



Parental condition affects early life-history of a coral reef fish

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ABSTRACT

Parents can exert a range of non-genetic effects on the growth and survival of their offspring. In particular, parents may modify the size or condition of their offspring depending on the amount of energy they have available for reproduction. In this study, the body condition of breeding pairs of the coral reef fish *Acanthochromis polyacanthus* was experimentally manipulated to test the effects of parental condition on reproductive output and offspring life history traits. Parents in good condition commenced breeding earlier, had higher reproductive output, and their eggs exhibited increased survival during embryogenesis, compared to parents in poorer condition. Increased reproductive output was attained through more reproductive bouts over the breeding season that contained both a greater number and an increased size of eggs. The offspring from parents in good condition were larger at hatching, with larger yolk reserves and increased survival on endogenous reserves. Larger size is expected to provide benefits to offspring through reduced susceptibility to size-selective mortality. The range of offspring characteristics modified by parental condition could result in a greater proportion of offspring from good condition parents recruiting to the population.

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1. Introduction

Parents can influence the phenotype of their offspring through both genetic and non-genetic means. Genetic effects involve the inheritance of traits through the transfer of genes from parents to their offspring (e.g., Miles et al. 2007). Parental effects may occur either directly, through the transfer of specific material to the offspring, or indirectly through parental behaviour (Bernardo, 1996a, Heath and Blouw, 1998). However, genetic and phenotypic parental effects are not discrete, and therefore evaluating the relative magnitude of each component is often difficult (Lacy, 1998). The importance of phenotypic effects on offspring life histories is likely to vary among species. Surprisingly, only a few studies of parental effects have attempted to distinguish between genetic and non-genetic components. The distinction is important because non-genetic effects have the potential to be adaptive and even oppose the direction of selection on heritable traits (Kirkpatrick and Lande, 1989, Bernardo, 1996a). It is believed that non-genetic effects should be most important for species that experience fluctuating environmental conditions because it gives parents the opportunity to modify offspring characteristics to suit different environmental conditions and thereby increase their fitness regardless of underlying genetic constraints (Bernardo 1996a).

Variations in the environmental conditions experienced by parents may affect their body condition and provide the opportunity for phenotypic effects on offspring. It is commonly believed that mothers have a greater potential to affect their offspring than fathers because of the difference in the size of male and female gametes (Bernardo, 1996a). Specifically, mothers may alter the number, size, energy content or biochemical composition of their eggs depending on the energy they have available to invest in their offspring. These initial modifications to the eggs can potentially have downstream effects on the growth, reproductive success and ultimately the survival of the offspring.

The energy available for reproduction is often limited and this can lead to trade-offs in the way that parents partition reproductive output (Smith and Fretwell, 1974, Bernardo, 1996b). Parents may adjust the number or quality of offspring produced depending on the total energy available for reproduction and the environmental factors that are likely to impact on the success of their offspring. The advantage of investing in either large numbers of offspring, or high quality offspring, may depend on the nature of the mortality agents experienced and the relative importance of size or other characteristics on offspring survival. Producing more offspring may be optimal for species where mortality is high and non-selective during the early life history (Stearns and Hoekstra, 2000). In contrast, producing better quality offspring may be optimal for species where mortality is highly selective, or where competition for key resources is important (Stearns, 1992, Einum and Fleming, 2000). In this case better quality

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offspring could have greater capabilities in escaping these selective pressures through more rapid growth, increased size or enhanced competitive ability.

In marine fishes, parental effects have been found to influence size at hatching (Panagiotaki and Geffen, 1992, Kerrigan, 1997, Green and McCormick, 2005a, Trippel et al., 2005, McCormick, 2006), hatching success (Laine, 1999, Laine and Rajasilta, 1999, Saillant et al., 2001, Rideout et al., 2004, Trippel et al., 2005), yolk reserves (Kerrigan, 1997, Gagliano and McCormick, 2007), swimming ability (Green and McCormick, 2005a) and growth rate of larvae (Green and McCormick, 2005a). However, the nature of these effects (genetic or non-genetic) has rarely been addressed (but see Kerrigan, 1997, Green and McCormick 2005a, McCormick, 2006, Gagliano and McCormick, 2007) and the potential importance of these effects on the survival of offspring is still poorly understood.

High mortality rates occur in the larval and early juvenile stages of most reef fishes (Sale and Ferrell, 1988, Bailey and Houde, 1989, Almany and Webster, 2006). Predation and starvation are thought to be two of the key agents of mortality in larval fishes and may be highly selective (Meekan and Fortier, 1996, Paradis et al., 1996, Leis and McCormick, 2002). There is increasing evidence that fast growth during larval life enhances survival through being larger for a given age and by reducing the time spent in this vulnerable life history stage (Searcy and Sponaugle, 2000, Bergenius et al., 2002, Wilson and Meekan, 2002). Larger offspring may possess characteristics such as enhanced swimming ability (Miller et al., 1988, Fisher et al., 2000), an earlier time of first feeding (Wallace and Aasjord, 1984) or greater predator avoidance (Miller et al., 1988). If parental effects influence the growth or development rate of larvae, and thus size at a given age, then these influences are likely to be of major importance to offspring survival.

We investigated the effect of a phenotypic factor, adult body condition, on patterns of reproductive allocation and offspring life-history traits of the spiny damselfish, *Acanthochromis polyacanthus*. Specifically, we experimentally tested if body condition influences the number or quality of offspring produced and if differences in offspring characteristics are likely to have significant consequences for early survival. Adult breeding pairs were reared on either a high or a low-quantity diet to produce parents that differed in overall body condition. Reproductive output of high and low condition parents was compared and offspring characteristics were monitored to identify the occurrence and magnitude of parental effects at hatching. The survival ability of newly hatched offspring on their endogenous reserves was then tested and related back to their physical characteristics at hatching.

2. Materials and Methods

2.1. Study Species

The tropical damselfish, *A. polyacanthus*, was used in this study because it is amenable to laboratory breeding and the offspring can be reared with high success, as they do not possess a planktonic larval stage. Difficulty rearing larvae of other coral reef fishes due to their extended planktonic phase limits their potential for cross-generational experiments of the type undertaken in this study. *A. polyacanthus* form breeding pairs, with most reproduction occurring over a 4 month period during the summer on the Great Barrier Reef (October to February, Robertson, 1973). Eggs are laid on substrate within caves (Thresher, 1983) and care is provided to the benthic eggs by both parents (Pankhurst et al., 1999). After hatching, the young remain with the parents for up to 45 days (Kavanagh, 2000).

2.2. Adult breeding pairs

2.2.1. Collection and experimental design

Adult pairs of *A. polyacanthus* were collected from Bramble Reef (146°41'E, 18°25'S) and Pelorus Island (146°29'E, 18°32'S) on the

central Great Barrier Reef, Australia, during June and July 2005. Care was taken to only collect established breeding pairs, which were identified by the behavioural interaction of individuals towards each other and defence of a common territory. Pairs were collected using a barrier net and hand nets.

Eleven adult pairs of *A. polyacanthus* were transferred to 70 l aquariums at the James Cook University Marine and Aquaculture Research Facility. Each aquarium contained a large shelter composed of half a terracotta pot and two bricks underneath to elevate it. *A. polyacanthus* readily lays eggs on the underside of terracotta pots, or on the side of the bricks, therefore, this shelter also served as a breeding site. All tanks were aerated and supplied with a constant flow of seawater at a rate of 2.5 to 3 l min⁻¹. During an acclimatization period all fish were fed 0.25 g of commercial fish flake food (Wardley Total Marine Flakes) per day until the start of the experiment.

Pairs were randomly assigned to either a high (6 pairs) or low (5 pairs) quantity diet on 5th August 2005. The high quantity diet was exactly three times the amount of the low quantity diet. Pairs on the high quantity diet were fed twice a day every day. Pairs on the low quantity diet were fed once a day, two out of every three days. Within a three day cycle pairs on the low quantity diet were fed the morning of day one, then the afternoon of day two and no food on day three. For the first three weeks of the experiment, each feeding consisted of 0.235 g commercial fish flakes (0.58% body weight; protein 45%, fat 8%, fibre 3%). Subsequently, morning feeding remained as 0.235 g commercial fish flakes while the afternoon feeding consisted of 0.21 g of INVE Aquaculture Nutrition NRD 12/20 pellets (0.51% body weight, protein >55%, fat >9%, fibre <1.9%). Previous work has estimated natural feeding rates in damselfish to be 1% body mass per day (Booth and Alquezar, 2002) and a preliminary analysis revealed that food levels described above were enough to increase adult condition (high quantity) and the minimum required to allow reproduction (low quantity) (Donelson, 2006). Pairs received either the low or high feeding treatment for at least 2 months before breeding occurred. Offspring not used in experiments listed below were removed from their parental tank at day 7 (earlier than they would naturally leave in the wild) to avoid parents eating the food given to the offspring from day 7 post-hatching, which would have confounded the adult food treatment.

Water temperature in the aquariums was gradually increased to simulate the increase in ocean water temperature during spring and to provide a natural stimulus to commence breeding (Hilder and Pankhurst, 2003). Water temperature was raised 0.5 degrees every 5 d from 23.4 °C on the 18th of August 2005, until it reached 28 °C. The temperature was then maintained between 27.3 °C to 28.9 °C for the remainder of the experiment, which is close to the average ocean temperature recorded at Orpheus Island in summer (average range 26.13 °C to 30.12 °C from October to February).

2.3. Measuring adult condition

To confirm that the two food treatments influenced the body condition of breeding pairs, the length and weight of each fish was estimated after 6 weeks on the high and low feeding levels and again at the end of the experimental breeding season. Importantly, the first estimate of body condition was taken before pairs started breeding, to ensure that pairs in the different treatments exhibited different body condition before they commenced breeding. Each fish was captured with a small hand net and sexed by visual inspection of their genital papilla. Males have a conical papilla and females have a rounded papilla that extrudes when gentle pressure is placed on the abdomen. Excess water was removed and wet weight was recorded to the nearest 0.01 g. Each fish was then placed in a transparent plastic bag containing enough water to cover the body and photographed against a grid. The calibrated photograph was used to estimate the length of each fish.

2.4. Egg production

2.4.1. Egg collection and monitoring

Potential nesting areas were checked in the early morning for the presence of eggs. On discovery of a clutch an underwater photograph was taken for later estimation of the number of eggs laid. To measure egg size a sample of 10 eggs was then collected from each clutch using tweezers. To reduce potential bias of egg size depending on its location within the clutch, 5 eggs were taken from the outer region and 5 from the inner region of the clutch. Eggs were preserved and stored in a 6% formaldehyde and seawater solution. *A. polyacanthus* eggs hatched from late morning to early afternoon and established nest sites were checked regularly throughout the day from 8 days post-laying until hatching.

2.4.2. Clutch size and egg dimensions

The number of eggs in each clutch at laying was estimated by viewing the digital photographs on a computer screen using Image Tool (UTHSCSA, San Antonio, USA). The number of eggs in each clutch was counted from 2 photographs and any discrepancies between the 2 counts were identified to gain the best estimate of the number of eggs present at each time.

Egg size was measured directly after collection or within 24 hours of preservation. With a pilot study we confirmed that minimal shrinkage or degradation of the eggs occurred within this period (<1%; t-test: $t=0.512$, $d.f.=0.004$, $n=50$, $p=0.611$). Egg length and width were estimated using dial vernier callipers (accurate to ± 0.02 mm) viewed under a stereoscopic microscope. The volume of each egg measured was then estimated by assuming that egg shape was a cylinder with a half sphere at each end, which was the best and easiest approximation of volume. To estimate the reproductive output of each spawning the number of eggs in each clutch was multiplied by the average egg volume for that clutch.

2.5. Juveniles

2.5.1. Number at hatching

Multiple digital photographs were taken of offspring groups shortly after hatching and the number of juveniles present was estimated from two separate photographs for each clutch. If these 2 counts differed another photograph was analysed.

2.5.2. Characteristics at hatching

20 haphazardly selected juveniles were removed from each clutch within 2 hours of hatching for measurement of physical attributes immediately after hatching. The sampled fish were euthanased with an overdose of clove oil and then preserved in 4% phosphate buffered formaldehyde solution. Weight, standard length (SL), yolk length and yolk height were measured after 2 days of preservation. Specimens were blotted dry and weighed to the nearest mg. They were then photographed in a lateral position under a stereomicroscope. SL and yolk length and height of each fish were estimated from the digital photograph using image analysis. All attributes were estimated 3 times and an average of the 3 values was calculated (estimates varied <2%). Yolk area was calculated from length and height measurements by assuming it was half an ellipse in shape.

2.5.3. Influence of parental condition on offspring survival

To determine if parental body condition influences the length of time offspring can survive on their endogenous resources, a random sample of 20 newly hatched individuals from each clutch were reared without food. Juveniles were haphazardly selected from each clutch within 2 hours of hatching and transferred to individual 2 l plastic aquariums (260×120×95 mm) in an environmentally controlled room. Juveniles were removed from the parental tank with a small plastic cup to minimise stress and dispensed into their aquariums by

gently tipping the cup close to the water surface. Each aquarium received seawater at a rate of approximately 47 ml min⁻¹ at a temperature between 27.9 °C and 29.4 °C. The light regime was 14 h light: 10 h dark. Each aquarium containing a juvenile was checked daily at approximately 09:00 and 17:00 h to determine if that individual was still alive.

To determine if the ability of juveniles to survive on their endogenous energy reserves could be predicted by their body size or yolk size, an additional group of 10 juveniles was collected from each of 9 clutches. Clutches were randomly selected from 3 low and 6 high food parents. Clutches from only 9 of the 11 parental pairs were tested due to space constraints in the environmentally controlled room. These fish were individually photographed through a stereomicroscope before being transferred to their individual containers for monitoring of survival on endogenous reserves as described above. Measurements were undertaken by placing each newly hatched fish into a small plastic bag with a limited amount of seawater and photographing it from above (dorsal view). A 5×5 mm grid was placed in the background of each photo for use in calibration. Standard length, yolk length and yolk width from above were estimated using image analysis.

To determine if the yolk area estimated in dorsal view correlated with the measurements of yolk area estimated at hatching in lateral view, a sub sample of 40 newly hatched larvae were photographed with a stereomicroscope from both the dorsal and side view. Since the offspring used to test survival potential was measured from the dorsal view, whereas yolk area at hatching was measured from the lateral view, the relationship between these two measures of yolk amount was investigated. The relationship was significant (regression: $r^2=0.58$, $p<0.001$), indicating that dorsal yolk area is a reasonable proxy for lateral yolk area, although some variability between the two estimates clearly exist.

2.6. Data analysis

2.6.1. Adult condition

The body condition of adults in the 2 food treatments was compared using ANCOVA. Weight was used as an indicator of condition, assuming that for a given body length, individuals in good condition would be heavier than those in poorer condition. Body condition prior to breeding (6 weeks after commencing the treatments) and at the conclusion of the experiment were analysed separately. Food level (high, low) was regarded as a fixed factor, weight (g) was the dependent variable and standard length (mm) was the covariate. Tests for homogeneity of slopes (all non-significant) were undertaken prior to ANCOVA.

To explore whether investing in reproduction influenced maternal body condition, the relationship between total reproductive output over the breeding season and the condition of females at the conclusion of the experiment was explored with Spearman rank order correlations. Female condition was calculated as the residuals of a linear regression performed on all female fish's standard length and weight. Correlations were performed on females from each feeding treatment separately. All statistical analyses were conducted using Statistica version 7 (Stat Soft, Tulsa, USA).

2.6.2. Egg production

A two-way orthogonal ANOVA was used to test whether the number of eggs produced differed between food treatments (fixed factor) and between first and second clutches (fixed factor). Nested ANOVA was then used to test if the volume of eggs produced by pairs in the first or second clutch differed between food treatments (fixed factor) or among parents (random factor). Egg volume was analysed separately for the first and second clutches. The data from the first clutch was balanced at the level of parents so a type III model was used. However, not all parents produced a second clutch, therefore the data from the second clutch was unbalanced and a type IV model was

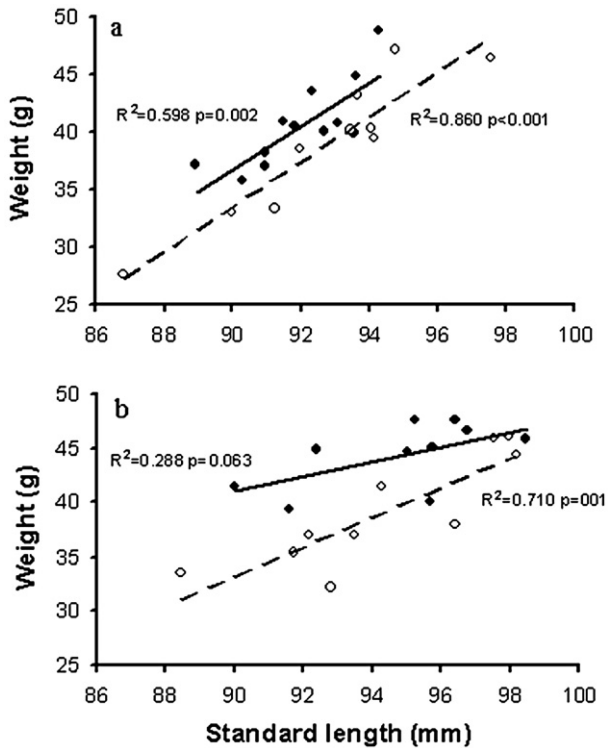


Fig. 1. Weight of all adult fish in high (●) and low food (○) treatments at: (a) 6 weeks after commencement of food treatments, and (b) at the conclusion of the experiment at 21 weeks. Separate linear regressions are shown for fish fed a high quantity diet (solid lines) and fish fed a low quantity diet (dashed lines).

used. Data from the second clutch was square root transformed in order to meet the assumption of normality.

Differences in embryonic duration between food treatments (high and low food) and clutch number (one and two) were investigated using factorial ANOVA. A type VI model was used due to the samples being unbalanced between food levels and clutches. A nested ANOVA, which would be able to test for differences between parents within a food level, was not used because each breeding pair had a single value of embryonic duration for each clutch.

Since parents within the food treatments were not all the same standard length or body condition, and egg size is known to correlate with both these factors, the relationship between egg size and maternal size and maternal condition for clutch 1 was investigated using a multiple regression. The measure of body condition used was the residuals of the standard length to weight relationship of females prior to breeding.

2.6.3. Offspring attributes at hatching

The size and development of offspring from parents (random) in the 2 different feeding treatments (fixed) were compared with type IV nested ANOVA. Where required, square root transformations were applied to satisfy assumptions of homogeneity of variance or normality. The ANOVA revealed significant variability in offspring attributes between parents within the feeding treatments, therefore,

we used multiple regression to determine if this variability was related to differences in the size or condition (residuals of SL to weight relationship) of individual mothers. In addition, the percentage variation explained by each level of the nested model was computed.

Since parental effects most prominently occur through modification of gametes, offspring characteristics at hatching are likely to be correlated with egg size. Partial correlations were used to determine which offspring attributes were most strongly related to average egg volume. This enabled differentiation between the variables that were significantly correlated with egg volume and those that were highly correlated with the other variables. The variables at hatching that were of interest were average standard length, weight and yolk area.

2.6.4. Influence of parental condition on offspring survival

The survival duration of offspring from parents on high and low food regimes was compared using a two-sample survival analysis (Cox's F-test). The relationship between individual's yolk area and their survival ability was then explored with simple linear regression. The two individual regressions were tested for homogeneity to determine if offspring from the two parental treatments had different relationships between yolk characteristics and survival time. This was found to be non-significant and subsequently offspring from both parental treatments were pooled to explore the overall relationship between survival and yolk characteristics.

3. Results

3.1. Adult condition

Body condition of adults differed between the 2 food treatments. Adults on the high quantity diet were heavier, for any given length, than those on the low quantity diet, both before they commenced breeding (Fig. 1a; ANCOVA: $F_{1,19}=10.60$, $p=0.004$) and at the conclusion of the experiment (Fig. 1b; ANCOVA: $F_{1,17}=15.01$, $p=0.001$). The same pattern was evident when only the female's weight was used in the analysis (ANCOVA: $F_{1,8}=5.66$, $p=0.045$ and $F_{1,7}=7.35$, $p=0.03$ for start and conclusion of the experiment respectively). The difference between the weight of individuals in the 2 food treatments was accentuated at the conclusion of the experiment compared to immediately before breeding commenced (Fig. 1).

The relationship between the total amount of energy invested in reproduction during the breeding season (as measured by the number and size of eggs produced) and the condition of females at the end of the experiment differed with food treatment. There was a negative but non-significant relationship between body condition and total reproductive output for females from the low food treatment (Spearman correlation: $r=-0.5$, $n=5$, $p>0.05$). In contrast, females from the high food regime exhibited a positive but non-significant relationship between body condition and reproductive output (Spearman correlation: $r=0.5$, $n=5$, $p>0.05$). This suggests that when food was abundant, females were more able to invest in reproduction without reducing their body condition. In contrast, when food was limited, the condition of females tended to be negatively affected by an increased investment in reproduction (Table 1).

Table 1

Comparison of the mean weight (+/- SE) and standard length (+/- SE) of adult fish after 6 weeks on low and high quantity diets and at the end of the experimental period

Date	Low Quantity Diet				High Quantity Diet			
	Female (n=5)		Male (n=5)		Female (n=6)		Male (n=6)	
	Weight	Standard length	Weight	Standard length	Weight	Standard length	Weight	Standard length
6 weeks (16th Sept 2005)	38.51 +/-1.141	92.23 +/-1.427	39.17 +/-2.673	93.31 +/-1.304	42.95 +/-1.559	92.78 +/-0.528	39.36 +/-1.365	91.64 +/-0.878
Termination (6th January 2006)	38.26 +/-2.096	93.70 +/-1.664	39.84 +/-2.633	94.91 +/-1.281	45.28 +/-1.389	95.29 +/-0.777	43.21 +/-1.247	94.17 +/-1.515

Table 2

Timing of first egg-clutch production for pairs of *A. polyacanthus* fed either a high quantity or a low quantity diet

Timing of 1st clutch	October	November	December	January
High food Pairs	4	1		
Low food Pairs	1	1	1	1

Numbers indicate the number of breeding pairs that produced their first clutch in each month.

3.2. Egg production

Adult pairs in the high food treatment produced egg clutches earlier than pairs in the low food treatment (Table 2). Four out of five breeding pairs from the high food regime laid their first clutch in October, compared to only one low food pair. Additionally, there was less variation in the date at which the first clutch was produced by high food treatment pairs compared to pairs in the low food treatment. Most high food pairs laid their first clutch before the end of October. In contrast, low food pairs laid their first clutch between October and January, with one pair laying each month and one pair not breeding at all. All pairs in the high food treatment laid 4 or 5 clutches, except for on pair that only laid 3 clutches. In contrast, only 1 pair in the low food treatment laid more than 2 clutches, and this pair laid a total of 4 clutches.

The reproductive output of pairs in the high food treatment was greater than that of pairs in the low food treatment for both the first and second clutch (Fig. 2). The mean reproductive output of the third and fourth clutches from high food parents was also greater than the reproductive output of the first clutch from low food parents (Fig. 2). The number of eggs laid (factorial ANOVA: $F_{1,12}=25.05$, $p<0.001$) and volume of eggs (nested ANOVA clutch 1: $F_{1,6}=12.91$, $p=0.011$ and clutch 2: $F_{1,6}=12.65$, $p=0.012$) differed between food treatments (Fig. 3). Differences in egg volume between the two food treatments were most apparent in the first clutch, with an average egg volume from high treatment pairs of 5.76 mm³ compared to 4.38 mm³ from fish in the low food treatment. Within parental treatments a reduction in the size of eggs produced was evident between the first and second clutches, although the number of eggs produced remained relatively constant (Fig. 3). Egg volume also differed significantly between parents within food treatments (nested ANOVA clutch 1: $F_{5,58}=8.64$, $p<0.001$ and clutch 2: $F_{6,73}=7.29$, $p<0.001$).

To determine if the differences in egg volume between parents within a treatment were related to maternal standard length, the relationship between these two variables was investigated for the first clutch. Since a relationship between maternal condition and reproductive output had also been identified (Fig. 2), both maternal variables were incorporated into a stepwise multiple regression. This allowed examination of the relationship of egg size to both maternal size and

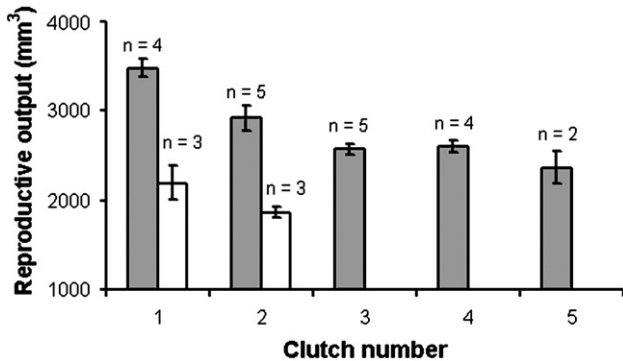


Fig. 2. Mean (+/- SE) reproductive output (egg number*mean egg volume) between breeding pairs on high (shaded) versus low (open) quantity diet. Sample sizes shown above bars.

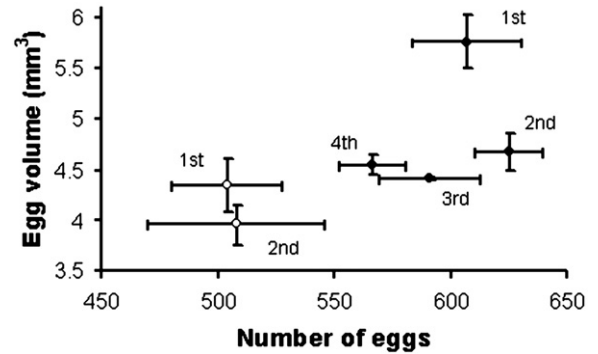


Fig. 3. Relationship between components of reproductive output for the first four clutches. Points represent the mean value (+/- SE), where (●) are high food parents and (○) are low food parents.

condition, with their covariance incorporated. This analysis revealed that neither maternal condition or maternal standard length significantly contributed to the variation in egg volume between breeding pairs within feeding treatments (regression: $F_{2,5}=2.07$, $r^2=0.234$, $p>0.05$).

Egg mortality in the period between laying and hatching was approximately 20% greater in clutches from low food parents (Fig. 4; factorial ANOVA: $F_{1,11}=6.08$, $p=0.031$) and there were no differences in the percentage survival between the first two clutches (factorial ANOVA: $F_{1,11}=0.293$, $p=0.60$). Embryonic duration ranged from 9 to 11 d, but did not differ between feeding regimes (factorial ANOVA: $F_{1,11}=1.286$, $p=0.283$).

3.3. Offspring characteristics at hatching

Offspring of parents in the high food treatment were heavier at hatching with a mean weight of 3.77 mg (+/- 0.088 SE), than offspring of parents in the low food treatment which averaged 2.975 mg (+/- 0.071 SE) (Table 3). Offspring from high food level parents also possessed larger yolk sacs than offspring from low food parents (Mean high: 1.80 mm² +/- 0.02 SE and low: 1.52 mm² +/- 0.022 SE; Table 3), and food levels accounted for 45.6% of the variation in yolk size. This strong difference in yolk size between treatments may have contributed to the difference in juvenile weight between treatments. There was also a clear trend for offspring from high food parents to be longer at hatching (Mean high: 5.59 mm +/- 0.038 SE and low: 5.27 mm +/- 0.032 SE; Table 3). Although this trend was not statistically significant at alpha=0.05, it is consistent with the effects seen in other offspring characteristics. Furthermore, similar amounts of variation in weight (26.7%) and standard length (22.8%) of offspring at hatching were attributable to parental feeding treatment (Table 3). Between 37 and 57% of the variation in the morphological attributes of the offspring was

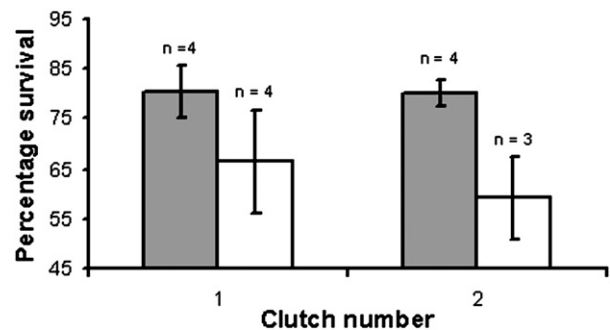


Fig. 4. Mean percentage of offspring surviving from laying until hatching (+/- SE). Shaded bars are offspring from high treatment parents and open bars are offspring from low treatment parents. Sample sizes shown above bars.

Table 3
Comparison of the standard length, weight (square root transformed) and yolk area (square root transformed) of newly hatched offspring from parents fed either a high quantity or a low quantity diet

	df	Standard Length				Weight				Yolk area			
		MS	F	p	% Variation	MS	F	P	% Variation	MS	F	p	% Variation
Food Treatment	1	4.552	3.762	0.094	22.8	1.773	7.131	0.032	26.7	0.531	11.761	0.011	45.6
Pair(Food Treatment)	7	1.210	17.136	<0.001	34.5	0.249	6.797	<0.001	16.5	0.045	10.064	<0.001	17.0
Error	171	0.071			42.0	0.037			56.9	0.005			37.4

explained by differences among offspring within a pair within treatments (Table 3).

Standard length, weight and yolk size of offspring differed among parents nested within food treatments (Table 3). Differences among pairs accounted for 34.5% of the variation in juvenile standard length, while these differences only accounted for 16.5 and 17% of the variation in juvenile weight and yolk size respectively (Table 3). To explore this variation, the relationship of offspring characteristics at hatching to maternal size and maternal condition prior to breeding was examined with stepwise multiple regression. Variation in offspring standard length among parents within treatments was not significantly associated with maternal length, but showed some association to maternal condition ($F_{2,177} = 18.45$, $r^2 = 0.163$, $p < 0.05$). Weight and yolk area at hatching exhibited a significant relationship to both maternal condition and size (weight: $F_{2,177} = 25.29$, $r^2 = 0.213$, $p < 0.05$; yolk area: $F_{2,177} = 30.12$, $r^2 = 0.245$, $p < 0.05$).

Offspring characteristics at hatching were correlated with egg size. A strong association was found between average egg volume and both mean hatching length (regression: $F_{1,18} = 23.13$, $r^2 = 0.338$, $p < 0.001$) and yolk area (regression: $F_{1,18} = 19.91$, $r^2 = 0.499$, $p < 0.001$). A similar association was evident between egg size and weight of newly hatched fish, and this relationship was strengthened by the removal of an extreme outlier (egg volume: 4.897, weight: 1.722; regression: $F_{1,17} = 25.29$, $r^2 = 0.574$, $p < 0.001$). Since these offspring characteristics were potentially correlated to each other, the interrelationship between the variable was further explored by calculating the variables' partial correlations with egg volume. This showed that egg volume was most strongly related to standard length (partial correlation: 0.588, $n = 20$, $p = 0.010$) and yolk area (partial correlation: 0.748, $n = 20$, $p < 0.001$) at hatching. Furthermore, the relationship between weight at hatching and egg volume was not significant (partial correlation: 0.223, $n = 20$, $p = 0.374$) because of the high correlation of weight to yolk area (correlation: 0.46, $n = 20$, $p < 0.05$) and standard length (correlation: 0.76, $n = 20$, $p < 0.05$). Food treatment of the parents did not influence the relationship between hatching characteristics and egg volume relationship (homogeneity of regressions SL: $F_{1,17} = 0.025$, $p = 0.877$ and yolk area: $F_{1,17} = 0.741$, $p = 0.401$).

3.4. Influence of parental condition on offspring survival

Offspring of parents in the high food treatment survived longer on their endogenous energy reserves than offspring from parents in the low food treatment (Fig. 5; Cox's F-test: $F_{198,158} = 2.12$, $p < 0.05$). Substantial mortality of progeny from the low food treatments began at 5.5 to 6 days

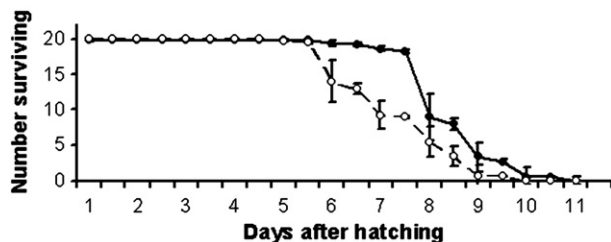


Fig. 5. Mean survival times of offspring on endogenous reserves from parents fed a high (solid lines) or low quantity (dashed lines) diet (+/- SE).

post-hatching compared to 7.5 to 8 days for the high food treatment (Fig. 5).

Differences in survival ability of offspring from high and low food parents were found to relate to yolk size at hatching (linear regression: yolk area: $F_{1,65} = 11.11$, $r^2 = 0.133$, $p = 0.001$; proportional yolk area (yolk area/SL): $F_{1,65} = 13.86$, $r^2 = 0.163$, $p < 0.001$), however, these factors explained only a relatively small amount of the variation in survival time. Offspring possessing the same survival time varied substantially in yolk area. The relationship between survival time and yolk area or proportional yolk area did differ between the parental feeding regimes. Progeny from parents on the low food diet survived up to 3 days less than those with the same yolk size from high food pairs.

4. Discussion

Non-genetic parental effects that could affect the survival prospects of juvenile *A. polyacanthus* were identified in relation to the food supply available to adult breeding pairs. Food availability affected the body condition of parents and this influenced both their total reproductive output and the phenotype of their offspring. Parents on the high food diet and in good condition produced larger eggs than parents on the low food diet and in poorer condition. Larger eggs yielded juveniles with larger endogenous reserves, which tended to be larger in size and weight at hatching. Offspring from parents in good condition also survived longer on their endogenous reserves than offspring of parents in poorer condition. Clearly, by directly influencing the key components of the juvenile phenotype, parental feeding conditions can have a major influence on adult fitness through its influence on the probability of offspring surviving during the highly vulnerable early juvenile life stage.

Parental feeding treatment influenced not only the total amount of reproductive products produced but also influenced the relationship between parental condition and total allocation to reproduction. Parents on the high food diet were in better condition at the start of the breeding season and remained in better condition over 15 weeks later, even after allocating substantially more energy to reproduction compared to parents in the low food treatment. The condition of females in the low food treatment was affected by their total reproductive output over the breeding season. The more energy allocated into reproduction, the poorer their condition. The opposite trend was seen for females in the high food treatment. This indicates that differences in food supply for breeding pairs can have far reaching consequences for their fitness.

Parents in the high food level exhibited a reproductive mode with a number of potential fitness advantages. First, well fed parents began breeding earlier and completed more reproductive bouts over the breeding season. While reproductive bouts were increased due to the abortion of broods after only 7 days, compared to approximately 45 days in nature (Kavanagh, 2000), this result gives an indication of reproductive potential. These findings are supported by field observations showing pairs of *A. polyacanthus* that breed earlier in the season are more likely to lay a second clutch (Thresher, 1983). In addition to the greater number of clutches produced, the clutches of well fed parents also contained more eggs. Since early life history stages of marine fish have been associated with high levels of mortality (Sale and Ferrell, 1988, Bailey and Houde, 1989, Almamy and Webster, 2006) the production of a greater number of gametes could equate to a

greater number surviving. Therefore, parents in better condition would likely have more offspring recruiting into the population. In addition to the premise of superior recruitment due to an earlier time of breeding and more gametes being produced, a greater level of survival during the embryonic stage was also observed in the high food treatment.

Offspring produced by parents with greater food availability were larger at hatching, which may improve their chance of survival in the natural environment (Ellis and Gibson, 1995, Sogard, 1997, Enum and Fleming, 2000, Vigliola and Meekam, 2002). Large size is thought to be beneficial because it imparts greater swimming ability (Miller et al., 1988, Fisher et al., 2000) and increased energy reserves (Blaxter and Hempel, 1963). Additionally, a larger size at hatching has been associated with an earlier time of first feeding and physical attributes, such as a larger jaw size, that may allow access of a greater range of prey types (Blaxter and Hempel, 1963). In addition to a size advantage, offspring from parents in better condition also possessed larger yolk sacs. This is consistent with research showing that *Pomacentrus amboinensis* mothers provided with greater food availability produced offspring with larger yolk reserves (Kerrigan, 1997, McCormick, 2003). Furthermore, in an experimental field study with *A. polyacanthus*, Booth and Alquezar (2002) found that juveniles in better condition had higher persistence on the reef.

Yolk size is often measured as a proxy for energy reserves and in previous studies has been found to correlate with larval survival (Blaxter and Hempel, 1963). In contrast to expectations, the survival of individual *A. polyacanthus* offspring on endogenous reserves was not well correlated with their yolk size. This suggests that yolk size alone does not necessarily increase survival potential. Nevertheless, offspring from high food parents exhibited greater survival on endogenous reserves. This indicates that yolk composition or yolk usage and metabolism, rather than yolk area alone, may be critically important to the survival of offspring on endogenous reserves. If offspring from different parents possess varying levels of energy stores, such as protein, carbohydrates and lipids, their survival time could be affected (Sargent, 1989, Rainuzzo et al., 1997). It seems likely that the yolk of offspring from parents in the high food treatment was nutritionally superior to that of offspring from parents in the low food treatment. Since yolk area from the dorsal view only explained 58% of the variation observed in the lateral view it is possible that measuring yolk area from just one view missed some of the complex yolk shape, thereby reducing the power to detect a relationship between yolk area and survivorship.

Generally it is believed that a trade-off exists between the size and number of eggs able to be produced in a clutch (Smith and Fretwell, 1974, Bernardo, 1996b). This study found that mothers in good condition were able to produce a larger number of bigger eggs compared to females in poorer condition. This suggests that these mothers were able to partition more energy into reproduction and were not limited to altering either the number or size of gametes. These results contrast with those of field studies on another damselfish, *P. amboinensis*, in which field manipulations of maternal condition influenced yolk-sac and oil globule size but had no effect on the number of eggs produced (McCormick, 2003, Gagliano and McCormick, 2007).

The identity of parents had a major influence on offspring attributes with significant differences often being found among parents within food treatments. Variation in egg size and offspring characteristics at hatching among parents within treatments may be genetically-based, or differences in the magnitude of non-genetic effects related to such factors as age (Berkeley et al., 2004) or prior feeding history. However, for both weight and yolk area more variation was due to feeding level than was attributed to differences among parents. Thus the important result remains that regardless of genetics, age, or other unaddressed non-genetic factors, the current parental condition modified through food level had a significant effect on aspects of offspring produced. These influences were substantial enough to cause measurable

differences in survival and physical attributes, which are known to produce benefits in a natural setting.

A. polyacanthus proved to be a useful model species for investigating non-genetic parental effects on a range of offspring characteristics. Importantly, because of the high level of development at hatching experiments were able to be conducted on survival abilities of juveniles immediately after hatching. Along with the benefits of using *A. polyacanthus* potentially come some limitations. Since this species does not possess a pelagic larval stage it could be argued that the results of this study are not relevant to other coral reef fishes. However, all efforts were made to limit the parental effects that could occur through post-hatching care. Furthermore, existing research on parental effects in reef fishes supports many of the physical differences observed in this study. For example, research on parental effects in *Amphiprion melanopus* showed modifications to egg length and offspring size at hatching (Green and McCormick, 2005a). Additionally, diet supplementation of breeding *P. amboinensis* caused an increase in the size of progeny's yolk reserves (Kerrigan, 1997, McCormick, 2003, Gagliano and McCormick, 2007). Consequently, it is concluded that the effects of parental condition on the observed offspring characteristics are likely to be relevant to other reef fishes.

It is generally believed that maternal influences to offspring are more prevalent than paternal because of the differential investment in gametes (Bernardo, 1996a). In this study, multiple offspring characteristics were found to correlate to the size of eggs produced, including offspring and yolk size at hatching. This clearly provides evidence for maternal influences, however some variation in offspring characteristics was not explained by egg size alone and survival of individual fish did not correlate well with yolk size. This suggests that additional maternal effects to offspring may occur through alternative means such as yolk composition (Sargent, 1989, Rainuzzo et al., 1997). However, there is also the potential for fathers to have affected the phenotype of their offspring. Paternal effects largely determined offspring growth rate in *A. melanopus* (Green and McCormick, 2005a) and this was suggested to relate to the increased investment by fathers through nest tending (Green and McCormick, 2005b). In *A. polyacanthus* males provide care during the nesting stage (Pankhurst et al., 1999) and, therefore, it is possible that they exerted some phenotypic effects on their offspring.

This study provides clear evidence that the food supply available to breeding pairs of a coral reef fish can have significant impact on their reproductive output and the success of their offspring. A range of offspring characteristics were found to be modified by parental body condition, including egg size, larval size and the size of yolk reserves. Size differences at hatching are likely to provide offspring from well-conditioned parents with substantial benefits that could lead to improved growth and survival. Furthermore, offspring of these parents have a better ability to survive on their endogenous reserves. This may be critically important in an environment where food is patchily distributed. Differences in timing of reproduction along with physical differences between the offspring of parents on high and low quantity diets indicates that variation in diet experienced by adults can have far reaching effects on individual fitness and ultimately on the dynamics of local populations.

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