Trade-offs obscure the relationship between egg size and larval traits in the diadromous fish *Galaxias maculatus*

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ABSTRACT: Egg size is one of the most frequently used surrogate measures of maternal investment and is strongly related to fitness-determining traits, such as offspring size in many marine animals, but the relationship is not universal. Because the amount of yolk allocated to eggs is finite, not all fitness-determining traits can be simultaneously maximised, and trade-offs should be expected. The results of the present study show that egg size (quantified as cross-sectional area) poorly predicts the size of larval morphological traits (length, yolk sac area, oil globule area, myomere depth, and eye area) in the native Australian fish *Galaxias maculatus*. Egg size was correlated with yolk sac size and larval body depth, but it explained <13% of the total variation in these traits. Moreover, egg size did not predict the time it took for larvae to hatch, nor did it predict the duration from hatching to starvation. However, when egg size and yolk sac size (i.e. yolk remaining at hatching) were both included as predictors, the fits with larval traits improved greatly ($r^2 = 0.02$ to 0.46 for larval length, $r^2 = 0.11$ to 0.17 for myomere depth, and $r^2 < 0.001$ to 0.14 for eye area). These findings indicate that there is a trade-off between the quantity of yolk at hatching and the size of larval morphological traits in *G. maculatus*. The remaining unexplained variation may be a consequence of trade-offs with other unmeasured traits, such as metabolic rate, and the potential that egg size is not a good proxy for maternal investment.

KEY WORDS: Life history theory · Maternal effects · Offspring size · Phenotypic plasticity

INTRODUCTION

The mortality rates of marine invertebrates and fishes in the early life history stages are very high, resulting in only a small fraction of larvae surviving to maturity (Thorson 1950). Starvation and predation are 2 major sources of mortality for feeding marine larvae, particularly soon after hatching (Bailey & Houde 1989, Morgan 1995). Both sources of mortality may be selective, such that particular larval phenotypes are more likely to survive until recruitment. For example, larger and faster growing larvae are frequently shown to have a higher probability of surviving the larval period than smaller and slower growing larvae (Hare & Cowen 1997, Allen 2008). There

are also a large number of other morphological, behavioural, and physiological traits that have been linked to increased larval survival (Morgan 1995). When and how strongly such traits are expressed depends not only on genetics and the environmental conditions experienced during development but also on the extent of maternal provisioning (Bernardo 1996, Rossiter 1996, Marshall & Keough 2007).

Maternal investment of resources to offspring is one of the most important and well-studied maternal influences on larval survival, particularly its effect on larval size (Chambers & Leggett 1996, Chambers 1997, Marshall & Keough 2007). Egg size is the most frequently reported surrogate measure of maternal investment and the most strongly correlated with the

size of larvae at hatching in a large number of marine species (reviewed by Duarte & Alcaraz 1989, Chambers & Leggett 1996, Marshall & Keough 2007). However, many of the studies that examine the relationship between egg size and larval traits compare among group means rather than individuals. When relationships between egg size and larval traits have been examined at the individual level, they tend to be weaker (reviewed by Chambers 1997). For instance, there is a significant relationship between egg size and larval length in Atlantic cod *Gadus morhua*. Egg size explains 58% of the variation in larval length when group means are compared (Marteinsdottir & Steinarsson 1998), but when individuals are compared, only 6% of the variation is explained (Pepin et al. 1997). Consequently, the current evidence for strong relationships between egg size and offspring size may be misleading.

Egg size also affects several other traits in marine larvae that are important for survival, such as energy reserves, growth rate, and duration of the embryonic and larval periods (reviewed by Duarte & Alcaraz 1989, Cowen & Sponaugle 1997, Marshall & Keough 2007). Because the maternal resources that can be allocated to eggs are finite, trade-offs should be expected among larval traits (i.e. not all fitness determining traits can be simultaneously maximised). Interspecific differences in the correlations between components of larval fitness suggest that species adopt different strategies to optimise fitness in response to different rates and sources of mortality (e.g. Herrera et al. 1996, Kavanagh & Alford 2003, Fisher et al. 2007). Fisher et al. (2007) showed that within the genus *Sebastes*, larvae of species that are at greater risk of starvation hatch at smaller sizes with larger energy reserves, and species that are at greater risk of predation and advection hatch at larger sizes with smaller energy reserves.

Evidence for intraspecific variation in the allocation of maternal resources among larval traits important for fitness is extremely rare. Because many studies compare group means, trade-offs at the individual level are likely to be obscured (cf. Chambers et al. 1989). When studies do examine variation at the individual level, trade-offs may be difficult to detect when there is variation in the resources available to invest in traits (van Noordwijk & de Jong 1986). For instance, larvae hatching from large eggs may be larger and have greater energy reserves than conspecific larvae hatching from small eggs, despite a trade-off between larval size and energy reserves. Therefore, studies that examine multivariate relationships among larval traits and egg size at the

individual level may be more likely to find evidence for trade-offs (e.g. Chambers et al. 1989, Heyer et al. 2001).

The common galaxias *Galaxias maculatus* is a species of diadromous fish that exhibits considerable variation in egg size and larval traits (Semmens 2009). During autumn and winter, adult *G. maculatus* migrate downstream towards the spawning sites in estuaries, where they form large shoals (Benzie 1968a, McDowall 1968). Females typically scatter their eggs onto the roots, stems, and leaves of densely growing vegetation inundated by peak spring tides (Barbee et al. 2011). The eggs adhere to the flooded vegetation, and the majority of the embryonic period occurs terrestrially (Benzie 1968a, McDowall & Charteris 2006). Embryonic development to the point of hatching normally takes 10 to 14 d, coinciding with the timing of the subsequent spring tide (Benzie 1968b). Embryos that are not inundated when they are ready to hatch remain metabolically active and able to hatch, but a long delay in hatching can negatively affect fitness-determining traits in larvae (Semmens & Swearer 2011). Once the eggs hatch, larvae move downstream and out to sea, where they remain throughout the 3 to 6 mo period of larval development (McDowall et al. 1994).

Newly hatched *Galaxias maculatus* larvae are precocial relative to the larvae of other species of fishes, possessing pigmented eyes, functional jaws, and large yolk sacs, and display active swimming behaviour (Benzie 1968b, McDowall 1968). These traits suggest that predator avoidance and starvation resistance are important components of larval survival in *G. maculatus*. The speed of hatching is also likely to be an important determinant of fitness because hatching is triggered by inundation on peak spring tides, which occurs infrequently and lasts for short periods. If embryos have not completed development in time, they must delay hatching for a number of weeks until the next spring tide, increasing the risk of starvation (Semmens & Swearer 2011).

The relationship between egg size, larval traits, and survival has not been investigated in *Galaxias maculatus* despite its commercial importance and the fact that it is arguably the most naturally widespread freshwater fish in the world (Berra et al. 1996, Waters & Burridge 1999). The aim of the present study was to examine whether egg size (quantified as cross-sectional area) is correlated with the morphology (length, body depth, eye area, yolk sac area, and oil globule area) and performance (hatching success, speed of hatching, and starvation resistance) of larval *G. maculatus*.

MATERIALS AND METHODS

Relationship between maternal investment and larval morphology

Samples were collected by uprooting vegetation with adhering eggs within 24 h of spawning on 2 occasions: 6 May 2003 from the Maribyrnong River (37° 46.35′ S, 144° 51.14′ E) and 9 May 2004 from the Cumberland River (38° 34.50′ S, 143° 56.96′ E) in southeastern Australia. Samples were taken from multiple locations across the entire spawning ground to ensure that many females were represented in the collections. Eggs were then transported in an insulated container and placed in a controlled temperature room at 17°C within 4 h of collection.

At the time of collection, the uprooted vegetation and adhered eggs were placed in a 2.5 l plastic locking bag, and to maintain humidity, the bag contents were misted with tap water weekly. Handling the eggs greatly increases the risk of mortality from fungal infections (D. Semmens pers. obs.). Therefore, 50 eggs from the Maribyrnong River and 120 eggs from the Cumberland River were individually marked with a labelled pin placed adjacent to each so that their identity was known. When eggs were found in clusters (1 or more eggs within ~5 cm of another) it was assumed that they had been spawned by the same female, and only 1 egg in the cluster was marked. Each marked egg was digitally imaged on the spawning substrate using a Spot Insight monochrome digital camera (model 3.1.0) mounted on an Olympus SZ60 dissecting microscope at 63× magnification. Image analysis software (Image-Pro v.4.5) was used to measure the mean diameter (to 1 μm) of each egg, calculated from automated diameter measurements taken every 2° of arc after manually outlining the chorion. The eggs were close to spherical, so egg size was quantified as the cross-sectional area, calculated using the formula $\pi(\mu \div 2)^2$, where μ is the mean diameter measurement.

Two weeks after they were spawned, the eggs from each collection site were individually hatched in 40 ml glass vials by inundation in water of salinity 6 so that the identity of all larvae was known. Newly hatched larvae were collected within 1 h of hatching and preserved in 10% buffered formalin. Each larva was digitally imaged at 20× magnification in the same manner as the eggs (Fig. 1). Standard length was measured from maxilla to the tip of the notochord. The depth of the myomeres was measured perpendicular to the long axis of the body, immediately behind the anus. Eye size, yolk sac size, and oil globule size were quantified as their cross-sectional areas, calculated from 2 perpendicular diameter measurements, using the formula $\pi[(a \div 2) \times (b \div 2)]$, where a and b are the 2 measurements.

The majority of the larvae were measured in profile. However, for larvae that were laterally flexed, a second image was taken so that their length could be measured by tracing the midline of the dorsal surface. Larvae that were flexed in the dorsoventral plane were measured by tracing the dorsal edge of the vertebral column. Larvae that were flexed in both planes were not measured (Maribyrnong River: n = 11; Cumberland River: n = 19).

Relationship between egg size and hatching success, speed of hatching, and starvation resistance

Eggs were collected on 6 May 2003 from the Maribyrnong River. The eggs were transported, marked, imaged, and maintained as described previously. As before, 50 eggs were hatched in individual 40 ml glass vials. Every 15 min for the first 5 h after inundation, the identity of hatched larvae was recorded and then once again after 24 h. Some eggs
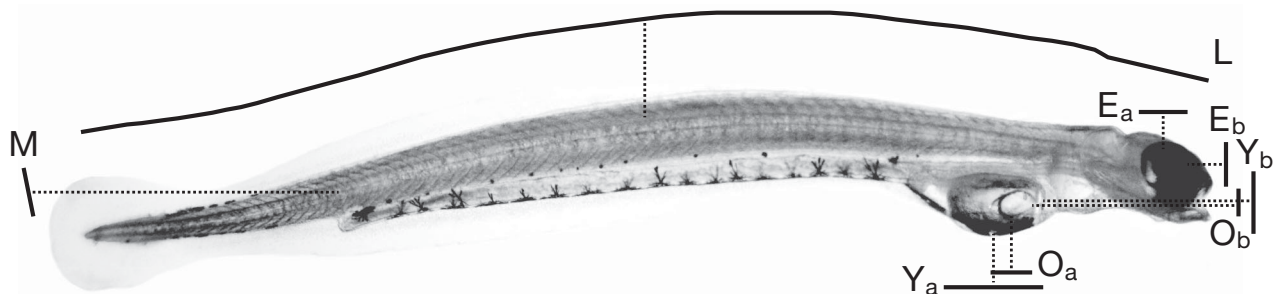

Fig. 1. *Galaxias maculatus.* A newly hatched larva, showing the points at which larval length (L), myomere depth (M), eye diameter (E_a, E_b), yolk diameter (Y_a, Y_b), and oil globule diameter (O_a, O_b) were measured. Because larvae were often flexed in the dorso-ventral plane, length was not measured as a straight line

(n = 8) never hatched and were removed from the starvation experiment.

After 24 h, the water within each vial was replaced with water of salinity 33, and larvae were maintained in their individual glass vials at 17°C until their demise. Larvae were provided with live newly hatched *Artemia* sp. nauplii daily (which they are reported to feed on, see Mitchell 1989), but these were never consumed. Each day, a 5 ml water change was performed, and unconsumed *Artemia* sp. nauplii were removed and replaced. At this time, larvae were observed for 30 s to assess swimming behaviour. When larvae were not swimming, the jars were gently shaken, and larvae that did not begin swimming were deemed to be dead. Dead larvae were removed with forceps and checked for a heart-beat to confirm that they had died. The identity of larvae that had died was recorded before they were preserved in 10% buffered formalin.

Statistical methods

Multivariate analysis of covariance (MANCOVA) was used to test for significant differences in the relationship between the larval traits and egg area between the 2 populations. The MANCOVA found no significant effect of population ($F_{3,133}$ = 1.455, p = 0.209), and the slopes were homogenous ($F_{3,133}$ = 1.158, p = 0.333). The MANCOVA was followed by univariate ANCOVAs to test the relationship between egg area and each trait separately. Because the consequences of making a Type II error were greater, no correction to the α-level was made to control for multiple comparisons (i.e. this increased the likelihood that population differences and heterogeneity of slopes would be found). The univariate ANCOVA tests also found that there were no population differences (p > 0.15 for all traits) and that regression slopes were homogenous (p > 0.25 for all traits), so population was dropped as a factor and the 2 populations were pooled, and the data were reanalysed using simple and multiple linear regression.

Linear regression analyses were used to test the relationships between egg area and the measured traits. Analysing the data this way involved multiple tests of the same data, inflating the familywise error rate. Therefore, the α-level for the tests was adjusted using the Bonferroni correction. Linear regression analyses were also used to test the relationship between egg area and time to starvation and between egg area and hatching speed. An analysis of variance (ANOVA) was used to test whether eggs that

hatched in the first 5 h, hatched after the first 5 h, or did not hatch were different in size.

The yolk sac contents are the residual maternal investment not allocated to embryonic development, and the yolk sac area and oil globule area varied considerably (see 'Results'), which might obscure relationships between egg size and larval traits. Consequently, multiple regressions were used to test the combined effect of egg area and yolk sac area on larval length, eye area, and myomere depth. Although the relationship between egg area and yolk sac area was significant (see 'Results'), egg area explained very little of the variation in yolk sac size (~13%), indicating that collinearity was not of concern. A substantial amount of the variation in oil globule area was explained by a significant relationship with yolk sac area (~63%); therefore, it was not included as a predictor in the multiple regression analyses. To control the familywise error rate, these tests were included when calculating the Bonferroni corrected α-level for all regressions. Comparisons between regression models were made using Akaike information criterion (AIC) scores and weights (w_i).

For all tests, data were first evaluated for normality and homogeneity of variances. In every instance, except for oil globule area, the assumptions were met, and therefore, analyses were performed on untransformed data. Oil globule area was cube root transformed to meet the assumption of normality. Inspection of the data and subsequent statistical analyses were conducted using R (2.13.0) statistical software (R Development Core Team 2011).

RESULTS

Relationship between maternal investment and larval morphology

Yolk sac area increased with increasing egg area, but the relationship explained a small proportion of the variation (r^2 = 0.129, $F_{1,137}$ = 20.19, p < 0.001; Fig. 2a). The relationship between egg area and cube root transformed oil globule area was not significant after Bonferroni correction (r^2 = 0.038, $F_{1,137}$ = 5.37, p = 0.022; Fig. 2b). Cube root transformed oil globule area increased with yolk sac area, and the relationship explained most of the variation (r^2 = 0.633, $F_{1,137}$ = 236, p < 0.001; Fig. 2c).

There was no relationship between egg area and larval length (r^2 = 0.016, $F_{1,137}$ = 2.16, p = 0.144; Fig. 3a). There was a significant positive relationship between egg area and myomere depth, but only a
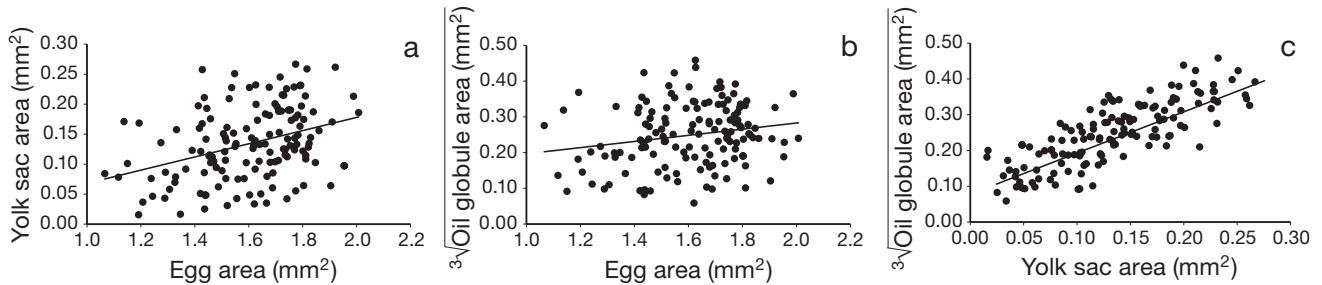
Fig. 2. *Galaxias maculatus.* Individual-level relationships of (a,b) egg size, (a,c) yolk quantity, and (b,c) oil globule size in recently hatched larvae of *G. maculatus.* Lines describe the least-squares regression fits
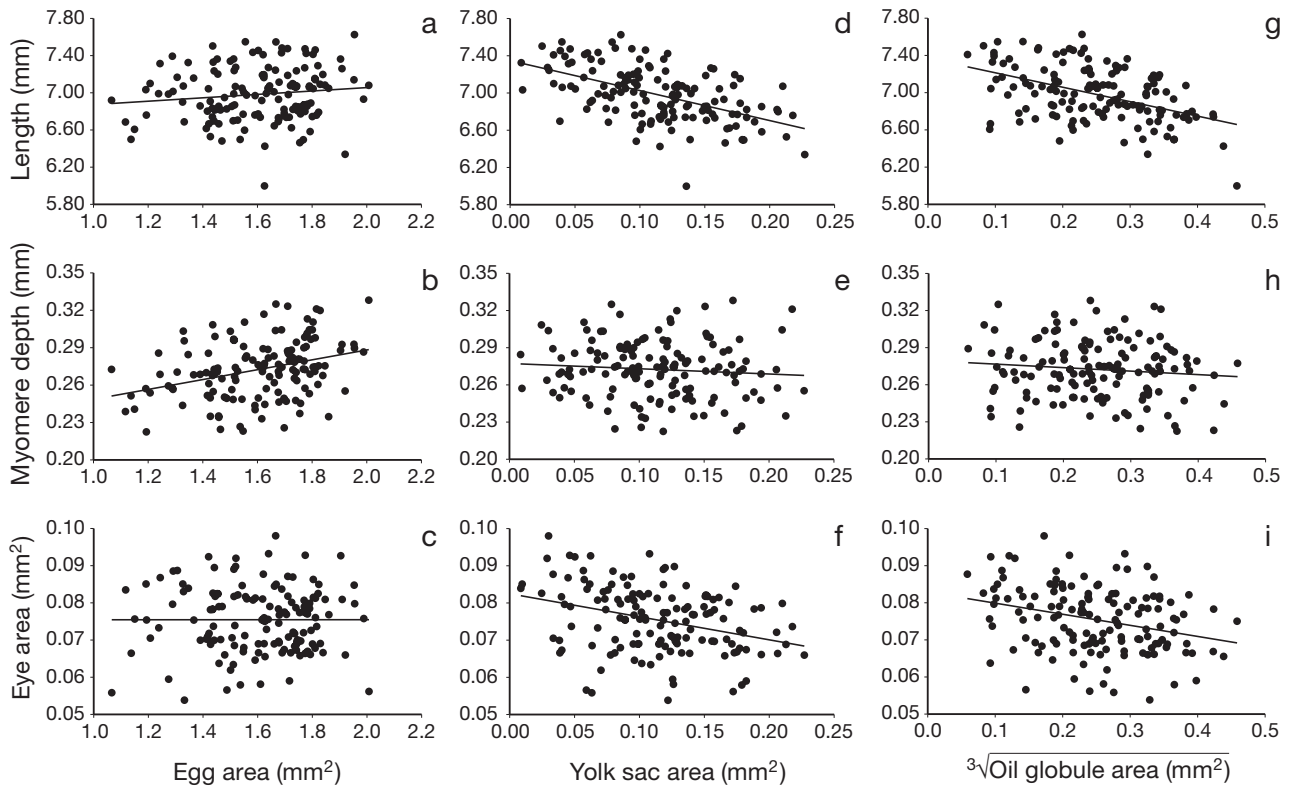

Fig. 3. *Galaxias maculatus.* Individual-level relationships of (a–c) egg size, (d–f) yolk quantity, and (g–i) oil globule size in recently hatched larvae of *G. maculatus* to (a,d,g) larval length, (b,e,h) myomere depth, and (c,f,i) eye size. Lines describe the least-squares regression fits

small portion of the variance in myomere depth was explained ($r^2 = 0.112$, $F_{1,137} = 17.21$, p < 0.001; Fig. 3b). There was no relationship between egg area and eye area ($r^2 < 0.001$, $F_{1,137} < 0.01$, p = 0.972; Fig. 3c).

There was a significant negative relationship between yolk sac area and larval length ($r^2 = 0.332$, $F_{1,137} = 68.03$, p < 0.001; Fig. 3d). The relationship between yolk sac area and myomere depth was negative but not significant ($r^2 = 0.013$, $F_{1,137} = 1.76$, p = 0.187; Fig. 3e). There was a significant, negative relationship between yolk sac area and eye area, but the amount of explained variation was small ($r^2 = 0.124$, $F_{1,137} = 19.34$, p < 0.001; Fig. 3f).

The cube root transformed oil globule area was negatively related to larval length, and the relationship explained about a fifth of the variation ($r^2 = 0.219$, $F_{1,137} = 38.38$, p < 0.001; Fig. 3g). There was no relationship between the cube root transformed oil globule area and myomere depth ($r^2 = 0.011$, $F_{1,137} = 1.56$, p = 0.214; Fig. 3h). There was a significant relationship between the cube root transformed oil globule area and eye area, but only a small portion of the variation was explained ($r^2 = 0.088$, $F_{1,137} = 13.91$, p < 0.001; Fig. 3i).

The multiple regressions of larval traits on egg area and yolk sac area were highly significant in all cases.

The multiple regressions explained close to half of the variation in larval length ($r^2 = 0.458$, $F_{2,136} = 57.35$, $p < 0.001$) but much less of the variation in myomere depth ($r^2 = 0.174$, $F_{2,136} = 14.36$, $p < 0.001$) and eye area ($r^2 = 0.138$, $F_{2,136} = 10.89$, $p < 0.001$). When the sources of maternal investment were compared, yolk sac area explained more variation in larval length and eye area than egg area, while the reverse was true for myomere depth (Table 1).

For all 3 traits, the regression model with the lowest AIC score included both egg area and yolk sac area as predictors (Table 2). For length and myomere depth, there was substantial support for the multiple regression model ($w_i > 0.9$). For the eye area, the multiple regression was only marginally better than the univariate yolk sac area model ($\Delta AIC < 2$), indicating that the simpler model should be preferred.

Relationship between egg size and hatching success, speed of hatching, and starvation resistance

There was no relationship between egg size and time to hatch for larvae that hatched within the first 5 h ($r^2 = 0.125$, $F_{1,15} = 2.138$, $p = 0.164$). There was very little size difference between eggs that hatched during the first 5 h (mean = 1480.992 ± 11.375 SE), eggs that hatched after 24 h (mean = 1481.598 ± 10.386 SE), and eggs that did not hatch (mean = 1478.577 ± 8.874 SE), and these differences were not significant ($F_{2,47} = 0.013$, $p = 0.987$). On average, larvae survived 16.6 d (SE = 5.3 d) without food, but the duration from hatching to starvation was unrelated to egg size ($r^2 = 0.002$, $F_{1,40} = 0.062$, $p = 0.805$).

DISCUSSION

The results of the present study show that the relationship between egg size and larval morphological traits is weak in *Galaxias maculatus*. Egg size predicted the yolk sac size and body depth of larvae at hatching but explained <13 % of the variation in these traits. Egg size was not related to larval length, oil globule size, eye size, hatching success, speed of hatching, or the duration that larvae persisted before starvation. These results contrast with the many studies that have found egg size to be highly correlated with fitness-determining traits in newly hatched larvae. However, this literature is biased towards salmonids, and the appropriate level of replication for many studies is group means (Reznick 1991, Heath & Blouw 1998). Studies that examine the relationship between egg size and larval traits at the individual level often find that the relationship is weaker than the correlations between group means suggest (reviewed by Chambers 1997), although the relationships are rarely as weak as those presented here (for similar findings, see Lagomarsino et al. 1988, Pepin et al. 1997, Kennedy et al. 2007).

Analysis of relationships between group means may obscure potentially important variation at the individual level. This implies that trade-offs may be easier to detect in comparisons among species than within species (e.g. Fisher et al. 2007). Regardless, trade-offs are expected to occur among individuals because the resources allocated to offspring are finite, and developing embryos must either allocate these resources to

Table 1. Multivariate regression results showing how much each factor contributed to the overall model

Trait	Variable (mm^2)	Beta coefficient	Partial r^2	*t*-value	p
Length (mm)	Egg area	0.394	0.055	5.841	<0.001
	Yolk area	−0.711	0.405	−10.532	<0.001
Myomere depth (mm)	Egg area	0.429	0.143	5.143	<0.001
	Yolk area	−0.266	0.030	−3.190	0.002
Eye area (mm^2)	Egg area	0.148	<0.001	1.741	0.084
	Yolk area	−0.405	0.142	−4.760	<0.001

Table 2. Regression models for each trait showing the number of parameters in the model (K), the difference in Akaike information criterion scores between models (ΔAIC) and the Akaike weight of each model (w_i)

Trait	Model (mm^2)	K	ΔAIC	w_i
Length (mm)	Egg area	1	80.907	<0.001
	Yolk sac area	1	29.117	<0.001
	Egg area + Yolk sac area	3	0.000	0.999
Myomere depth (mm)	Egg area	1	8.031	0.002
	Yolk sac area	1	22.703	<0.001
	Egg area + Yolk sac area	3	0.000	0.982
Eye area (mm^2)	Egg area	1	19.422	<0.001
	Yolk sac area	1	1.064	0.370
	Egg area + Yolk sac area	2	0.000	0.630

growth and metabolic demands or retain them in the yolk sac to increase resistance to starvation. The results presented here show a negative relationship between yolk sac size and larval length, providing evidence that there may be a trade-off between these 2 traits. Indeed, when yolk sac size and egg size were considered together, close to half of the variation in larval length was explained, which was greater than the amount of variance explained when the traits were considered independently. A multiple regression relationship also explained more of the variance in myomere depth than single predictors.

Variation in the relative risk of mortality from different sources might select for different optimal distributions of resources among competing traits. Fish larvae suffer mortality from a number of sources that are likely to exert selection pressures on a variety of larval traits (Bailey & Houde 1989, Holmes & McCormick 2009). Traits that enhance larval survival in one situation may be neutral or even disadvantageous in another situation. For example, the more yolk that larvae have at hatching is likely to improve their ability to resist a period of starvation. In contrast, a large quantity of yolk may impair their ability to escape predators, by decreasing swimming speed through increased weight and drag. The potential for larvae to survive during their early life history is, therefore, likely to depend on the sources of mortality and whether cues about relative mortality risks are used to optimise investment of maternal resources among competing traits.

Some research suggests that mothers may transmit information about the relative risk of mortality from particular sources to larvae. Larval phenotypes are influenced by the food environments that their mothers previously experienced during their own early life history (Taborsky 2006), recent life history (Reznick & Yang 1993), and shortly before spawning (Kerrigan 1997, Gagliano & McCormick 2007). Maternal exposure to predator cues in early life (Segers & Taborsky 2011) and shortly before spawning (Giesing et al. 2011) can also induce shifts in the larval phenotype. Variation in the environment that is experienced by mothers within a population may therefore lead to differences among larvae in resource allocation to competing traits. Moreover, discrepancies between the information provided by mothers and cues in the offspring environment may contribute to differences among individuals (cf. Rossiter 1996).

Starvation is likely to be an important source of mortality for *Galaxias maculatus* larvae because they must drift down estuaries and out to sea before they reach areas where they can reliably find food. The risk of starvation during the downstream migration has been linked to the distance travelled and the transit time to the sea in another amphidromous fish (Iguchi & Mizuno 1999). The 2 populations sampled here differ substantially in the distance of the spawning site from the sea (Hicks et al. 2010, Barbee et al. 2011). It might therefore be expected that the size of the yolk sac or oil globule should differ between the populations to reflect the risk of starvation, but no differences were found. However, local adaptations to the risk of starvation may be prevented by high gene flow among populations (Waters et al. 2000).

The larvae of *Galaxias maculatus* are at increased risk of starvation compared to other amphidromous fish because hatching can only occur on spring tides, which can mean an extended period of incubation leading to reduced yolk reserves at hatching (Semmens & Swearer 2011). If embryos are unable to hatch before spring tides recede, they may be trapped on the riverbank for 2 or more weeks before they get another opportunity to hatch. Such delays in hatching can result in smaller yolk sacs and shorter time to starvation (Semmens & Swearer 2011). Therefore, the ability to hatch quickly when provided with an opportunity may be an important component of fitness in *G. maculatus.* Smaller eggs might be expected to hatch more quickly because they can less afford a period of extended incubation. Alternatively, larger larvae may hatch more quickly because their size helps them to escape the egg. Contrary to these expectations, speed of hatching was unrelated to egg size. However, there was a trend towards larger larvae hatching more quickly, and a larger sample size may have found a significant relationship.

The duration of time that larvae persisted before starvation was also unrelated to egg size in the present study. However, egg size also explained little of the considerable variation in yolk sac size (CV = 45%) and oil globule size (CV = 35%). These traits, particularly the oil globule, are important for starvation resistance in a wide range of fish species (e.g. Chambers et al. 1989, Fisher et al. 2007, Gagliano & McCormick 2007) and are likely to be better predictors of starvation resistance than egg size. These traits were not measured here because earlier attempts to measure newly hatched, live *Galaxias maculatus* larvae resulted in extremely high mortality rates. Other studies have reported successfully measuring older larvae using MS-222 as an anaesthetic to reduce movement (Mitchell 1989). This might be a fruitful method for future studies wishing to identify the factors that determine starvation resistance in *G. maculatus.*

Variation in metabolic rate may have contributed to the observed high variation in the size of the yolk sac and oil globule and may explain why egg size was poorly predictive of larval traits and resistance to starvation. The metabolic rate of embryos can vary substantially among individuals within species (Docker et al. 1986, Bang et al. 2004) and is important in determining growth rate and survival during starvation (Bochdansky et al. 2005, Bang et al. 2007). Most of the variation in metabolic rate among individuals is attributable to differences among families (Docker et al. 1986, Pakkasmaa et al. 2006, Régnier et al. 2010), suggesting that genetic and parental effects largely determine metabolic rate. Studies that can link the metabolic rate of individual embryos to their traits as hatchlings are extremely rare (but see Bang & Gronkjaer 2005). It would be interesting for future studies to examine the effect of metabolic rate on the traits of *Galaxias maculatus* larvae after hatching to see if variation in metabolic rate can explain the poor fit between egg size and larval traits.

In addition to variation in metabolic rate, the strength of the relationship between egg size and larval traits may be weak or absent if egg size does not accurately predict energetic content. For example, maternal diet can significantly influence the allocation of lipid and protein to offspring, without a corresponding change in egg size (Gunasekera et al. 1996, Afzal Khan et al. 2005, Gagliano & McCormick 2007). Lipids have a greater energy density than both carbohydrates and proteins, such that for a given volume, embryos and larvae have more available energy when lipids make up a greater proportion of the yolk. Differential hydration of eggs may also affect the energy density of eggs. The assumption that egg size is a reliable predictor of energetic content is rarely tested, and some studies find that there is a substantial amount of variation in energetic content that is not explained by egg size (McEdward & Carson 1987, McEdward & Coulter 1987).

The data presented here show that the relationships between egg size and larval traits are complex. Although there was a trend towards larval traits being larger in larvae hatching from large eggs, only yolk sac size and body depth were significantly related to egg size. However, there was evidence that trade-offs among larval traits might exist and may have obscured simple relationships between those traits and egg size. Accounting for a trade-off with yolk sac size significantly improved the fit of the data for all of the traits considered, but a considerable amount of unexplained variation remained. Some of the unexplained variation may be a result of

maternal effects, genetic factors, or a poor relationship between energy reserves and egg size. The present study demonstrates that egg size is not always strongly related to proxies of larval quality and highlights the need to consider multiple larval traits when evaluating the relationship between egg size and the larval phenotype.

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