentially explain less of the variance in the data set. Trends in the original variables (SL, head depth, yolk, etc.) are represented as vectors given by correlations of these variables with the canonical variates (also known as total structure coefficients). These vectors are plotted on the first two canonical axes, together with treatment centroids and their 95% confidence clouds (Seber 1984). The strength or importance of each of the original variables in discriminating among groups is displayed graphically as the length of these vectors. The assumption of multivariate normality was validated prior to analysis.

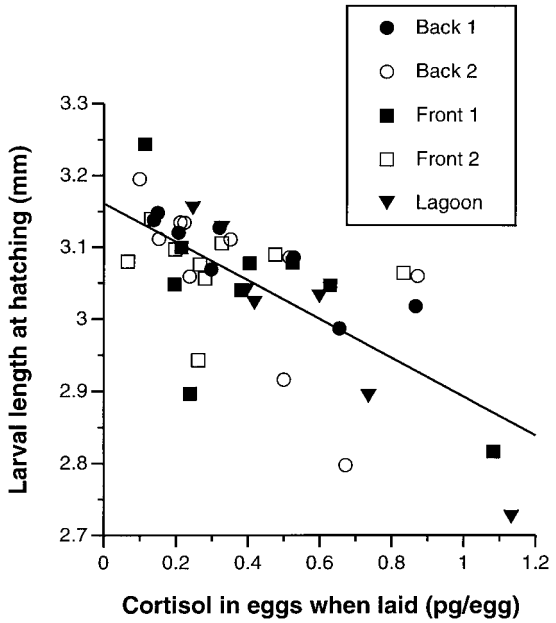

FIG. 1. The negative relationship between levels of cortisol in the eggs of *P. amboinensis* (pg/eggs) within 4 h of spawning and the mean standard length of the larvae at hatching from the same clutch. Equation: Length = 3.163 − 0.272(cortisol), $P > 0.001$, $r^2 = 0.479$.

RESULTS

Natural variability in stress hormone levels of eggs and larval quality

Levels of cortisol varied markedly among females, ranging from 0.3 to 76.0 ng/g ovary mass ($n = 150$). When eggs laid by the monitored females were assayed, levels of cortisol in the eggs strongly correlated with levels in the maternal ovaries (Pearson's $r = 0.538$, $n = 30$, $P < 0.01$), suggesting a strong connection between maternal ovary and embryo cortisol levels. Moreover, this correlation is likely to underestimate the relationship for two reasons. Firstly, females were collected at the end of the monitoring period while egg samples used in the analysis were collected up to 5 d prior to female collection. Secondly, females other than the primary female may have contributed egg clutches to a nest.

Levels of cortisol in the eggs just after laying were strongly correlated with the length of larvae at hatching (Fig. 1) and explained 48% of the variability in the larval length ($P < 0.001$). High levels of the hormone in the eggs resulted in short larvae. Data did not allow rigorous statistical comparison; however, the variability in both larval length and egg cortisol was evenly spread over all locations sampled (Fig. 1). Neither egg nor ovarian cortisol levels were significantly correlated with any other aspect of larval morphology measured.

Experimental test of stress hormone impact on progeny quality

There was high variability in cortisol concentrations in the ovaries of females within locations, to the extent that there were no differences among the five monitored locations in mean cortisol content ($F_{4,42} = 1.38$, $P = 0.256$, Fig. 2). Injection of cortisol elevated ovarian cortisol in the females breeding on patch reefs to the highest mean levels occurring in local populations around Lizard Island (Fig. 2). The mean cortisol levels in the breeding females injected with the 50 μg/g body mass cortisol were significantly higher than either the transplantation-control or injection-control females, with the latter not differing from one another ($F_{3,17} = 7.26$, $P = 0.004$; Fig. 2 shows means comparisons). Thus the injection treatments succeeded in elevating ovarian cortisol in the experimental breeding females.

The experimental treatments had no effect on the levels of testosterone in the ovaries (Fig. 3). Testosterone levels were low in the females from all experimental treatments and similar to those monitored in back reef and lagoonal sites. Females from the reef front sites had more than twice the levels of testosterone than females from other sampled localities. This suggests that the experimental manipulation of cortisol levels did not also indirectly alter ovarian testosterone levels, which otherwise could have influenced larval morphology.

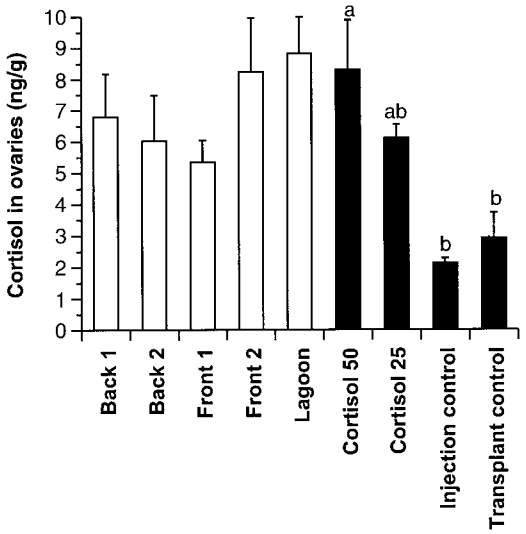

FIG. 2. Mean (+1 SE) ovarian cortisol levels (ng/g ovary mass) of females from the four experimental treatments and primary females from monitored nests at five locations around the Lizard Island fringing reef (2 back-reef, 2 reef-front, and a lagoonal location). Experimental treatments were: cortisol injected with a cocoa butter vehicle at two concentrations (25 and 50 μg/g body mass) and two controls (transplantation and injection). Data show that cortisol was elevated by injection to the upper levels naturally occurring in local populations of *P. amboinensis.* Means of experimental treatments that are statistically different by Tukey's (HSD) tests have different superscripts.

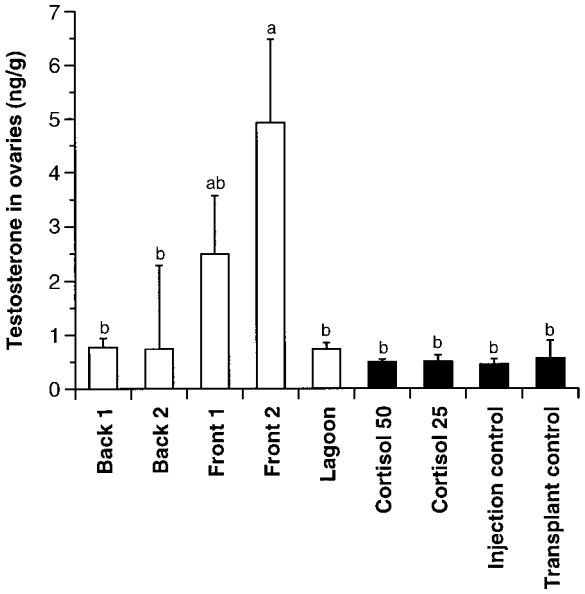

FIG. 3. Mean (+1 SE) ovarian testosterone levels (ng/g ovary mass) of females from the four experimental treatments and primary females from monitored nests at five locations around the Lizard Island fringing reef. Analysis of variance showed a significant difference among localities in ovarian testosterone (log transformed, $F_{8,56} = 5.43$, $P > 0.0001$), and Tukey's (HSD) groupings are displayed as superscripts.

Manipulation of maternal cortisol directly influenced the morphology of the larvae those females produced (Fig. 4). A multivariate analysis of variance found that larvae at hatching produced by females from the four treatments significantly differed in their morphometrics (Pillai's Trace = 0.666, $P < 0.0001$). Control females had the lowest levels of cortisol and produced larvae that were significantly longer, had larger eyes and larger yolk sacs than females from any of the other three treatments. Females from the highest cortisol treatment (50 μg/g body mass) produced larvae that were shortest at hatching and had the smallest yolk sacs. The lower cortisol treatment and injection control spanned the range between these two extremes. The significant difference between the larvae produced by the injection-control and transplantation-control females suggests that injection also influenced larval morphometrics. This field experiment, therefore, concurs with the negative relationship between egg cortisol content and larval length described earlier for natural populations (Fig. 1).

Influence of the behavioral interaction regime on female stress levels

The density of the most abundant egg predator, the wrasse *Thalassoma lunare,* together with other known egg predators, accounted for >23% ($P < 0.01$) of the variability in female cortisol levels (Table 1). These egg predators consisted of five other wrasses and damselfishes which prey regularly on eggs of *P. amboinensis* (M. Emslie and G. Jones, *unpublished manuscript*). Furthermore, densities of the highly territorial damselfish, *Dischistodus perspicillatus,* other female *P. amboinensis* (competitors for the nest site), other demersal damselfishes and juvenile *P. amboinensis* (competitors for resources), together with male *P. amboinensis* (competitive dominant in the system) accounted for an additional 14.5% ($P < 0.05$) of the variation in female cortisol levels.

DISCUSSION

Stress is a ubiquitous feature of vertebrate life and reproduction is particularly sensitive to its disruptive effects. Chronic stress suppresses reproductive hormones in most vertebrates including mammals (Carlstead et al. 1993, Berga 1995, Von Borell 1995), amphibians (Rose et al. 1995), reptiles (Elsey et al. 1990, Wilson and Wingfield 1994, Summers et al. 1995), birds (Astheimer et al. 1995), and fishes (Pickering

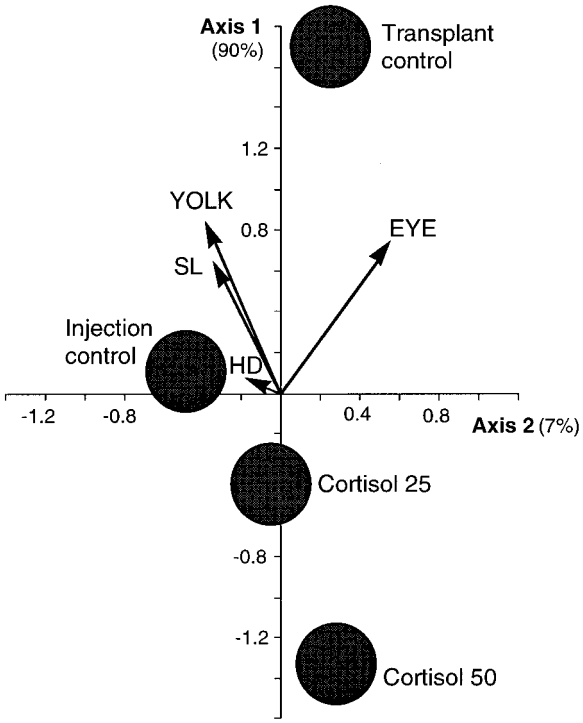

FIG. 4. Comparison of the morphology of larval *Pomacentrus amboinensis* at hatching among four experiments; two of which modified the levels of female cortisol by injection (Cortisol at 25 and 50 μg/g body mass), and two of which represent controls for manipulation (injection and transplantation controls). A canonical discriminant analysis (a type of multivariate means comparison; CDA) displays the difference in larval morphology among the four treatments. Treatment centroids and 95% confidence clouds are plotted together with the direction and importance (as indicated by vector length) of trends in measured larval attributes: standard length (SL), eye diameter (EYE), yolk sac area (YOLK), and maximum head depth (HD). The position of the injection control suggests that the disturbance associated with injection has elevated stress levels without the supplementation of cortisol.

TABLE 1. Percentage of variance in ovarian cortisol levels in primary female *P. amboinensis* explained by the densities of various fish species within a 5-m radius of the nest site.

Species	Cumulative percentage prediction of cortisol levels (r^2)	Relationship to primary female
Other egg predators	11.9	Egg predator
Thalassoma lunare	23.2	Egg predator
Secondary female *P. amboinensis*	27.2	Competitor for nest
Dischistodus perspicillatus	31.0	Competitor (space/food)
Male *P. amboinensis*	33.4	Competitive dominant
Other damselfishes	35.7	Competitor (space/food)
Juvenile *P. amboinensis*	37.7	Competitor (space/food)

Note: Results are from a stepwise linear regression that gives a model that best describes the variability in female cortisol levels.

1993, Pankhurst and Van Der Kraak 1997). The ultimate impact of stress on the reproductive system in terms of the number and quality of surviving offspring has seldom been explored (Campbell et al. 1994 for exception). This is particularly true for the effect of low-level, sub-chronic stress typically found in natural systems. The present study suggests that low-level maternal stress derived from interactions with other fishes and mediated through maternal physiology, influenced the morphology and quality of offspring at dispersal. The number of egg predators in the vicinity of the nest, together with the density of fishes competing for the nest site appear to elevate levels of the stress-associated hormone, cortisol, in the females. This leads to the changes in the size of the larvae at hatching and the size of the nutritive yolk sac that provides the energy for early development.

Maternal stress and the quality of progeny

There are few comparable studies which have explored the link between an organism's behavioral interaction regime, maternal stress, and progeny quality. However, living in high density populations (i.e., crowding) has deleterious effects on the growth and reproductive function of females of a range of animals (e.g., birds: Craig and Craig 1985; monkeys: Saltzman et al. 1994; reptiles: Elsey et al. 1990, Summers et al. 1995). For instance, ovarian recrudescence is slower in captive and crowded lizards, and the presence of a dominant individual inhibits reproductive activity in subordinate males (Summers et al. 1995). These effects may be due to reduced food intake due to competition and decreased efficiency of food utilization (Fagerlund et al. 1981). Campbell et al. (1992, 1994) simulated crowding stress in rainbow trout and found that stressed females produced eggs that were smaller and had lower survival to hatching than progeny from unstressed females, supporting the results of the present study. A small number of studies have examined the effect of stress caused by pollutants on oogenesis and offspring quality. In fish, the experimentally induced stressor has been sublethally low pH levels, which simulates the effects of acid rain. This type of stress appears to have a similar physiological action to crowding stress, elevating cortisol levels and reducing growth and fecundity (e.g., Tam et al. 1990). In a similar way to crowding stress, Weiner et al. (1986) showed that progeny of acid-exposed female trout had reduced survival, indicating that embryonic development was adversely affected by stress.

Many of these studies are confounded by differences in the nutritional intake of stressed vs. less stressed groups. Stress, especially in crowded conditions, is partly brought on and exacerbated by lower levels of nutrition. In the present study there was a poor relationship for the monitored fishes between the mass or size of the females and the mean size of the larvae she produced ($r^2 = 0.111$, $P = 0.04$; $r^2 = 0.046$, $P = 0.19$ respectively) or ovarian cortisol levels exhibited ($r^2 = 0.025$, $P = 0.29$; $r^2 = 0.028$, $P = 0.26$ respectively), suggesting that food availability is less likely to be a confounding factor in this field study. Adult *P. amboinensis* principally eat benthic algae and zooplankton (M. I. McCormick, *personal observation*), and although there may be more zooplankton impinging on the windward side of the reef, there is no evidence to suggest that either food supply is limiting to the point of causing stress.

Direct or indirect effects of stress on progeny quality?

Evidence presented is strongly suggestive of a link between maternal cortisol and larval development. Cortisol levels in the eggs are strongly determined by levels of cortisol in the females, and the cortisol supplementation experiment suggests that cortisol plays a role in determining larval morphology. It is unknown, however, whether cortisol is directly influencing larval morphology through cortisol in the egg influencing subsequent development, or whether maternal cortisol is indirectly influencing larval morphology through a secondary effect of high cortisol on the females reproductive system (e.g., Foo and Lam 1993). Elevations in cortisol levels after chronic stress often coincide with

a slow decline in reproductive hormones, such as testosterone and estradiol. These hormones control oocyte development and the production and allocation of the protein, vitellogenin, to the egg. The effect of stress on the allocation of nutrients to the egg during oogenesis may influence the offspring, rather than the direct effect of cortisol (Pankhurst et al. 1995). A recent study has shown that despite the coincidence of changes in sex steroids and cortisol during stress, elevated cortisol levels do not directly influence ovarian steroid production in fishes (Pankhurst et al. 1995). In the present study, testosterone levels in the female ovaries were not influenced by the cortisol treatments, suggesting that the effect of enhanced female cortisol on the offspring phenotype may not be through the action of sex steroids on oogenesis.

An alternative, supported by this study, is that the elevated levels of cortisol in the egg directly influence the development of the offspring. In flatfishes and salmonids cortisol interacts with the thyroid hormones that govern development, directly influencing developmental schedules of larvae (De Jesus et al. 1990). Similar hormonal interactions influence tadpole development (Galton 1990). Conclusive demonstration of a direct effect of cortisol on larval development requires an experimental manipulation of cortisol environment of the eggs after ovulation and fertilization so that other maternal factors (e.g., vitellogenin production) can be controlled for.

Benefits of producing large larvae

Despite some uncertainty of the details of the underlying mechanism, this study suggests that the behavioral interaction regime of a female pomacentrid influences the morphology of her larvae. There is little doubt that the production of large larvae with large yolk sacs is beneficial to the survival of offspring (Leggett and Deblois 1994), although this can be a complex function of the predator field into which the larvae enter (Paradis et al. 1996). Large fish larvae have a locomotory advantage that enhances their ability to escape predators and capture prey (Blaxter 1986, Bailey and Houde 1989). Large yolk reserves and oil globules confer early growth advantages and enhance the ability to survive periods of starvation after hatching (Rothschild 1986, Chambers et al. 1989). This variability in the quality of individual larvae is of critical importance, because small changes in the mortality schedules of larvae can have major effects on recruitment rates (Houde 1987, Underwood and Fairweather 1989). Variability in larval quality at hatching therefore may be a critical determinant of the survival of individuals through to adulthood. Implications for the present study are that the larger larvae produced by non-stressed females may have a greater probability of survival through to recruitment than larvae from stressed females. At an individual level, the interaction regime to which breeding pairs are exposed will potentially determine which adults contribute most to the next generation of reef fish.

Maternal size and nutritional status also influence progeny characteristics in *P. amboinensis.* Kerrigan (1996) experimentally manipulated food availability to two sizes of females (small and large) placed in breeding pairs on isolated patches of coral. She found that small females that were not supplementary fed produced the largest larvae at hatching, and these had the smallest yolk reserves. In contrast, large, supplementary-fed fish produced moderate sized larvae, but these had by far the largest yolk reserves. Food availability and the assemblage characteristics that promote maternal stress are likely to vary among sites and through time, and this will lead to the release of larvae that vary in quality on these spatial and temporal scales. Interannual variability in the size of the females in the breeding population will further increase this variability in larval phenotype.

Maternally driven larval quality and its ramifications for selection

The situation described in this paper, where maternal stress gives rise to a quality response in the larvae, highlights the complexity of the factors that drive variability in traits at specific life stages. The variability in the traits at a particular life stage may not be related to the necessity for variability at that stage (i.e., adaptive phenotypic plasticity) but rather the product of events prior to that life stage. Phenotypes are not simply a product of a complex interaction between genotype and the organism's external environment. Nongenetic maternal effects add to this complexity. Bernado (1996*b*) stressed that to understand variance in propagule size and its implications for both maternal and offspring fitness, it is necessary to consider explicitly the ecological context in which a mother is producing offspring, not just that into which the offspring will enter. The environment of the female will influence the amount of energy she invests into each propagule. Her ability to compete for resources, efficiently process them into reproductive products, and then liberate them to maximize her reproductive success will all influence the size and quality of the individual larvae at hatching.

For temperate fisheries stocks it is argued that egg and larval sizes are adaptive ''solutions'' to the expected mortality, which relate to the concentration and sizes of zooplankton prey for these larvae (Ware 1975, Wootton 1994). However, this relationship is weaker than generally surmised (Leggett and Deblois 1994), and the relationship is likely to be strongly influenced by maternal effects (Chambers and Leggett 1996). In the present study, the phenomenon of unstressed females producing larger offspring may not necessarily be an adaptive solution to a facet of the larval environment perceived by the female, but rather may simply

represent an important source of some of the variability in propagule quality that has individual benefits.

Maternal influences complicate the consequences of selection regardless of whether they have a heritable basis (Kirkpatrick and Lande 1989). A number of studies have recently suggested that some reef fish populations are replenished to a large extent by propagules originating from the same population (Planes 1993, Brogan 1994, Schultz and Cowen 1994). G. Jones and M. Milicich (*unpublished manuscript*) tagged embryos of *P. amboinensis* at Lizard Island and collected the recruits as they came back to the reef to settle at the end of their larval stage. They determined that the reef population was not a completely open system as parsimoniously assumed, but rather one in five individuals recruiting may be returning to their natal reef. This further complicates the impact of maternal influences on the drift of traits in the population.

If much of the variability in larval characteristics and performance is maternally derived, as this and other studies suggest (Kerrigan 1996; B. A. Kerrigan and M. I. McCormick, *unpublished manuscript*), then ecologists are challenged to address the question of how persistent are these maternal influences, and do they influence growth, fitness, and survival in later life stages. Future studies may show that the maternally derived hormonal and nutritive aliquot in the yolk may greatly influence, not only larval events, but post-recruitment events, such as time to maturity. Such long-lasting effects have been shown in higher vertebrates. For instance, in many litter-bearing rodents the level of exposure of the fetus to testosterone in the uterus influences adult androgen levels, which govern aggression, sexual attractiveness, and the length of the estrus cycle in females (Clark and Galef 1995).

Maternal effects represent an interesting and important aspect of population quality that has received little attention in marine environments. This study suggests that the social structure of a local population may influence characteristics of progeny that influence survival. Recent reviews stress the ubiquitous nature of maternal influences on progeny characteristics and the potential of these effects to have important consequences for adult life stages (Bernardo 1996*a*, *b*, McNamara and Houston 1996). Our understanding of fish population dynamics will be greatly advanced by exploring the mechanisms underlying the quality of progeny and their flow-on effects to reef populations.

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