Temperature-mediated trade-offs and changes in life-history integration in two slipper limpets (Gastropoda: Calyptraeidae) with planktotrophic development

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Intraspecific variation in egg size and hatching size, and the genetic and environmental trade-offs that contribute to variation, are the basis of the evolution of life histories. The present study examined both univariate and multivariate temperature-mediated plasticity of life-history traits, as well as temperature-mediated trade-offs in egg size and clutch size, in two planktotrophic species of marine slipper limpets, *Crepidula*. Previous work with two species of *Crepidula* with large eggs and lecithotrophic development has shown a significant effect of temperature on egg size and hatching size. To further examine the effect of temperature on egg size in *Crepidula*, the effects of temperature on egg size and hatching size, as well as the possible trade-offs with other the life-history features, were examined for two planktotrophic species: *Crepidula incurva* and *Crepidula* cf. *marginalis*. Field-collected juveniles were raised at 23 or 28 °C and egg size, hatching size, capsules/brood, eggs/capsule, time to hatch, interbrood interval, and final body weight were recorded. Consistent with results for the lecithotrophic *Crepidula*, egg size and hatching size decreased with temperature in the planktotrophic species. The affects of maternal identity and individual brood account for more than half of the intraspecific variation in egg size and hatching size. Temperature also showed a significant effect on reproductive rate, with time to hatch and interbrood interval both decreasing with increasing temperature. However, temperature had contrasting effects on the number of offspring. *Crepidula* cf. *marginalis* has significantly more eggs/capsule and therefore more eggs per brood at 28 °C compared to 23 °C, although capsules/brood did not vary with temperature. *Crepidula incurva*, on the other hand, produced significantly more capsules/brood and more eggs per brood at the lower temperature, whereas the number of eggs/capsule did not vary with temperature. The phenotypic variance–covariance matrix of life-history variables showed a greater response to temperature in *C. incurva* than in *C.* cf. *marginalis*, and temperature induced trade-offs between offspring size and number differ between the species. These differences suggest that temperature changes as a result of seasonal upwelling along the coast of Panama will effect the reproduction and evolution of life histories of these two co-occurring species differently. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 763–775.

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INTRODUCTION

Intraspecific variation in egg size and hatching size, and the genetic and environmental trade-offs that contribute to variation in these features, are the basis of the evolution of life histories. Despite an eloquent call for more detailed studies and a greater awareness

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and appreciation of propagule size or offspring size as a complex phenotype of both the mother and her offspring (Bernardo, 1996), these factors remain poorly studied especially for marine invertebrates (Moran & McAlister, 2009). Much of the literature on marine invertebrate egg size has focused on interspecific variation and a single, mean egg size or hatching size is often used to represent each species. Where intraspecific variation has been examined it is

often in the context of increased variance and the possibility of bet-hedging in species with nurse eggs (Spight, 1976; Rivest, 1983) or other extraembryonic nutrition (Rivest, 1986). Where intraspecific variation in eggs size and hatching size have been examined in species lacking nurse eggs, the focus has been on trait means and plasticity has received limited attention. With the exception of specific trade-offs predicted by life-history theory (e.g. trade-offs between offspring size and number), little attention has been given to the variances and covariances of these characters. Measures of both trait means and the phenotypic variance–covariance structure are necessary to understand phenotypic evolution because the evolutionary response to selection is a function of the phenotypic variance–covariance matrix ($\mathbf{P}$). The common simplifying assumption that $\mathbf{P}$ is constant within a species is not always true because $\mathbf{P}$ can vary as a result of both environmental and genetic factors (Pigliucci, Cammell & Schmitt, 1999; Roff & Mousseau, 2005). If $\mathbf{P}$ varies with temperature, then the response to selection will also vary with temperature.

The present study aimed to document the temperature-mediated phenotypic plasticity in life-history features and the response of the phenotypic variance–covariance matrix to changes in temperature in two species of marine gastropods. Egg size and hatching size for both marine and terrestrial ectotherms show a phenotypic plastic response to temperature. In most cases, both decrease with increasing temperature (e.g. molluscs: Honkoop & Van Der Meer, 1998; Collin & Salazar, 2010; insects: Ernsting & Isaaks, 2000; Fischer, Brakefield & Zwaan, 2003; Liefting et al., 2010; fishes: Bengtson, Barkman & Berry, 1987; Kokita, 2003; nematodes: Van Voorhies, 1996; Gutteling et al., 2007; rotifers: Stelzer, 2002; reviewed by Atkinson et al., 2001). This almost ubiquitous pattern of temperature-mediated plasticity in offspring size is similar to and may be related to the 'temperature-size rule' (TSR); the plastic response of adult body size to temperature (Arendt, 2007; Kingsolver & Huey, 2008). Several alternate explanations have been proposed for the temperature-size rule (Arendt, 2010; Forster, Hirst & Atkinson, 2011; Forster & Hirst, 2012; Zuo et al., 2012), although few of them explicitly include models of egg or offspring size, and many of them rely on differences in temperature dependence of growth rate and development rate. This cannot apply to eggs but could apply to hatchlings (Zuo et al., 2012 but see also Forster et al., 2011).

Temperature-mediated plasticity in body size is alternately suggested to be an adaptive response, which is the result of some kind of physiological constraint (Atkinson, Morley & Hughes, 2006; Forster et al., 2011), and/or the result of trade-offs with other life-history traits (Arendt, 2010). Detailed optimality models and reciprocal rearing experiments often (but not always) show that offspring have highest fitness at the temperatures at which the eggs were produced (insects: Landa, 1992; Fischer et al., 2003; Fischer, Bauerfeind & Fiedler, 2006; fish: Bownds, Wilson & Marshall, 2010). These experiments have been conducted on a limited number of species and it is not clear how natural selection could almost always result in large eggs doing better at lower temperatures. The physiological constraint hypothesis receives support from studies that suggest many cells (and not just egg cells) are larger at cooler temperatures (fish and nematodes: Van Voorhies, 1996; insects: Azevedo, French & Partridge, 2002; Blanckenhorn & Llaurens, 2005). Eutelous species such as rotifers and nematodes often show decreases in body size with increased temperature and, by inference, this must also be the result of decreased cell size with temperature (Stelzer, 2002). A recent review, however, concluded that the TSR is the result of both changes in cell size and number (Arendt, 2010). This could also be true of differences in hatching size, although the only study to examine this in marine invertebrates showed that the cell size in hatchling bryozoan larvae did change with temperature (Atkinson et al., 2006). Because embryos do not feed, it is likely that differences in hatching size ultimately reflect the size of the single egg cell. Decreases in egg size with temperature, but without a trade-off with clutch size, have sometimes been interpreted as additional support for the physiological constraint hypothesis (Blanckenhorn, 2000).

The integrated organismal life-history phenotype includes trade-offs between offspring size and other life-history traits such as clutch size or clutch frequency. These trade-offs may also influence the evolution of offspring size. For example, demographic models of the evolution of egg size based on the trade-off between egg size and number, as well as temperature-dependent growth rates, predict the observed pattern of larger eggs at cooler temperatures (Yampolsky & Scheiner, 1996). This trade-off between egg size and clutch size is also used as a key assumption of many models stemming from life-history theory (Stearns, 1992; Bernardo, 1996). Intraspecific trade-offs between egg size and clutch size have indeed been reported for a variety of invertebrates (insects: Stam, Isaaks & Ernsting, 1998; Fischer et al., 2003; bivalves: Honkoop & Van Der Meer, 1998; amphipods: Skadsheim, 1989). However, there are cases where egg size varies in response to temperature without a change in clutch size (Joosse, Brugman & Veld, 1973; Van Straalen & Joosse, 1985; Blanckenhorn, 2000), or cases where a trade-off is only observed under certain environmental conditions. The well-studied trade-off between reproductive effort and life span is similarly plastic and can be uncoupled under certain environ-

mental conditions (Flatt & Schmidt, 2009; Grandison, Piper & Partridge, 2009). When uncoupled from reproduction, lifespan may continue to show trade-offs with other fitness-related traits (Stearns, 2011). The complex web of relationships between life-history variables suggests that examining multivariate phenotypic plasticity, reflected by the stability of the variance–covariance relationships (Pigliucci *et al.*, 1999), could be a fruitful route to understanding temperature effects on life histories.

The present study examined both univariate and multivariate temperature-mediated plasticity of life-history traits and the possibility of temperature-mediated trade-offs in egg size and clutch size in two planktotrophic species of marine slipper limpets in the genus *Crepidula*. The questions to be addressed were: (1) what are the univariate responses of egg size, hatching size, clutch size, clutch frequency, and brood duration to two temperatures typical of seasonal variation in sea temperature; (2) how stable is **P** to changes in temperature; and (3) is there evidence for temperature-mediated changes in the trade-offs in egg size and egg number in the two species? Because at least three broods were measured from each female for each variable, it was also possible to determine how egg size and offspring size vary among females and among broods from a single female, with such data rarely being available for marine invertebrates.

MATERIAL AND METHODS

Two species of *Crepidula* with planktotrophic development were collected from the intertidal of the Pacific coast of Panama, near Panama City, where they reproduce year round. *Crepidula* cf. *marginalis* were collected from the undersides of rocks and *Crepidula incurva* from the shells of gastropods at Chumical (8.874°N, 79.644°W) near the town of Veracruz in 2009. Small juveniles were collected, assigned to pairs and placed in 350-mL cups. Thirty to thirty-five replicates of each species were maintained at 23 and 28 °C (temperatures normally experienced by these animals in the field), fed 50×10^3 cell mL^{-1} *Isochrysis galbana* daily and the water was changed three times a week. Because *Crepidula* are all protandrous, one individual in each cup became male, whereas the other became female. Females deposit thin-walled transparent capsules that they brood between the substrate, neck, and propodium (not in the pallial cavity as is often reported). Broods consisted of 20–40 capsules each with 30–40 eggs that were laid over the course of 24 h and hatched synchronously when the mother ejected them from under the shell. The female in each cup was checked twice a day for eggs or hatchlings. Broods were visible through the transparent walls of the containers and hatchlings could

be seen swimming in the water column. Because hatchlings were measured after they hatched naturally and because cleavage is not synchronous, measurements were not attributed to individual capsules. The number of days to hatching and the number of days between hatching and the deposition of the subsequent brood were recorded where possible. Ten to 20 eggs and hatchlings were photographed and measured with IMAGE J (Abramoff, Maelhaes & Ram, 2004) for three broods from each female in acordance with methods described by Collin & Salazar (2010) and Collin (2010). Because veliger shells are plan-spiral in lateral outline, a plug-in was designed to measure the Feret's diameter (the longest axis of the object), which is approximately equivalent to shell length as traditionally measured for gastropod larvae.

After three broods of eggs and three broods of hatchlings had been collected, the total number of capsules, and the number of eggs per capsule for a subsample of five capsules were counted for the next three broods. Because counting or measuring eggs requires the removal of the brood from the female, and because many broods were collected after cleavage had begun, 'brood number' in the statistical analyses does not reflect every brood produced; rather, it reflects the relative order of the broods included in that analysis.

STATISTICAL ANALYSIS

Effects of temperature, maternal identity, and individual brood on egg size and hatching size were examined with a mixed model analysis of covariance with maternal identity ('female') as a random effect nested within the fixed temperature treatment effect, with brood as a random effect nested within female, and with female length as a covariate. The time that hatchlings were held in ethanol before being measured was also included as a covariate to eliminate possible preservation effects. Effects of temperature and maternal identity on capsule number, eggs per capsule, total eggs per brood, time to hatching, and interbrood period for three broods from each female were analyzed in a similar way, with female as a random effect nested within temperature and with length as a covariate. All statistical analyses except for those for CPC (see below) were conducted using JMP, version 9 (SAS Institute).

Phenotypic variance–covariance matrices were calculated for each species at each temperature using egg size, hatching size, total clutch size, and clutch frequency. Because all of the variables could not be measured for the same brood, the **P**-matrices were constructed using the mean values for each female and compared using the Flury method implemented in CPC – Common Principle Component Analysis

software (CPC, version 0.96; Phillips & Arnold, 1999). This method compares matrices at different levels of similarity so that equality, proportionality, and shared partial principle components can be tested. **P**-matrices were compared for the two different temperatures within each species to determine how temperature affects the multivariate plasticity or phenotypic integration of life-history characters (Pigliucci *et al*., 1999). The multivariate analysis of variance (MANOVA) jackknife method of Roff (2002) was also applied to compare the **P**-matrices. In this method, the data matrix from which the variance–covariance matrix is calculated is jackknifed and $N-1$ pseudoreplicate **P**-matrices are constructed. Each variance or covariance value is then subjected to MANOVA.

RESULTS
EGG SIZE AND HATCHING SIZE

Egg size was measured for 3538 eggs for *C. incurva* and 3180 eggs for *C. cf. marginalis*. For *C. incurva*, this included 78 broods from 29 females at 28 °C and 80 broods from 28 females at 23 °C. For *C. cf. mar-*ginalis, this included 66 broods from 30 females at 28 °C and 82 broods from 33 females at 23 °C. In both species, egg size was significantly smaller at 28 °C than at 23 °C (Fig. 1, Tables 1, 2;) and egg size increased significantly with maternal shell length (Fig. 2, Table 2). In addition, in both species, 20–25% of the variation in egg size was a result of the random effect of female identity nested within treatment and 45–50% of the variation in egg size was a result of the random effect of brood nested within female.

Hatching size was measured for 3536 hatchlings for *C. incurva* and 3984 for *C. cf. marginalis*. For *C. incurva*, this included 86 broods from 29 females at 28 °C and 85 broods from 29 females at 23 °C. For *C. cf. marginalis*, this included 107 broods from 38 females at 28 °C and 98 broods from 33 females at 23 °C. In both species, hatching size was significantly smaller at 28 °C than at 23 °C (Fig. 1, Tables 1, 2). Unlike egg size, hatching size did not vary significantly with maternal shell length (Table 1). In both species, similar percentages of variation in hatching size were the result of the random effect of female identity nested within treatment and of brood nested within female.
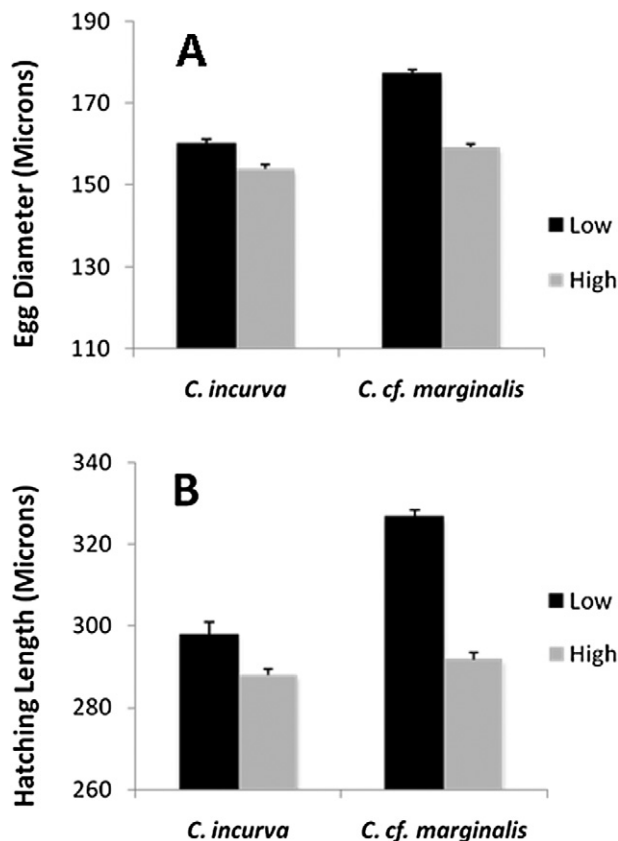

Figure 1. Effect of temperature on (A) egg diameter and (B) hatching length of *Crepidula incurva* and *Crepidula* cf. *marginalis*. Error bars represent the SE.
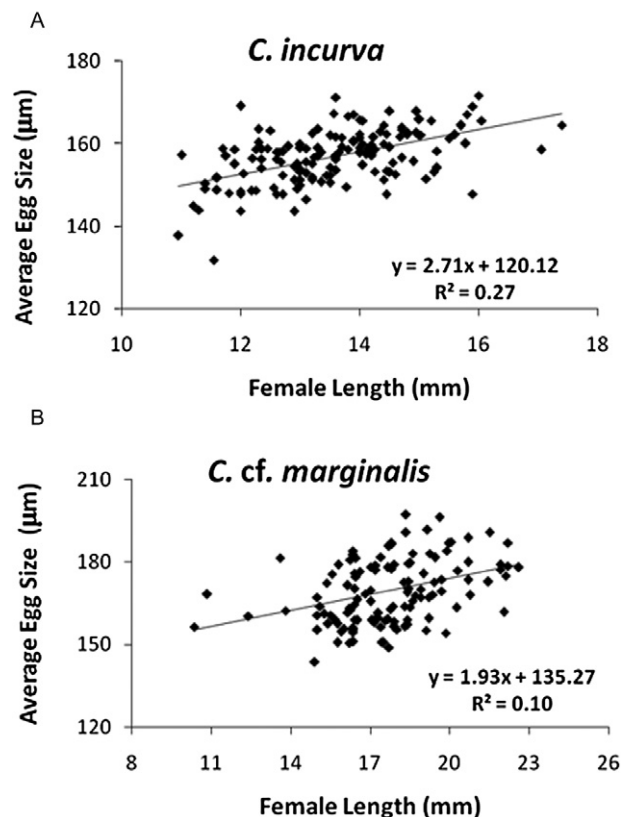

Figure 2. Relationship between egg size and maternal shell length for (A) *Crepidula incurva* and (B) *Crepidula* cf. *marginalis*.

Table 1. Summary of egg size and hatching size for *Crepidula incurva* and *Crepidula* cf. *marginalis*

	Crepidula incurva		*Crepidula* cf. *marginalis*	
Egg size (effective diameter)	High (28 °C)	Low (23 °C)	High	Low
Mean egg size (mean ± SE)	153.07 ± 0.15	161.12 ± 0.12	158.48 ± 0.15	177.83 ± 0.23
Minimum brood (mean)	131.8	151.3	143.7	159.4
Maximum brood (mean)	166.0	171.7	169.7	197.2
Hatching size (Feret length)				
Mean hatching size (mean ± SE)	287.51 ± 0.33	298.92 ± 0.36	292.2 ± 0.4	326.9 ± 0.5
Minimum brood hatching (mean)	251.4	243.3	261.7	252.3
Maximum brood hatching (mean)	318.3	321.6	332.8	391.5

Table 2. Mixed model analysis of egg size and hatching size

	d.f.	F	P	% of total REML variance
Crepidula incurva				
Egg size				
Temperature	1	32.73	**< 0.0001**	
Female (temperature) random				25.80
Brood (temperature, female) random				48.84
Female length	1	12.51	**0.0004**	
Hatching size				
Temperature	1	17.68	**< 0.0001**	
Female (temperature) random				17.13
Brood (temperature, female) random				40.97
Female length	1	1.90	0.17	
Days in ethanol	1	0.003	0.95	
Crepidula cf. *marginalis*				
Egg size				
Temperature	1	139.31	**< 0.0001**	
Female (temperature) random				21.11
Brood (temperature, female) random				45.97
Female length	1	4.65	**0.03**	
Hatching size				
Temperature	1	108.51	**< 0.0001**	
Female (temperature) random				25.29
Brood (temperature, female) random				44.93
Female length	1	0.0003	0.98	
Days in ethanol	1	1.30	0.25	

Significant effects are shown in bold. REML, restricted maximum likelihood.

The number of days the hatchlings had been stored in ethanol before measurement did not contribute significantly to variation in hatching size (Table 2).

NUMBER OF EGGS AND CAPSULES

Reproductive output was significantly affected by temperature treatment in both species (Table 3). Unlike the results for egg size and hatching size, the two species showed different relationships between fecundity and temperature.

For *C. incurva*, the number of capsules and the total number of eggs per brood decreased with temperature (Fig. 3). There was no effect of temperature on eggs per capsule but, after stepwise removal of nonsignificant factors, there was a significant increase in eggs per capsule with maternal length (Table 3). Overall, there was a significant decrease in the total number of eggs per brood with increasing temperature, with 987 eggs per brood at 28 °C and 1356 eggs per brood at 23 °C (Table 3).

For *C.* cf. *marginalis*, eggs per capsule increased with temperature (Fig. 3). Eggs per capsule also increased significantly with maternal length, although there was no effect of temperature on capsule number (Table 3). This resulted in the increase in the total eggs per brood with temperature, with 1145 eggs per brood at 28 °C and 842 eggs per brood at 23 °C (Table 3).

DAYS TO HATCHING AND INTERBROOD PERIOD

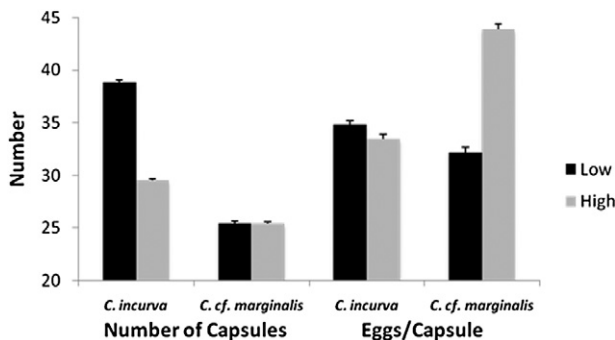In both species, development is completed in significantly less time at 28 °C than at 23 °C ($P < 0.0001$ in both species; Fig. 4). In neither species did the random effect of female nested within treatment contribute much to the variation in time to hatching (< 8%). In *C. incurva*, brood order had no effect on time to hatching ($P > 0.3$). However, in *C.* cf. *marginalis*, there was a significant effect of brood; the third brood took significantly longer to hatch (10.2 days) than the first and second broods (9.2 and 8.9 days) ($P = 0.0002$).

Unlike the time to hatching, the random effect of female contributed to the variation in time between broods (33% of total variation in *C. incurva* and 27% in *C.* cf. *marginalis*). In *C. incurva*, the interbrood
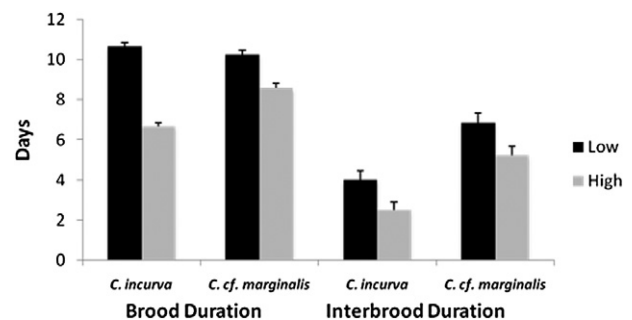

Figure 3. Effect of temperature on number of capsules per brood and number of eggs per capsule for *Crepidula incurva* and *Crepidula* cf. *marginalis*. Error bars represent the SE.

Figure 4. Effect of temperature on brood duration and interbrood period for *Crepidula incurva* and *Crepidula* cf. *marginalis*. Error bars represent the SE.

Table 3. Effects of temperature, maternal identity, brood order, and maternal length on eggs per capsule, number of capsules per brood and total eggs per brood for both *Crepidula incurva* and *Crepidula* cf. *marginalis*

		Eggs/capsule			Number of capsules			Total eggs/brood		
	d.f.	F	P	% of total REML variance	F	P	% of total REML variance	F	P	% of total REML variance
Crepidula incurva										
Temperature	1	0.21	0.65		9.47	**< 0.004**		7.32	**0.01**	
Female (temperature) Random				76.10			48.7			56.3
Brood number	2	0.04	0.96		1.25	0.29		1.05	0.36	
Length	1	2.18	0.14*		0.35	0.55		0.48	0.49	
Length *Temperature	1	0.11	0.74		0.35	0.70		0.02	0.89	
Crepidula cf. *marginalis*										
Temperature	1	27.26	**< 0.0001**		0.0007	0.98		6.90	**0.01**	
Female (temperature) Random				67.70			35.67			49.10
Brood number	2	0.57	0.57		0.087	0.92		0.26	0.77	
Length	1	11.85	**0.0009**		0.0019	0.96		3.45	0.07	
Length *Temperature	1	0.26	0.61		0.0008	0.97		0.33	0.56	

*Becomes significant with stepwise removal of nonsignificant effects. Significant effects are shown in bold. REML, restricted maximum likelihood.

interval was significantly shorter at 28 °C than at 23 °C ($P < 0.03$; Fig. 4) and there was no significant effect of brood order. In *C.* cf. *marginalis*, the inter-brood period was almost significantly shorter at 28 °C than 23 °C ($P = 0.08$; Fig. 4). Interbrood period was significantly shorter for earlier broods (4.9 days for the first brood, 6.4 days for the second brood, and 6.9 days for the third brood; $P = 0.03$).

WEIGHT

In both species, dry body weight of the animals at the end of the experiment increased with shell length (*C. incurva*: $r^2 = 0.49$, $N = 51$, $P < 0.0001$; *C. marginalis*: $r^2 = 0.87$, $N = 80$, $P < 0.0001$) but in neither species was there an effect of temperature treatment ($P > 0.05$). Likewise, shell weight increased with shell length (*C. incurva*: $r^2 = 0.74$, $N = 51$, $P < 0.0001$; *C.* cf. *marginalis*: $r^2 = 0.49$, $N = 80$, $P < 0.0001$) but there was no effect of temperature treatment on shell weight. In neither species was there a significant interaction between temperature treatment and shell length.

P-MATRIX COMPARISONS

The **P**-matrices (Table 4) were less similar for *C. incurva* at the two temperatures than they are for *C.* cf. *marginalis*. For *C. incurva*, the Jump-up, Step-up, and Akaike information criteria (AIC) criteria from the CPC analysis showed that **P** at the two temperatures were 'unrelated' (Table 5). However, because of the limited number of models tested in a CPC analysis, a finding of unrelated matrices should be interpreted to indicate only that the lowest model tested should be rejected, and not that all possible kinds of similarity can be rejected (Houle, Mezey & Galpern, 2002). By contrast, *C.* cf. *marginalis* showed significant similarities between **P** at 23 and 28 °C. The Jump-up, Step-up, and AIC analyses criteria favoured different models (Table 5) ranging from proportional to CPC (3). Even though CPC tends to underestimate the shared structure of the matrices (Houle *et al.*, 2002), it is clear that *C.* cf. *marginalis* showed less temperature-mediated differences in **P** than did *C. incurva*. Because the large value of variances and covariances involving the total number of

Table 4. P-matrices for both species

	Egg diameter	Hatching size	Shell length	Total eggs per brood	Brood frequency
Crepidula cf. *marginalis* – 28 °C					
Egg diameter	19.26				
Hatching size	24.57	113.83			
Shell length	1.50	−0.65	4.31		
Total eggs per brood	−457.78	−96.54	41.68	139 937.84	
Brood frequency	7.91	**9.85**	−0.37	−474.25	10.51
Crepidula cf. *marginalis* – 23 °C					
Egg diameter	36.16				
Hatching size	50.46	284.64			
Shell length	2.29	7.97	3.25		
Total eggs per brood	736.36	1506	133.89	119 190.56	
Brood frequency	11.58	**10.54**	0.99	13.42	18.53
Crepidula incurva – 28 °C					
Egg diameter	16.64				
Hatching size	16.35	85.55			
Shell length	1.78	−2.00	0.94		
Total eggs per brood	−75.31	−637.64	89.77	92 139.78	
Brood frequency	**0.94**	0.48	−0.50	−447.28	6.99
Crepidula incurva – 23 °C					
Egg diameter	15.38				
Hatching size	11.11	62.23			
Shell length	1.18	4.68	1.14		
Total eggs per brood	−265.64	302.75	42.06	175 193.01	
Brood frequency	**0.75**	−1.98	0.34	−33.11	10.04

Values that are not detected as being different in the multivariate analysis of variance of the jackknifed dataset are shown in bold.

Table 5. Results of Flury's Hierarchy test using CPC (Phillips & Arnold, 1999)

	Crepidula incurva				*Crepidula* cf. *marginalis*			
	χ^2	d.f.	*P*	AIC	χ^2	d.f.	*P*	AIC
Step-up model								
Equal-proportional	3.00	1	0.08	34.41	4.71	1	0.03	25.64
Proportional-CPC	6.59	4	0.16	33.41	10.40	4	**0.03***	22.93
CPC – CPC (3)	1.23	1	0.27	34.83	1.58	1	0.21	**20.53**
CPC (3) – CPC (2)	4.45	2	0.11	35.59	0.59	2	0.75	20.94
CPC (2) – CPC (1)	8.38	3	0.04	35.14	1.47	3	0.69	24.36
CPC (1) – unrelated	10.76	4	**0.03**	32.76	6.89	4	0.14	28.89
Unrelated	–			**30.00†**				30.00
Jump-up model								
Equal – unrelated	34.41	15	0.003		25.64	15	**0.04**	
Proportional – unrelated	31.42	14	0.005		20.93	14	0.10	
CPC – unrelated	24.83	10	0.006		10.53	10	0.40	
CPC (3) – unrelated	23.59	9	0.005		8.94	9	0.44	
CPC (2) – unrelated	19.14	7	0.008		8.36	7	0.30	
CPC (1) – unrelated	10.76	4	**0.03**		6.89	4	0.14	

The 'Step-up' method starts with unrelated model and first compares with CPC (1) and then moves up the hierarchy, comparing each model with the slightly more complicated model above it, as long as each test is nonsignificant. The 'Jump-up' method also starts at bottom of the hierarchy and tests each against unrelated until a significant difference is detected. Alternately, the maximum likelihood approach supports the selection of the model with the lowest Akaike information criteria (AIC) value. The model selected by each method is shown in bold.
*Step-up supports the Equal model when repeated without total eggs.
†AIC supports the Equal model when repeated on log-transformed data.

eggs per brood could mislead the CPC analyses, they were repeated with log-transformed data and with the **P** calculated without the total eggs. This analysis produced overall similar results (Table 5).

MANOVA analysis of the jackknifed datasets supported the CPC result that the matrices for *C. incurva* differ (overall model $P < 0.001$); all but the covariance between egg diameter and brood frequency differed significantly ($P < 0.0001$). *Crepidula* cf. *marginalis*, showed a similar pattern, with MANOVA analysis of the jackknifed datasets showing that they differed significantly ($P < 0.001$), with all but the covariance between hatching size and brood frequency differing in the univariate tests ($P < 0.0001$).

DISCUSSION

Compared to studies of plants, birds or mammals, there are still large gaps in our understanding of the microevolutionary processes acting on offspring size in marine invertebrates. In particular, within-species variation in offspring size and the contribution of environmental-mediated plasticity to this variation have been the focus of few studies. A recent review (Marshall & Keough, 2008) concluded that the lack of studies that explicitly examine different levels of variation limits our ability to identify patterns in offspring size for marine invertebrates. The same point (i.e. that information is lacking for intraspecific variation in egg size and quality) was made by Moran & McAlister (2009).

In the two species of *Crepidula* with planktotrophic development examined in the present study, significant variation in egg size and hatching size was a result of the effects of temperature and maternal identity. Consistent with previous studies of *Crepidula* with large eggs (Collin, 2010; Collin & Salazar, 2010) and other studies of marine invertebrates (Moran & McAlister, 2009), these two species showed decreased egg size and hatching size with increasing temperature. A large proportion of the observed variation was a result of differences among females (see below). Also consistent with expectations from other studies, intracapsular development rate increased with temperature. Increased rates of growth and development with increased temperature have also been reported for the larvae of planktotrophic *Crepidula* (Pechenik, 1984, 1985).

Multivariate analysis showed that the overall response of life histories to different temperatures was more complex than they appeared from univari-

ate analyses. Analyses of trait means showed that the two species respond to temperature in the same way for all variables, except the components of eggs/brood. The trait variances and covariances summarized in **P**, however, varied significantly with temperature for *C. incurva* but retained some similarities across temperatures in *C.* cf. *marginalis*. In addition, the two species showed different trade-offs between offspring size and number (see below).

TEMPERATURE-MEDIATED TRADE-OFFS BETWEEN OFFSPRING SIZE AND OFFSPRING NUMBER

Every character measured in the present study except for relative body weight and relative shell weight showed temperature mediated plasticity. Similar to the results reported for the direct developer *Crepidula atrasolea* and the lecithotroph *Crepidula ustulatulina* (Collin & Salazar, 2010), in both *C. incurva* and *C.* cf. *marginalis*, egg size and hatching size decreased with increasing temperature. Similar to the situation in other marine invertebrates, the duration of development decreased with increasing temperature (Hoegh-Guldberg & Pearse, 1995). Despite these similarities, the total eggs per brood showed contrasting patterns in the two species: eggs per brood increased with increasing temperature in *C.* cf. *marginalis* but decreased with increasing temperature in *C. incurva*.

A common assumption of life-history theory is that the finite energy available for reproduction can result in direct trade-off between egg size and number (van Noordwijk & de Jong, 1986; Yampolsky & Scheiner, 1996). If there is a direct trade-off in egg size and number, and if energy available for reproduction is fixed for a single reproductive bout, the total number of eggs per brood × egg volume should remain constant. If the energy available is fixed per unit time, the total egg volume per brood/(brood duration + interbrood period) should remain constant. The present study used the mean values calculated for each species at the two different temperatures to see whether the temperature-mediated differences in egg size are compensated by a direct trade-off with offspring number (Table 6). In *C.* cf. *marginalis*, the egg volume per brood remained constant across the two temperatures but this species produced more egg volume per unit time at high temperatures than at low temperatures. In *C. incurva*, the egg volume produced per unit time remained constant, whereas the egg volume allocated to each brood was 50% higher at the lower temperature. When hatchling size was substituted for egg volume, the results were similar (Table 6).

The trade-off between egg size and number is part of a complicated multivariate network of trade-offs between current and future reproductive output, and current and future growth or survival. If temperature affected the total energy available for growth and reproduction, it would be possible to see a marked increase or decrease in total reproductive output, as has been observed for some species of butterfly (Steigenga & Fischer, 2007; Geister *et al.*, 2008; Gibbs, van Dyck & Karlsson, 2010). Unexpectedly, the two *Crepidula* species seem to maintain the same allocation to reproduction across the two temperatures, although *C. incurva* maintains a constant allocation per unit time, whereas *C.* cf. *marginalis* maintain a constant allocation per brood. The increase in allocation per unit time with increasing temperature in *C.* cf. *marginalis* could be a result of more energy overall being available to this species at the high temperature. This pattern is opposite of what is expected if the cost of somatic maintenance is

Table 6. Trade-offs in eggs size and egg number in *Crepidula incurva* and *Crepidula* cf. *marginalis* at 23 °C and 28 °C

	Egg volume/brood	Egg volume/day	Hatchling volume*/brood	Hatchling volume*/day
Crepidula incurva				
High	1.89×10^9	2.05×10^8	4.40×10^{10}	4.79×10^9
Low	2.93×10^9	1.99×10^8	6.78×10^{10}	4.61×10^9
Low as % of high	155.2%	**97.0%**	152.0%	**95.2%**
Crepidula cf. *marginalis*				
High	2.42×10^9	1.75×10^8	4.65×10^{10}	3.36×10^9
Low	2.46×10^9	1.44×10^8	5.14×10^{10}	3.00×10^9
Low as % of high	**101.5%**	82.0%	**103.2%**	83.3%

*The volume of hatchlings = $4/3 \times \pi \times$ (length) × (width) × (height) with length = Feret's length; width = Feret's length × top aspect ratio; height = Feret's length × side aspect ratio. Top and side aspect ratios were determined to be 0.68 and 0.66 for *C. incurva* and 0.61 and 0.66 for *C.* cf. *marginalis* respectively, by K. McDonald. Values that remain constant across the temperature treatments are shown in bold.

lower at cooler temperatures and therefore more energy would be available for reproduction at cooler temperatures (Steigenga & Fischer, 2007). Differences in the total pool of energy available to individuals can result in positive correlations among life-history traits that are expected to show trade-offs (van Noordwijk & de Jong, 1986). Alternately increased allocation to reproduction per unit time could be offset by a reduction in energy allocated to growth or survival, which is a possibility that these data cannot address.

Recent studies of model systems show that trade-offs that were considered to be the necessary consequence of the dichotomous allocation of a limited pool of resources to alternate, mutually exclusive aspects of survival or fitness (Y-type allocation decision) can actually be uncoupled. For example, reproductive output and life span can be uncoupled under certain environmental conditions (Flatt & Schmidt, 2009; Grandison *et al.*, 2009). Studies of endocrine and other signaling pathways suggest that 'trade-offs' may be controlled by regulatory feedback loops that can be uncoupled (Edward & Chapman, 2011; Stearns, 2011). These types of regulatory pathways are possible alternatives to traditional Y-type decisions as candidate mechanisms of the observed trade-off between egg size and egg number. Some biophysical models of how reproduction responds to temperature could also produce patterns consistent with trade-offs without Y-type energy allocation. In butterflies, egg production/differentiation and growth have different temperature sensitivities (Steigenga & Fischer, 2007; Geister *et al.*, 2008) resulting in both lower numbers of eggs and larger eggs at low temperatures (Steigenga & Fischer, 2007). A similar mechanism could be at play in *Crepidula*, which lay broods less frequently at cool temperatures.

MULTIVARIATE PLASTICITY

Stearns (2011), when making concluding remarks about a recent book on mechanistic approaches to life-history evolution (Flatt & Heyland, 2011), pointed out that the common view that evolution should work to reduce pleiotropy in features important to fitness does not fit our observations of life-history evolution. Life-history features are integral to the fitness of an organism but they are rife with trade-offs and correlations: changes in one feature often or usually affect some other feature of an organism's life history. The results of the present study showed that these trade-offs themselves can display phenotypic plasticity. The phenotypic integration (i.e. the network of trade-offs reflected by **P**) was more resilient to temperature change in *C*. cf. *marginalis* than in *C. incurva*. Because **P** is an important component of the multivariate selection gradient (Lande & Arnold, 1983),

temperature-mediated plasticity in **P** could alter selection gradients and significantly impact evolutionary responses to selection at different temperatures.

This temperature-mediated plasticity of **P** is highly relevant to the evolution of life histories of *Crepidula* species living along the Pacific coast of Panama where they are exposed to significant periods of the two different temperatures tested here. The Bay of Panama experiences seasonal upwelling. Non-upwelling (approximately May–December) sea surface temperatures are approximately 28 °C and the more variable upwelling (approximately January–April) sea surface temperatures range from 18 °C to 24 °C (D'Croz & O'Dea, 2007; Miglietta, Rossi & Collin, 2008). *Crepidula incurva* and *C*. cf. *marginalis* reproduce all year, and therefore their life-histories are exposed to selection at both 28 and 23 °C. If the brood frequencies measured in the laboratory are representative of brood frequencies in the field and if one-third of the year is under upwelling conditions (a generous estimate), then approximately 24% of the broods of *C. incurva* and 29% of broods of *C*. cf. *marginalis* are produced during low temperatures.

VARIATION AMONG AND WITHIN FEMALES

Variation in egg size and offspring size among females can be a result of genetic differences among females or maternal effects mediated by maternal condition, size or age. Therefore, it is not surprising that variation among females is common (e.g. echinoids: Lessios, 1987; Berger & Jelinski, 2008; bivales: Phillips, 2007; gastropods: Collin, 2010). Lessios (1987) found significant differences in egg size between females in each of 13 species of tropical echinoids that were spawned immediately after collection. Broods with the largest mean female egg size ranged across species from 1.15 times the smallest mean egg size to as much as two times the volume. Phillips (2007) found that 20% of the variation in egg size and 86% of variation in egg energy content in mussels was a result of variation among field-collected females, although little of the variation among hatchling larvae was a result of variation among females. Collin & Salazar (2010) found that 65% of the variation in egg size and hatching size of two species of laboratory-raised slipper snails (*C. atrasolea* and *C. ustulatulina*) was a result of variation among females when, similar to the other studies, only a single brood was measured for each female.

When broods from a single female vary, the among-female component of variation can be considerably overestimated if only a single brood is examined. For example, when multiple broods were measured for *C. atrasolea* and *C. ustulatulina* raised in incubators at the same temperatures, approximately 40% of the

variation in egg size was a result of variation among broods from the same female (Collin, 2010). A similar allocation of variation in egg size and hatching size was observed in the present study: 40–50% of variation was a result of among brood variation, whereas 15–25% was a result of variation among females in *C. incurva* and *C*. cf. *marginalis*. Why offspring size varies among and within clutches from the same female raised under constant conditions is not clear and needs to be more fully understood before a comprehensive understanding of egg size can be reached. However, it is clear that this pattern needs to be taken into account in interpreting variation detected from single broods per female.

FUTURE DIRECTIONS

More than a decade after Bernardo's (1996) call for a more detailed microevolutionary approach, our understanding of the evolution of offspring size in marine invertebrates has made some significant advances. Major steps have been made in our appreciation of the evolutionary implications of the influence of egg size on fertilization success in free-spawners (Farley & Levitan, 2001; Levitan, 2008), as well as the carry-over effects of egg size, larval experience, and settler size on juvenile performance (Pechenik, 2006), amongst others. However, a recent review of egg size in marine invertebrates (Moran & McAlister, 2009) concluded that, among other things, we still know very little about the causes of variation in offspring size among females; about how genetic, environmental, or genetic × environmental factors contribute to variation; and about how widely the observed patterns can be generalized across taxa and different modes of development. Work with calyptraeids supports the generalizations from other studies of marine invertebrates that offspring size decreases with increasing temperature and that there is significant variation among females. Too few studies exist to generalize across taxa on the variation among broods from the same female or to draw any generalizations about the variance–covariance structures of life-history phenotypes. Even in the relatively well-studied calyptraeid gastropods, we do not yet know how egg size reflects biochemical composition, the mechanism by which temperature affects changes in egg size, or the heritability of egg size.

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