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Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish

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ABSTRACT: Larval mortality in marine fishes is strongly linked to individual life history traits such as size and growth, but the processes that influence variability in these traits are poorly understood. We explore the relative importance of maternal and paternal influences and water temperature on the larval growth and performance characteristics of the tropical clownfish *Amphiprion melanopus* (Pomacentridae). Larvae were reared from an incomplete 4 male × 4 female diallel breeding cross at 2 temperatures (25 and 28°C). Paternity interacted with maternity and affected traits immediately prior to and after hatching. Size of larvae at metamorphosis was primarily affected by maternal and paternal influences, but not by rearing temperature. Paternity explained 52% of the variance in growth rates to metamorphosis, while the combination of paternity, maternity and temperature explained 30%. This strong paternal influence may be due to the extensive role males play in nest-tending coupled with the relatively long embryonic duration of the species. A negative relationship between larval growth rate and mortality within a tank emphasised the importance of these parental effects to larval survival and also provides support for the 'bigger is better' hypothesis.

KEY WORDS: Larval growth · Maternal effects · Mortality · Paternal effects · Swimming ability · Temperature

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INTRODUCTION

The population dynamics of marine fishes are characterised by high larval mortality (Ferron & Leggett 1994), which is predominantly driven by size- and growth-selective processes (Meekan & Fortier 1996). Size at hatching and pre-settlement growth strongly influence survival before and after settlement (Bergenius et al. 2002, Shima & Findlay 2002); however, the seminal causes of variation in larval size and growth are poorly understood. Intrinsically, variation in pre-settlement growth, and the body characteristics that co-vary with growth, are derived from 2 sources: parental influences and environmental fluctuations (Chambers & Leggett 1996). The interaction between these factors is also likely to be important for larval growth, but has rarely been examined (but see Benoit & Pepin 1999).

Environmental factors encompass the physical and biotic processes acting on developing eggs and larvae. Condition and growth in fish larvae are influenced by fluctuations in food (Green & McCormick 1999), temperature (Green & Fisher 2004), salinity (Sponaugle & Pinkard 2003), turbidity (Cobcroft et al. 2001), and turbulence (Utne-Palm & Stiansen 2002). Similarly, the oxygen and temperature environment into which eggs are laid may influence early developmental rates and size at hatching (e.g. Collins & Nelson 1993, Cancino et al. 2003, Green 2004). As the larva exhausts its yolk-sac reserves and begins exogenous feeding, water temperature (Houde 1990, Houde & Zastrow 1993) and the timing of prey production cycles (Cushing 1990) become particularly important determinants of survival. However, in wild fish populations, environmental factors explain less than 40% of variation in larval growth (Wilson & Meekan 2002, Caldarone et al. 2003),

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suggesting that other processes, such as parental effects, must account for a substantial amount of variability in growth.

Maternal contributions are generally considered more important than paternal contributions, due to nutritional provisioning of the embryo (Bernado 1996). In fishes, embryo and larval characteristics such as egg size, developmental rate, metabolism, growth and viability are affected by the body condition and genotype of the female parent (Chambers et al. 1989, Chambers & Leggett 1996, Kerrigan 1997, Marteinsdottir & Steinarsson 1998). Non-genetic maternal contributions take many forms that directly influence survival probabilities of larvae, including nutritional reserves (Kerrigan 1997), levels of developmental and metabolic hormones (Brown et al. 1988, McCormick 1998, 1999), and parental care (Bernado 1996). While paternal effects have been identified in fishes (Heath et al. 1993, Herbinger et al. 1995, Hoie et al. 1999a, Yamamoto & Reinhardt 2003), they are not detectable in all larval traits (Hoie et al. 1999b), and are often not considered.

Research on maternal effects in marine fishes has focused on commercially important species, which generally spawn pelagic eggs or benthic eggs without parental care (e.g. Altantic cod *Gadus morhua*, capelin *Mallotus villosus*; Chambers & Leggett 1996). In species that lay benthic eggs, males are generally responsible for nest-tending and nest-site selection (Clutton-Brock 1991). Parental care enhances offspring development (Sargent 1988, 1997), and is therefore a mechanism whereby males can invest in the survival potential of their offspring. Consequently, it is likely that variation in the size and condition of offspring from parentally tended eggs will reflect the male's contribution (Bernado 1996).

The present study experimentally examined the relative importance of paternal, maternal and temperature influences on early life history traits from conception to metamorphosis in a marine fish with paternal egg care. Temperature was selected since it strongly influences metabolism and growth in ectotherms, and the incubation and larval environments can differ markedly in temperature. We employed a diallel breeding design as it allows the variance attributed to the maternal, paternal and temperature components to be partitioned (Heath & Blouw 1998). Larvae were reared through to metamorphosis under experimental manipulations of parentage and water temperature and their growth and performance were assessed.

MATERIALS AND METHODS

Study species and brood stock. This study focused on the anemonefish *Amphiprion melanopus* (Pomacentridae), a common damselfish on coral reefs in the Indo-Pacific. This species was an ideal model for this study as it lays benthic eggs with a relatively long embryonic duration (8 to 9 d, Job & Bellwood 2000) and exhibits parental egg tending. While nest care involves both parents, males invest more time (40 to 70%) in tending embryos than females (20 to 30%) (Green 2004).

Brood stock pairs of *Amphiprion melanopus* were collected by professional aquarium fish collectors from mid-shelf reefs in the northern section of the Great Barrier Reef, adjacent to Cairns (16° 8′ S, 145° 7′ E), where they would naturally experience annual temperature fluctuations from 25 to 30°C (see www.aims.gov.au/pages/facilities/weather-stations/weather-index.html). Pairs were maintained in 100 l, round, flow-through tanks at 28°C at the James Cook University research aquarium for 2 yr and provided with hollow cement blocks (internal dimensions 16 × 16 × 19 cm) as a nesting substratum, simulating a protected nest. At the commencement of the experiment, adult fish were anaesthetised with clove oil, weighed for wet weight and measured for total length, body depth and body area. A measure of adult condition was estimated by Fulton's K condition factor, i.e. weight (g)/standard length $(mm)^3 \times 100$. Sex of each individual was determined by the size of the fish: as a protandrous species, the females are generally considerably larger than the males. This was cross-checked with the shape of the genital papillae, which is short and round in females and long and triangular in males (B. Green pers. obs.). Nesting behaviour and egg-laying was also used to cross-reference sexual identity (Green 2004). We resolved that size was an accurate proxy of sex.

Partitioning maternal, paternal and environmental effects. To test for parental and environmental influences on larvae, we employed a 4 female × 4 male diallel cross, producing 16 clutches from 16 unique parent combinations. A diallel breeding design is powerful in estimating the variation in offspring phenotype due to parental and environmental determinants (Heath & Blouw 1998). Within a diallel cross design, each female is mated with each male, creating a series of full and half-sibling crosses that replicates clutches produced by each female and by each male. Only 14 clutches were available from the current design because of problems with freezer storage. At the commencement of this experiment, brood stock comprised established breeding pairs, so to avoid effects of pairwise breeding history, each male was moved into a tank with a female that was not previously its breeding partner, commencing with the first cross. Females remained in the same tank and the males were moved for each new mating. Low numbers of sparsely distributed eggs were generally laid in the first clutch from a newly

matched pair of *Amphiprion melanopus* (B. Green pers. obs.); therefore, only the second brood from every new mating was sampled. This generally took 4 to 6 wk, as *A. melanopus* produce a clutch every 2 wk, and new pairs generally took 2 to 4 wk to produce their first clutch.

Each clutch of eggs resulting from the diallel cross was left with the parents until the night of hatching, 8 d after they were laid. Prior to dusk on the night of hatching, clutches were removed from their natal tank, taken into a temperature-controlled laboratory, photographed under running water and then hatched at dusk in a 70 l tank. The hatching tank was a closed system and aeration was used to simulate parental tending, and stimulate hatching (Green & McCormick 1999).

Temperature manipulation. Immediately after hatching, all larvae from a clutch (between 300 and 700 larvae) were transferred at random into ten 70 l glass aquaria held at 28°C. The temperature in 5 tanks was gradually adjusted to 25°C overnight, following hatching, and 5 tanks were maintained at 28°C. Through the remainder of the larval phase, 5 tanks were maintained at 28°C and 5 tanks at 25°C for each experimental clutch, resulting in 10 tanks testing 2 replicate temperatures per clutch. All 14 clutches of larvae resulting from the 4×4 diallel cross design were divided into the 2 rearing temperatures, with 5 replicate tanks of each in this manner. Differences in larval density within tanks were assumed to be negligible: based on hatchling weight of 0.001 g, the density difference equated to a maximum initial mass difference of 0.006 g l^{-1}.

Larval rearing. Larvae reared for the temperature manipulation experiment were maintained in 'green water' in an indoor laboratory following the methods of Green & McCormick (1999), whereby a *Nannochloropsis* sp. algal culture was added to the tanks each morning. Tanks were lit by fluorescent lights simulating a 14 h light:10 h dark summer light-cycle, and maintained as a semi-closed system, flushed nightly with temperature controlled water (at either 25 or 28°C, depending on experimental treatment) when the lights were off. Larvae were fed the rotifer *Brachionus* sp. at a density of approximately 5 ind. ml^{-1} for 1 to 3 d after hatching, and on Day 3 after hatching, *Artemia* sp. nauplii were added at a concentration of 1 to 2 ind. ml^{-1} (Green & McCormick 1999).

Sampling and morphometric measurement. ***Clutch size and density:*** Parental influence on clutch dimensions and densities per diallel combination were measured prior to the temperature-manipulation experiment. All egg clutches were photographed prior to hatching, and clutch dimensions were later measured using the UTHSCSA Image Tool graphics package (University of Texas, San Antonio). Egg-clutch area, perimeter and total number of eggs within the clutch were measured. Egg density was calculated as egg density = number of eggs/clutch area.

Egg size: To compare mean egg size between diallel clutches prior to temperature manipulation, 10 eggs from each clutch were sampled immediately prior to hatching and preserved in alcohol. Images of the preserved eggs were made with a digital camera (Olympus DP12) connected to a stereo dissector microscope and egg length, width and area were measured using the UTHSCSA image tool.

Post-hatching larval size: To compare maternal and paternal influences on mean size of newly hatched larvae between diallel clutches prior to temperature manipulation, 10 larvae were left in the hatching tank and maintained at 28°C overnight. They were sampled the following morning (1 d after hatching) and preserved in 70% ethanol for at least 2 mo. Newly hatched larvae were blotted dry with tissue paper, and their wet weight was measured to 3 decimal places. Following this, standard length and total length were measured under a dissecting microscope with a graduated eyepiece.

Size at metamorphosis: Temperature-manipulated rearing experiments were terminated at metamorphosis, when all fish were sampled. Metamorphosis was defined as when more than 70% of individuals from a tank had their post-orbital white strip and settled to the bottom of the tank (Green & McCormick 1999, 2001). Fish were preserved and measured for standard length and total length under a dissecting microscope with a graduated eyepiece. Fish were blotted to remove excess water, and their wet weight was measured to 3 decimal places. As growth of *Amphiprion melanopus* was linear during the larval phase under similar artificial experimental conditions (plotted from Green & McCormick 2001) growth rates were estimated according to the formula: $R_g = (L_s - L_h)/T_s$, where R_g is the rate of growth (mm d^{-1}), L_s is the length (mm) at sampling, L_h is the length (mm) at hatching, and T_s is the time (d) from hatching to sampling.

All specimens were preserved in ethanol and treated in the same manner so that any shrinkage due to ethanol storage would be consistent among samples.

Quantifying swimming performance. Critical swimming speed (*U*-crit, following Brett 1964) was used to determine the effects of rearing temperature and parentage on the functional swimming capabilities of larvae from 8 clutches from the diallel design. *U*-crit is a measure of the maximum sustainable swimming speed of larvae. Temperature affected developmental rate, and the effect of temperature treatment on swimming on absolute versus developmental age has been previously addressed (Green & Fisher 2004). For the purpose of the current study, swimming measurements were taken for fish of the same developmental age, at

7 d after hatching (dah) for fish reared at 28°C, and at 11 dah for fish reared at 25°C. This was an *a priori* sampling decision to coincide with 1 d prior to the average time taken to metamorphosis for the 28°C and 25°C treatments respectively (Green & Fisher 2004). We measured the swimming speed of 2 fish from each of the 5 replicate tanks at each temperature treatment (25 and 28°C), making a total of 10 larvae per clutch at each temperature. Details of all swimming trials are given in Green & Fisher (2004).

Table 1. *Amphiprion melanopus*. Correlations (r) of brood stock and egg clutch attributes. No correlations were significant at the Bonferroni corrected α-value of $p < 0.007$; *: significant correlations at $p < 0.05$, n = 11. TL: total length

Variable	No. of hatchlings	Clutch perimeter (mm)	Clutch area (mm^2)	Egg count	Egg density (mm^{-2})
Female weight	0.39	0.06	0.32	0.24	<0.01
Female TL	0.61*	0.23	0.49	0.68*	0.22
Male TL	−0.40	0.33	0.09	−0.19	−0.43
Male weight	−0.31	0.00	−0.09	−0.28	−0.33
Female + male size	0.20	0.41	0.45	0.40	−0.13
Female Fulton's *K*	−0.52	−0.25	−0.46	−0.69*	−0.24
Female + male weight	0.14	0.06	0.22	0.03	−0.22

RESULTS

Parental influence on clutch size and density

No pre-hatch clutch traits were correlated with parental characteristics at the Bonferroni corrected α-value of 0.0071; however, at the uncorrected α-value of <0.05, female total length and Fulton's *K* correlated with clutch attributes immediately before and after hatching. Female total length was positively correlated with the number of eggs in a clutch and the number of hatchlings, but not with clutch size or dimensions, and Fulton's *K* was negatively correlated to egg count (Table 1). Male size, and combined female and male size were not correlated with any of these clutch characteristics.

Parental influence on egg size

A multivariate analysis of variance (MANOVA), exploring trends in the individual egg characteristics (egg length, width and area) simultaneously, showed significant effects of male ($F_{8,398} = 15.65$, Pillai's trace, $p < 0.001$), female ($F_{4,198} = 15.7$, Pillai's trace, $p < 0.001$) and the interaction of male and female (m × f), ($F_{20,804} = 16.3$, Pillai's trace, $p < 0.001$) on all 3 egg characteristics. Females produced clutches that differed in mean egg length when mated with different males. Female f3 had the largest eggs when paired with 3 of the 4 males. In contrast, Female f1 produced the smallest eggs when paired with 2 males, but produced some of the largest eggs when paired with Male m1 (Fig. 1A).

Parental influences on larval size

The standard length of larvae 1 d after hatching differed when females were mated with different males

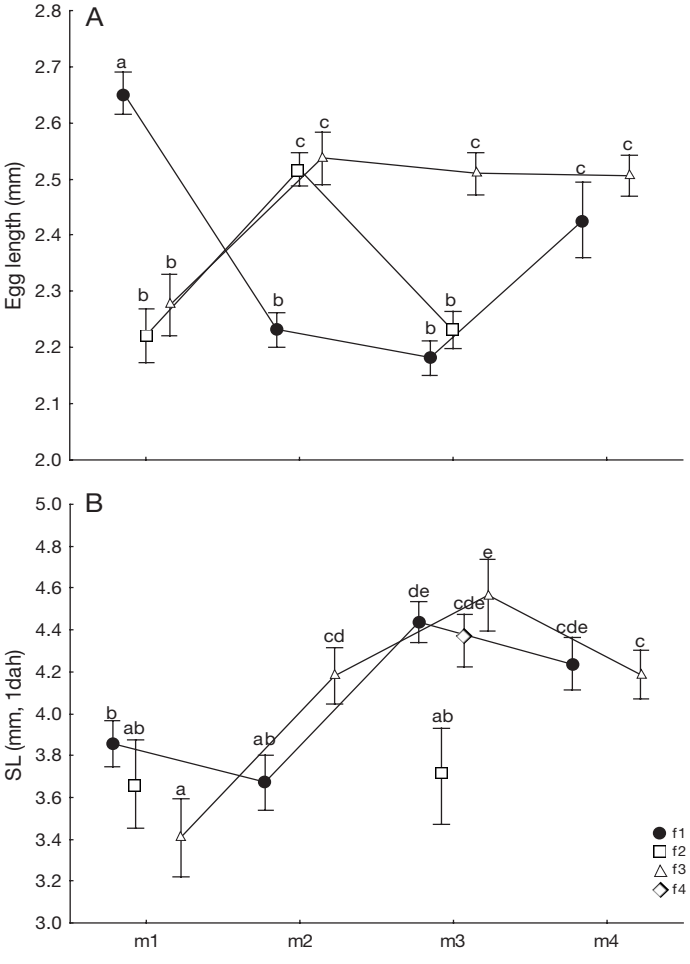

Fig. 1. *Amphiprion melanopus*. Combined effects of male (m1 to m4) and female (f1 to f4) identity on offspring size. (A) Pre-hatch egg length; (B) standard length (SL) of larvae 1 d after hatching (dah). Letters above error bars indicate Tukey's HSD (honestly significant difference) homogenous groups ($p < 0.05$). Tukey's HSD identify where differences lie and, as such, each letter represents significantly different subgroup. Error bars are 95% confidence intervals

Table 2. *Amphiprion melanopus*. Comparison of effects of female and male indentity on mean standard length (SL) of offspring 1 d after hatching: 4 × 4 diallel breeding design, using Type IV SS. Significant differences in **bold**

Variable	df	MS	*F*	p
Male	4	1.21	2.20	0.230
Female	4	1.07	0.35	0.723
Female × male	161	0.08	15.019	**<0.001**
Error	180	0.08		

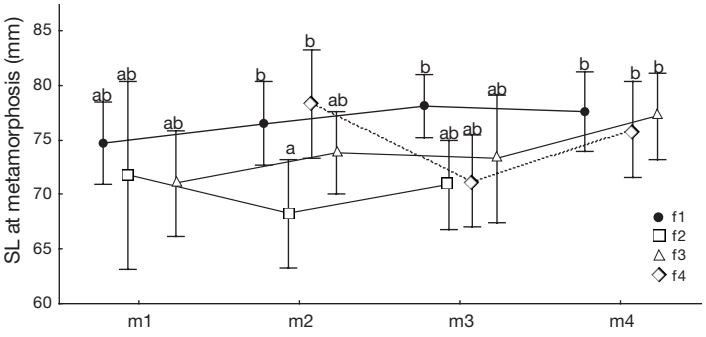

Fig. 2. *Amphiprion melanopus*. Standard length (SL) of metamorphosed larvae from a 4 × 4 diallel cross of males (m1 to m4) and females (f1 to f4). Letters a and b indicate Tukey's homogenous groups ($p < 0.05$). Error bars are 95% confidence intervals

(Table 2, Fig. 1B). Sizes of hatchlings from Female f3 were significantly different when she was mated with 3 of the 4 males. Larvae produced by f3 were smallest when sired by Male m1 and largest with Male m3. Hatchlings produced by Female f1 were significantly different between 3 males, while hatchings produced by Female f2 showed no difference between males. The influence of the male on his offspring differed, depending on the identity of the mated female (Fig. 1B).

Parental and temperature effects on offspring at metamorphosis

The standard length of larvae at metamorphosis differed as a function of female identity and between the various female/male combinations, while temperature did not have an effect (Table 3, Fig. 2). Larvae from Female f1 were significantly larger at metamorphosis than larvae from Female f2 when both females were crossed with Male m2, but not when crossed with the other males. There were no significant differences in size of larvae at metamorphosis in any larvae from females crossed with Male m4 (Fig. 2).

Table 3. *Amphiprion melanopus*. Comparison of effects of female and male identity and temperature on standard length and weight measured at metamorphosis of offspring from diallel breeding design. Results of multivariate ANCOVA controlling for time of clutch hatching are given. Significant results in **bold**

Variable	Effect df	Error df	Pillai's trace	*F*	p
Temperature (*T*)	2	1346	0.00	0.57	0.567
Female	6	2694	0.01	2.70	**0.012**
Male	6	2694	0.01	1.22	0.290
T × Female	6	2694	0.01	1.17	0.319
T × Male	6	2694	0.01	1.57	0.151
Female × Male	14	2694	0.02	1.74	**0.042**
T × Male × Female	14	2694	0.01	1.20	0.262
Tank (*T* × Male × Female)	204	2694	0.16	1.13	0.109

While larvae raised at the lower temperature (25°C) metamorphosed at a similar size to larvae raised at ambient temperature (28°C) (non-significant temperature effects, Table 3), they required more days of development to reach metamorphosis (Fig. 3; temperature × male × female (*T* × m × f): $F_{7,1348} = 4069$, $p < 0.001$). Time to metamorphosis did not vary in offspring from Females f1 at 28°C and f4 at 25°C with all males, while for Female f2, time to metamorphosis varied with all males at 25°C, and with Males m1 and m2 at 28°C, and Female f3 varied with multiple males at each temperature (Fig. 3). Although there were significant differences as a function of parentage, offspring reared at 25°C always took longer to reach metamorphosis than those reared at 28°C. The mean time to metamorphosis, regardless of parentage, was 8.9 ± 0.06 d at 28°C and 11.6 ± 0.09 d at 25°C.

Individual tanks within temperature treatments and m × f combinations were sampled as units at metamorphosis when at least 70% of the visible fish had metamorphosed; however, the actual number of fish metamorphosed showed more variability. Temperature did not affect the proportion of fish metamorphosed (temperature: $F_{1,103} = 2.515$, $p = 0.11$; *T* × m × f: $F_{7,103} = 0.931$, $p = 0.48$); however, parentage did affect the proportion of fish metamorphosed at the time of sampling (male: $F_{3,103} = 4.348$, $p < 0.05$; female: $F_{3,103} = 3.453$, $p < 0.05$; m × f: $F_{7,103} = 3.42$, $p < 0.05$).

Growth rate and mortality

Paternity and maternity also influenced the growth rate and mortality of larvae raised at the 2 temperatures (Table 4, Fig. 4). Larval growth rate varied significantly at all levels in a factorial analysis of temperature, female and male identity (Table 4). The combination of

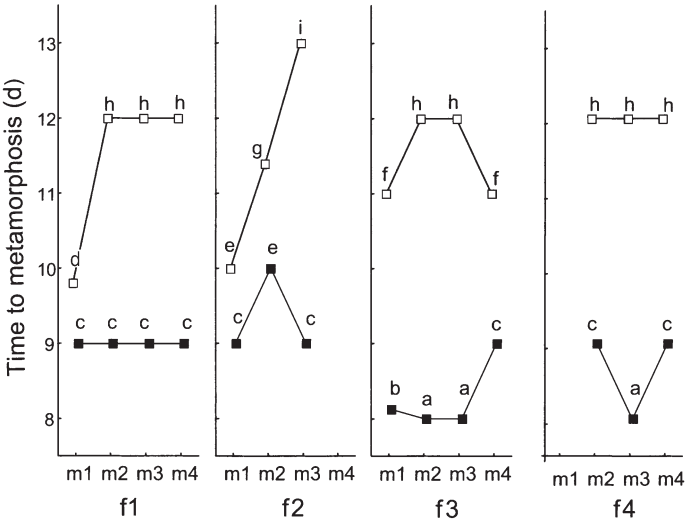

Fig. 3. *Amphiprion melanopus*. Mean time to metamorphosis of larvae from a 4 female × 4 male diallel cross raised at 25 (□) and 28°C (■). Means of 5 replicate tanks per temperature. Letters a to i indicate Tukey's homogenous groups ($p < 0.05$); 95% confidence intervals were included, but are not visible as they are smaller than the data points

these 3 factors accounted for 30% of the total variability in larval growth rate, while male identity alone accounted for 52% (Fig. 4A). Growth rates were generally higher at 28°C than at 25°C (Fig. 4A).

Mortality rates differed as a function of male and female identity, $T \times$ f and a combination of $T \times$ f × m (Table 4, Fig. 4B). More than 50% of the variability in mortality was due to differences among replicate tanks, while a further 28% was attributable to the combination of temperature and male and female identity (Fig. 4B).

Mortality rate within a tank was negatively correlated to growth rate averaged for that tank ($r = -0.30$, $p < 0.005$), and there was little change when the effects of temperature were excluded (partial correlation, $r = -0.29$, $p < 0.05$).

Table 4. *Amphiprion melanopus*. Mean growth rate and mortality of larvae; full factorial ANOVA of 4 female × 4 male diallel cross raised at 2 temperatures, measured at metamorphosis. Significant results in **bold**

Variable	df	MS	*F*	p
Growth rate				
Temperature (*T*)	1	0.326	582.65	**<0.001**
Female	3	0.002	3.36	**0.023**
Male	3	0.053	96.10	**<0.001**
T × female	3	0.003	4.56	**<0.005**
T × male	3	0.006	10.63	**<0.001**
Female × male	4	0.003	4.46	**0.002**
T × female × male	4	0.006	11.62	**<0.001**
Error	77	0.00056		
Mortality				
Temperature (*T*)	1	155.1	0.32	0.572
Female	3	3486.0	7.21	**<0.001**
Male	3	1775.4	3.67	**0.015**
T × female	3	587.8	1.21	0.310
T × male	3	1550.0	3.20	**0.028**
Female × male	4	697.4	1.44	0.228
T × female × male	4	1545.9	3.19	**0.017**
Error	77	483.8		

Maternal and temperature effects on swimming performance

Swimming performance, measured as critical swimming ability (*U*-crit), was affected by rearing temperature and female identity (Table 5, Fig. 5). Differences in the swimming ability of larvae from different females were not directly linked to the size of the female, as larvae from the smallest Female f3 achieved the fastest swimming speeds, while larvae from the medium-sized Female f4 achieved the slowest swimming speeds. However, larvae raised and tested at 28°C (ambient temperature) were capable of faster speeds than larvae raised and tested at 25°C (Fig. 5).

DISCUSSION

Maternal and paternal influence on clutch, egg and larval size

Maternal and paternal effects explained a large proportion of variation in offspring traits in *Amphiprion melanopus*, from conception to metamorphosis. Maternal influences were apparent in clutch traits prior to hatching, and maternal and paternal traits were impor-

Table 5. *Amphiprion melanopus*. Comparison of effects of female and male identity and temperature on mean critical swimming performance. (A) Results from full factorial cross; (B) summary of female and temperature effects only. Significant results in **bold**

Variable	df	MS	*F*	p
(A) Female	1	343.90	11.34	**<0.001**
Male	1	0.24	0.01	0.929
Temperature (*T*)	0	0		
Female × male	3	68.98	2.27	0.081
Female × *T*	1	4.99	0.16	0.685
Male × *T*	1	7.41	0.24	0.621
Female × male × *T*	3	50.18	1.65	0.178
Error	222	30.32		
(B) Female	2	349.93	11.28	**<0.001**
Temperature (*T*)	1	661.48	21.33	**<0.001**
Female × *T*	2	17.75	0.57	0.565
Error	232	31.01		

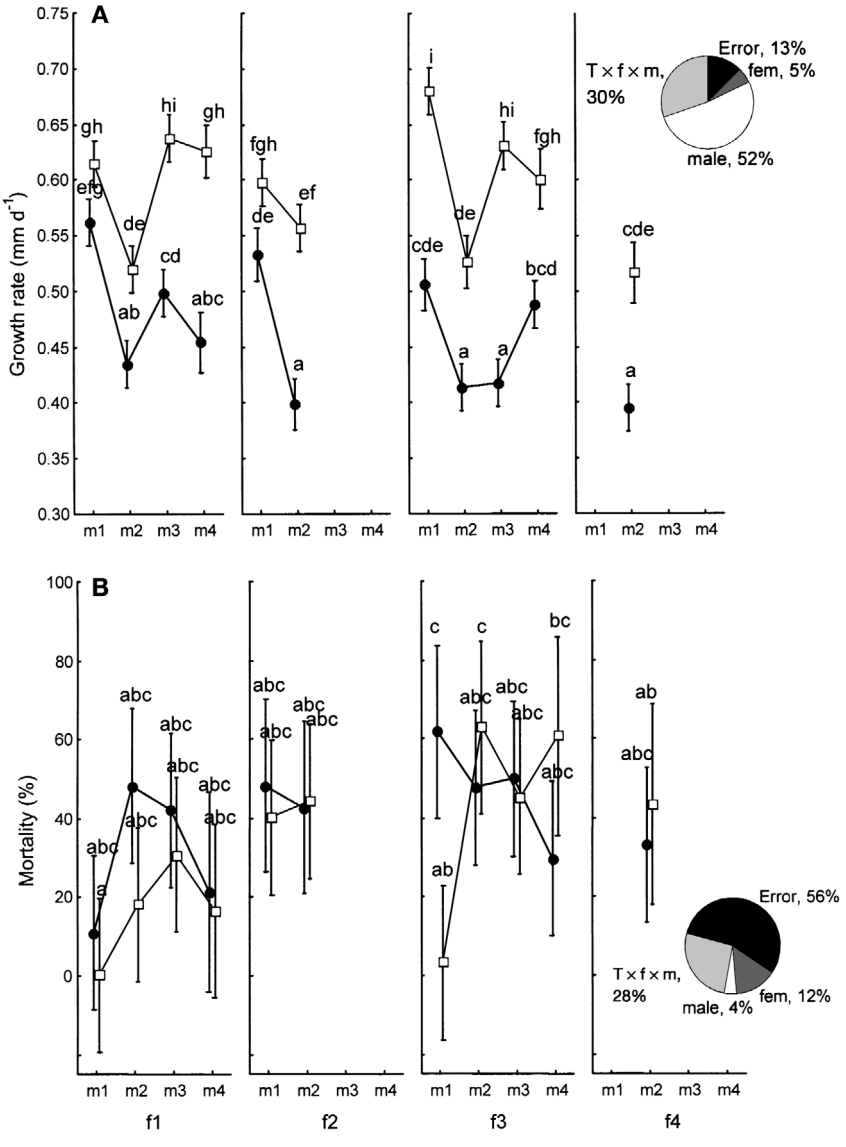

Fig. 4. *Amphiprion melanopus*. (A) Growth rate and (B) mortality of larvae raised at 2 temperatures (25 ● and 28°C □) from a 4 female × 4 male diallel cross, determined at metamorphosis. Letters a to i indicate Tukey's homogenous groups ($p < 0.05$). Error bars are 95% confidence intervals. Pie-charts represent variance components calculated with time of experiment as a covariate; where *T* × m × f: interaction temperature × male × female, fem: female

tant immediately after hatching through to metamorphosis. Within the 14 clutches resulting from the 4 × 4 diallel cross of males and females, clutch size and dimensions were more closely related to characteristics of the female parent than to those of the male; however, males influenced the pre-hatch trait egg size. Interestingly, after hatching, the male × female interaction became apparent in all larval fish traits measured. This is interesting, as most studies have shown that maternal heritabilities are higher than paternal heritabilities in early life (Heath & Blouw 1998)—if the sire component is characterised at all (Heyer et al. 2001). However, a strong paternal component is expected in organisms with nest-care behaviour (Bernado 1996).

The important male contribution to early life history traits can be interpreted in the light of the differential parental contributions from conception to hatching in *Amphiprion melanopus*. The territorial male prepares a nest site, where the female lays a monolayer of between 300 and 700 eggs on the substratum (Allen 1980). The male follows, fertilising the eggs and contributing 50% of the genetic material, but little else, to the initial egg allocation. *A. melanopus* has a long embryonic duration compared to many tropical species (i.e. 8 d, compared to 4 d for other pomacentrids [Job & Bellwood 2000] and 24 h for a pelagic spawning serranid [Masuma et al. 1993]) and therefore an extended dependence on maternally derived yolk supply. Consequently, the maternal investment in the endogenous resources of the larvae is high. While male defence and maintenance of the eggs are critical for their survival (Tyler 1995), his nest-care has no detectable effect on clutch size, but may be responsible for the paternal effect apparent in pre-hatch egg size.

Paternal influences were detected in eggs immediately prior to hatching and in newly hatched larvae. As the embryos develop, incorporating the male genetic contribution, the male actively tends them, fanning to oxygenate the eggs, and mouthing to remove dead eggs (Green & McCormick 2005). The male ensures maintenance of the embryonic environment by keeping the eggs well-oxygenated in a cryptic habitat that typically has poor water flow. He initially spends 40% of his time tending, increasing this to 70% towards hatching, compared with only 20 to 30% time tending by females (Green & McCormick 2005). Parental care enhances offspring development (Sargent 1988, 1997), and increases the chance of survival in offspring of fishes (Sabat 1994, Kuwamura 1997). Paternal fanning that enhances growth, development and survival, distinguishes *Amphiprion melanopus* from many of the species commonly targeted for the study of parental effects (e.g. Atlantic cod: Chambers & Leggett 1996, Marteinsdottir & Steinarsson 1998). Most studies addressing parental influences that did not detect a paternal effect used fish species that do not display parental care of the eggs, where the opportu-

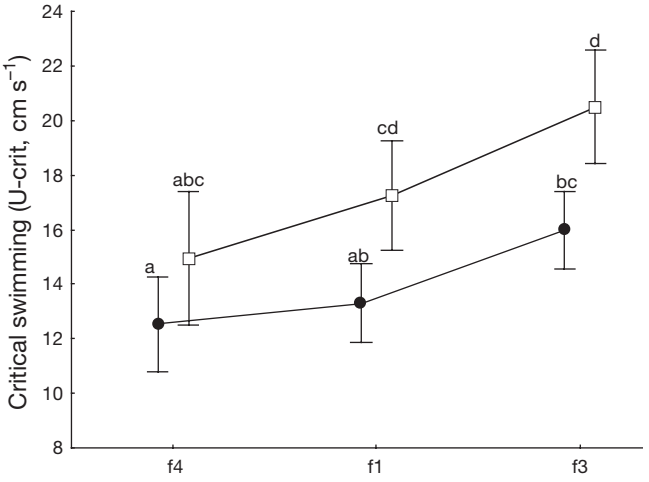

Fig. 5. *Amphiprion melanopus*. Critical swimming performance of larvae raised at 2 temperatures from 3 females measured 1 d prior to settlement. Error bars are 95% confidence intervals. Superscripts [a] to [d] indicate Tukey's homogenous groups ($p < 0.05$). (●) 25°C; (□) 28°C

nity for a significant male contribution was restricted (e.g. Benoit & Pepin 1999). The predominately male tending, coupled with long embryonic duration and ensuing dependence on the maternal contribution, increases the prospect for parental contributions to the larval characteristics of *A. melanopus*.

Temperature effects on larval traits

Time to metamorphosis

Temperature did not affect size at metamorphosis, but it did affect the time until metamorphosis and also larval growth rates. *Amphiprion melanopus* larvae raised at 25°C (representing the lower limit of their temperature range) metamorphosed at similar sizes to larvae raised at ambient temperature (28°C); however, fish raised at 25°C took longer to reach metamorphosis. The strong influence of water temperature on larval duration rather than size at metamorphosis suggests that metamorphosis is dependent on a size-related threshold rather than age, similar to starry flounder (Policansky 1983), winter flounder (Chambers & Leggett 1987) and a tropical goatfish (McCormick & Molony 1995).

Temperature and parental influence on swimming performance

Rearing temperature and maternity each affected maximum sustained swimming speed, while a paternal component was not identified. As the water temperature within the swimming chamber has no detectable influence on swimming speed (Chambers & Leggett 1996, Green & Fisher 2004), the reduction in swimming performance with decreased rearing temperature is not explained by the fish's metabolic response to the immediate water temperature. Swimming performance is most probably related to larval body condition, which is a sum of its genotype, modified by environmental history (Brett 1967, Angilletta et al. 2003). In young fishes, environmental effects can significantly modulate the maternal effect, causing variation in traits at the end of the larval period (Chambers & Leggett 1996).

Growth rate and mortality

While there were differences in larval growth rate at all levels of the male × female × temperature experimental design, the majority of variance in growth rate was attributable to paternity, followed by the interaction of male and female identity and temperature. We found some evidence that initial parental influences are maintained until metamorphosis, by following sibling groups rather than individual growth trajectories. Growth rates were highest in larvae from the Females f1 and f3, which also had the largest eggs and larvae at hatching. These growth rates and sizes identify a maternal and paternal contribution to initial offspring size that is maintained, although in weakened form, throughout the larval period to metamorphosis. The persistence of parentally derived traits throughout larval development is rare, although egg size has predicted larvae and juvenile size (Reznick 1991, Roff 1992, Chambers & Leggett 1996), and hatching size has predicted juvenile size in wild populations (Vigliola & Meekan 2002). Generally, environmental variation and favourable conditions after hatching can cause parentally derived growth differences to disappear (De March 1991, Hoie et al. 1999a). While the results from the present study reflect similar significant patterns in wild populations (Vigliola & Meekan 2002), they should be interpreted carefully due to the small sample size of this diallel design.

The interaction of paternity, maternity and temperature influenced mortality rate and, more importantly, experimental units (tanks) with fast growth had lower mortality than units with slow growth. Such a relationship between growth and survival has been coined the 'bigger is better' hypothesis (Miller et al. 1988, Bailey & Houde 1989) and is based on the reduced likelihood of predation for bigger individuals. Subsequent support for this hypothesis has shown that fishes that were larger at hatching and had subsequent higher growth, generally survived selective mortality (Anderson 1988, Meekan & Fortier 1996, Vigliola & Meekan 2002). Our

study suggests that there are parental origins for this relationship that may determine survival chances for the offspring in the absence of predators.

More than half of the variability in mortality was attributable to unknown sources among replicate rearing tanks. Contrary to most studies on maternal effects (see Heath & Blouw 1998 for discussion), there were minimal tank- or rearing-environment effects in the current study, with the exception of mortality. Particular attention was paid to maintaining identical conditions within all rearing units.

This study addressed the 'within population' variability in offspring traits that have maternal and paternal influences, and found significant effects from both these sources for the species *Amphiprion melanopus*. Maternal effects also exist between populations and between species (Chambers & Leggett 1996), and therefore it is likely that paternal effects do so also, at least in species with paternal nest-care. To determine the influences of natural variation in recruitment to wild populations, studies of the variation in parental effects between populations is important. Identifying that maternity, paternity and their interaction are critical determinants of larval size and performance is a vital first step in looking for sources of variation in populations that has previously been overlooked as ecological noise.

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