Ectotherms with a calcareous exoskeleton follow the temperature-size rule — evidence from field survey

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ABSTRACT: The temperature-size rule, an increase in body size of ectotherms at lower developmental temperatures and vice versa, prevails across taxa. Most studies in this context have focused on either terrestrial or freshwater species, and little is known about marine organisms with calcareous exoskeletons, in which a slower calcium carbonate precipitation may constrain somatic growth at lower temperatures. Further, as the vast majority of data stems from laboratory experiments, little is known about the applicability of the temperature-size rule under field conditions. If applicable, individuals that grow in warmer seasons should metamorphose at a smaller size than those that grow in cooler seasons. Based on this premise, we monitored size at metamorphosis in 2 natural populations of the intertidal gastropod with determinate growth, *Monetaria annulus*, for 46 mo in Okinawa. Metamorphosis in summer resulted in significantly smaller sizes compared to other seasons. There was a negative relationship between (summer) temperature and size at metamorphosis across populations. These findings suggest that higher temperatures induce a smaller metamorphic size in this species under natural conditions.

KEY WORDS: *Monetaria annulus* · Phenotypic plasticity · Seasonality · Size at metamorphosis

INTRODUCTION

Changes in life history traits often occur in response to environmental conditions, and such plasticity may or may not be of adaptive significance (e.g. Padilla & Adolph 1996). One prominent example is the negative relationship between developmental temperature and adult size in ectotherms, termed the temperature-size rule (Atkinson 1994, Berrigan & Charnov 1994). This recurrent pattern has received wide interest from both proximate (Partridge et al. 1994, van der Have & de Jong 1996, Kozłowski et al. 2004, Atkinson et al. 2006, Kammenga et al. 2007, Karl & Fischer 2008) and evolutionary perspectives (Partridge & French 1996, Angilletta & Dunham 2003, Walters & Hassall 2006).

Under laboratory conditions, most ectotherms follow the temperature-size rule (Atkinson 1994). However, whether it is also applicable under natural conditions is less clear, because patterns observed in the field may deviate from those obtained in the laboratory, for instance due to genetic differences across populations or environmental factors other than temperature (Partridge & French 1996, Fischer & Fiedler 2002, Walters & Hassall 2006, Kingsolver et al. 2007). Difficulties arise particularly when environmental factors spatially and/or temporally covary or when they interact in a complex manner. For example, at least in some groups, life history decisions are predominantly regulated by photoperiod rather than temperature (Masaki 1972, De Block & Stoks 2004).

In addition to the scarceness of field data, many studies have focused on terrestrial animals such as insects (Partridge & French 1996, Nylin & Gotthard 1998, Karl et al. 2008). To our knowledge, no study has examined whether marine ectotherms with calcareous exoskeletons follow the temperature-size rule. However, examining the temperature-size rule in calcifying organisms is particularly interesting, because crystallization of calcium carbonate is slower at lower temperatures (Vermeij 1987, Irie & Iwasa 2003), which may restrain somatic growth (Palmer 1981).

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The gold-ringed cowry *Monetaria annulus* (Linnaeus 1758) is an intertidal gastropod distributed throughout the Indo-Pacific tropics and subtropics. Like holometabolous insects, *M. annulus* exhibits determinate growth in the sense that somatic growth ceases at the end of the juvenile stage (but shell construction continues thereafter; see Irie & Adams 2007, Irie & Morimoto 2008). The juvenile stage begins with the metamorphosis from planktonic to benthic lifestyle, and continues until the second metamorphosis, during which shell coiling stops (see Fig. 1).

In the present study we explored whether this species follows the temperature-size rule under natural conditions, focusing on shell size at the end of the juvenile stage (referred to as 'size at metamorphosis'). Explicitly, we tested the hypothesis that *Monetaria annulus* that grow in the warmer season metamorphose at a smaller size than those that grow in the colder season, by investigating the temporal correlation between metamorphic size and ambient temperature across seasons and years. To control for effects of other environmental factors such as photoperiod, metamorphic size was also compared between 2 neighboring populations differing in thermal environment.

MATERIALS AND METHODS

Study sites. *Monetaria annulus* is the most abundant cypraeid species in the intertidal zone of Okinawa Island, Japan, being found at various types of coasts (Irie 2006). In the present study, we focused on 2 Okinawan populations, Yamada and Sesoko, which contrast with each other in terms of their benthic environments and species composition. Yamada is a north-facing, shallow shore, situated on the west coast of Okinawa Island. It is characterized by a broad, shallow reef-flat that partially emerges every low tide. *M. annulus* is distributed patchily on the reef-flat, co-occurring with nassariids, sea cucumbers, and other decomposers occurring on the sandy bottom. The study site was close to the sandy beach, in which coralline sand and angular pebbles almost completely cover the limestone base. Sesoko is a small island adjacent to Okinawa Island, approximately 25 km from Yamada. *M. annulus* has a zonal distribution parallel to the shoreline, and *M. moneta* and *M. caputserpentis* are found in vermicular depressions excavated by the rock-boring urchin *Echinometra mathaei*. It has a bare limestone ground, and is exposed to the air only during the ebb of spring tides. In Okinawa, reproduction and larval recruitment occur throughout the year (Katoh 1989), with a seasonal peak from late autumn to spring (T. Irie, unpubl. data). The duration of the planktonic stage is currently unknown either under field or laboratory conditions.

Sampling. We defined 'size at metamorphosis' as the shell width (mm) at the end of the juvenile stage (Fig. 1). Since a yellow ring is formed on the dorsal surface after no more than 5 d after metamorphosis (T. Irie pers. obs.), we limited our investigation to post-juvenile individuals without a ring (the variation of this transient period is obviously negligible but was incorporated into the error variance in our analysis; see 'Data analysis'). During low tides, living individuals were collected and shell width was measured using a caliper; they were then released at the same spot. All measured individuals were marked to avoid double counting. Measurements were taken from February 2005 to November 2008 at Yamada (n = 239) and Sesoko (n = 188). To record ambient temperature (res-
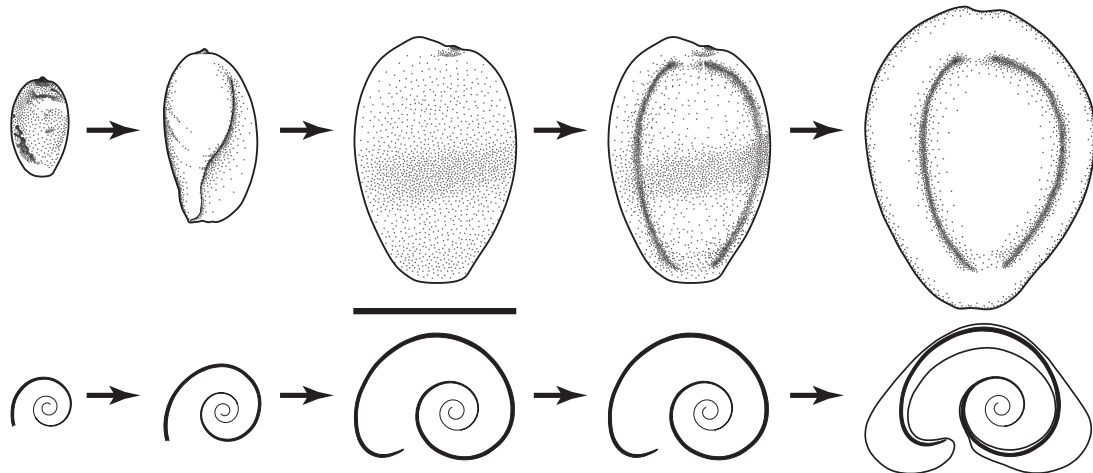

Fig. 1. *Monetaria annulus*. An ontogenetic series of external shell appearance (upper row: dorsal view; lower row: anterior view). From the left: younger and older juveniles, metamorphosing, callus-building, and adult individuals. Horizontal bar indicates the shell width measured at metamorphosis. The juvenile stage, which lasts for a few months in laboratory-reared individuals, is said to end when the shell aperture narrows by the outer lip turning inward (see Irie & Morimoto 2008)

olution: 0.5°C; recording interval: 1 h), digital data loggers (DS1921G-F50, Thermocron) were anchored to stones at each location.

Data analysis. Nonparametric regression curves were applied to examine the relationship between shell width at metamorphosis and measurement date. A series of randomization tests was performed to statistically evaluate the spatial/temporal patterns of metamorphic size. In addition, we calculated the sample cross correlations between the daily mean temperature and the expected metamorphic size (n = 365), an index of temporal covariation between 2 time-series with a possible time lag, for each of the 2 populations. This index is informative when considering that metamorphic size is likely to depend on the temperature during the juvenile stage rather than on the day of metamorphosis (see 'Discussion'), although temperature effects should be cumulative in reality. See Appendix 1 for the mathematical details of the statistical approach.

RESULTS

Daily mean and maximum temperatures were, as expected, highest in summer and lowest in winter at both localities (Fig. 2A), whereas size at metamorphosis exhibited the opposite pattern (Fig. 2B). Hence, cowries metamorphose at a smaller size in warmer seasons (n = 427, p < 0.0001, see Appendix 1). Cross correlation between the daily mean temperature and the expected metamorphic size for Yamada was strongest (r = −0.87) with a time lag for the metamorphic date of 27 d, and for Sesoko (−0.88) with a 56 d time lag. Fig. 3 illustrates the relationship between temperature and metamorphic size based on these time lags. Similar results were obtained for the correlations with daily maximum temperature and daily temperature amplitude (i.e. differences between daily maximum and minimum temperatures).

Summertime maximum temperatures were higher at Yamada than at Sesoko (Fig. 2A), reflecting that the sunshine warms up the seawater in tide pools during
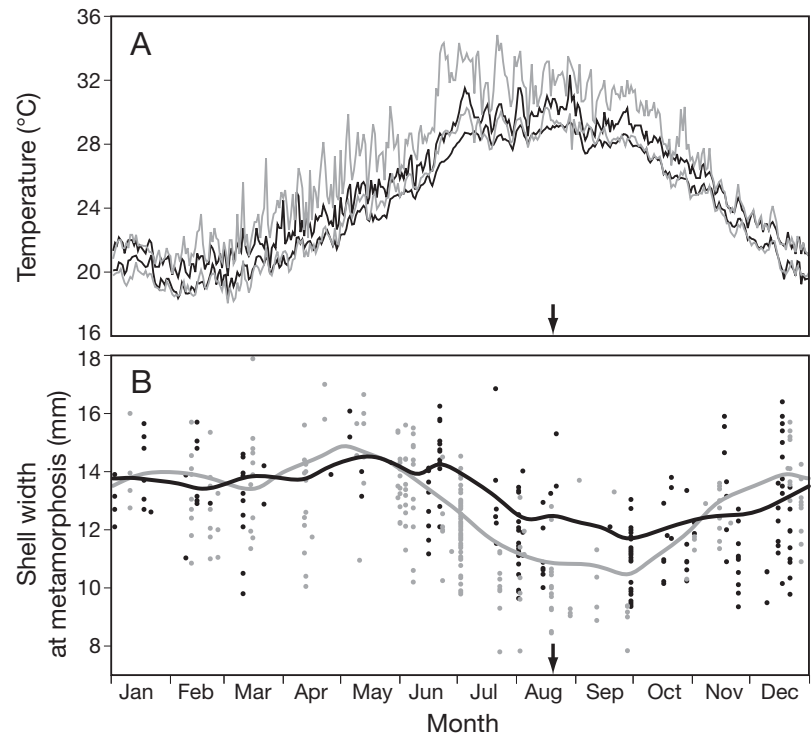

Fig. 2. *Monetaria annulus*. (A) Daily maximum and mean temperatures (grey line: Yamada; black line: Sesoko) averaged across years. (B) Seasonal variation in shell width at metamorphosis (dots) and expected values (curves) calculated by fitting nonparametric regression models (see Appendix 1) for Yamada (grey) and Sesoko (black). Data were pooled across years (year-to-year variation in metamorphic size not shown). Downward arrows indicate the date on which the difference of expected size at metamorphosis between the 2 populations was largest
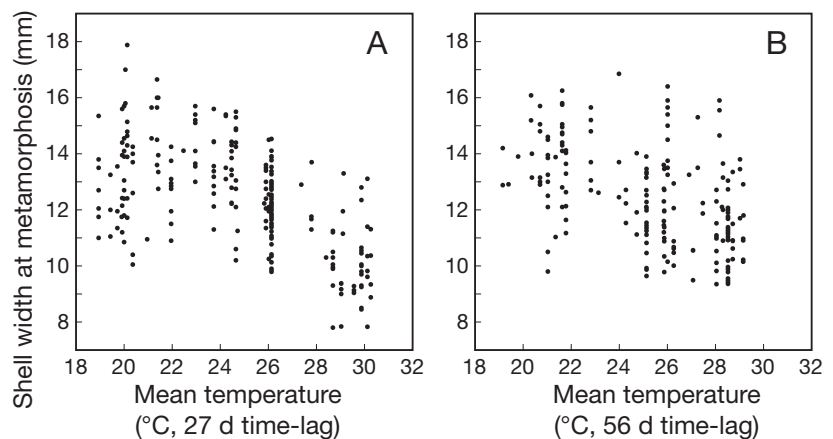

Fig. 3. *Monetaria annulus*. Scatterplots of observed shell width at metamorphosis against daily mean temperature with time lags of (A) 27 d for Yamada and (B) 56 d for Sesoko

ebb. Concomitantly, mean metamorphic size was smaller at Yamada compared to Sesoko throughout summer (n = 427, p < 0.0001, see Appendix 1) with a maximum size difference of 2.46 mm (indicated by arrows in Fig. 2).

DISCUSSION

In line with the temperature-size rule, our study demonstrates that *Monetaria annulus* that grow in warmer seasons metamorphose at a smaller size than those that grow in cooler seasons. There was no evidence that limitations in exoskeletal calcification affected seasonal variation in metamorphic size. Although potentially confounding effects of other environmental factors cannot be entirely ruled out (see below), 2 lines of evidence suggest a causal relationship between developmental temperature and body size in *M. annulus*. (1) Correlations between temperature and size were strongest when there was a time lag between metamorphic date and ambient temperature of 26 d for Yamada and 56 d for Sesoko, in accordance with the notion that size at metamorphosis is determined during development, which roughly estimated takes a few months (T. Irie, pers. obs.). (2) The differences between the two study sites, with higher summer temperatures at Yamada being accompanied by smaller body sizes compared to Sesoko, further support this interpretation.

Mechanistically, the observed seasonal variation in size at metamorphosis might in part be related to stressfully high maximum temperatures. The daily maximum temperatures reached 37.5°C at Yamada and 35.5°C at Sesoko. Such high temperatures may cause physiological disorders through protein denaturation and coagulation, and may further induce the up-regulation of heat shock proteins to regulate and repair protein folding (e.g. Hofmann 2005). Optimal energy allocation theory predicts that cowries, at high temperatures, may spend more energy in defense (e.g. heat resistance), potentially slowing down their somatic growth and exoskeletal construction, in turn resulting in smaller size at metamorphosis (Irie & Iwasa 2005). Testing this hypothesis would require controlled laboratory experiments using a full range of developmental temperatures.

Photoperiod, in contrast, is unlikely to cause the patterns found, because size at metamorphosis was significantly different between 2 populations experiencing equal photoperiodic conditions. Plastic responses to photoperiod are common among insects occurring in temperate and high latitudes (Nylin & Gotthard 1998, De Block & Stok 2004). However, photoperiod is not a reliable cue for organisms occurring at low latitudes, in which the difference in day length between the winter and summer solstices is small (only 3 h and 20 min in Okinawa).

In conclusion, the observed seasonality in metamorphic size in *Monetaria annulus* is most likely caused by temperature, although our survey cannot entirely rule out the possibility that other environmental factors are also involved. For example, longer tidal dry time in Yamada, possibly accompanied by lower food availability and salinity, might cause slower growth and resultant smaller metamorphic size in summer. To what extent the patterns described here were influenced by thermal stress occasionally experienced during summer could be elucidated by further experiments in which temperature and other conditions are controlled.

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Appendix 1. Statistical analysis

We fitted nonparametric regression curves to the scatterplot of the shell width at metamorphosis on the measurement date in order to statistically test for the hypotheses concerning the spatial/temporal patterns of metamorphic size. Nonparametric regression analyses include a family of variants in combination with smoothing functions, kernel weight functions, bandwidth and fitting criterion. The suitability of a method depends on the structure of the data to be fitted. Below, we briefly describe the model used in the present study and its computation procedure to assure the reproducibility of analysis.

Letting n be the number of observations and x be the regressor value at which regression coefficients are estimated, a locally weighted linear regression function (so called 'loess') is formulated by $Y = W(x)X(x)\beta(x) + \varepsilon(x)$, where $Y = [y_1, ..., y_n]^T$ is an $n \times 1$ vector of observed metamorphic size, $\varepsilon(x) = [\varepsilon_1(x),...,\varepsilon_n(x)]^T$ is an $n \times 1$ vector of the residuals, $\beta(x) = [\beta_0(x), \beta_1(x)]^T$ is a column vector consisting of the intercept and slope coefficients, and

$$X(x) = \begin{bmatrix} 1 & x_1 - x \\ \vdots & \vdots \\ 1 & x_n - x \end{bmatrix}$$

is an $n \times 2$ design matrix. $W(x)$ is an $n \times n$ diagonal matrix of weights, of which jth diagonal element is given by

$$W_{jj}(x_i) = w_j(x_i) \Big/ \sum_{i=1}^{n} w_j(x_i)$$

where w_j are the weights specified below. The regression curve, $b_0(x)$, is defined as the least-square estimator of the intercept coefficient β_0 as a function of the regressor x, and is calculated as

$$b_0(x) = e_1^T \left(X(x)^T W(x) X(x) \right)^{-1} X(x)^T W(x) Y,$$

where $e_1 = [1, 0]^T$ is the 2×1 unit vector. As kernel weight function we chose the normal density function with variance λ^2 and thus the weight w_j takes

$$w_j(x_i) = f(x_j; x_i, \lambda) = \frac{1}{\sqrt{2\pi\lambda^2}} \exp\left[-\frac{(x_j - x_i)^2}{2\lambda^2} \right]$$

in which λ was set at 14.6 d (= 4% of a year) throughout all analyses.

We first tested for seasonal variation in mean metamorphic size by performing a randomization test on the regression curves calculated in the foregoing method. Prior to computation, data were rearranged to be circular by pooling across survey years to improve the estimation particularly at both ends of the regressor axis (this procedure is statistically justified below). To compare the goodness-of-fit of the regression curve model (abbreviated as 'curve', below) to that of the horizontal line model (abbreviated as 'horizon') in which the expected metamorphic size takes the same value throughout the year, we calculated the difference of residual sums of squares (RSS) between the 2 models:

$$\Delta RSS = RSS_{horizon} - RSS_{curve}$$

for the original data (i.e. $\Delta RSS_{original}$). A randomization distribution was constructed by repeating the following steps 100 000 times: (1) randomly reassign y-values (i.e. metamorphic size) to the fixed x-values (i.e. date) within populations; (2) calculate ΔRSS for the newly generated data after fitting regression curves. The null hypothesis (no seasonality in metamorphic size) is rejected at the 5% level of significance, if $\Delta RSS_{original}$ is in the top 5% tail of the randomization distribution.

This analysis potentially suffers from pseudoreplication by ignoring the year-to-year variation in metamorphic size. To rule out this possibility, we tested the null hypothesis that the regression curves do not differ among years, using a randomization test as follows. We first computed the residuals from the regression curve model defined above. The residuals were fitted by regression curves again after setting the corresponding elapsed number of days from the initial survey date (instead of the circular date) as the independent variable, and then calculated the residual sum of squares for the residuals (RSS$_{curve}$). The curves should not deviate from the x-axis (i.e. a horizontal line with $y = 0$) if the null hypothesis is true. The statistic was set as

$$\Delta RSS = RSS_{horizon} - RSS_{curve}$$

in which RSS$_{horizon}$ is the sum of squared original residuals. To construct a randomization distribution, we then created 100 000 new data sets from the original residuals by randomly reassigning y-values (i.e. original residual) to the fixed x-values (i.e. date) within populations. As a result of computation, the year-to-year variation in metamorphic size was found to be nonsignificant ($p > 0.05$).

Finally, we tested the null hypothesis that the regression curves are statistically identical between the 2 populations. In this case, a statistic was defined as the residual sum of squares from the regression curves of metamorphic size on the circular date. The statistic for the original data (RSS$_{original}$) was compared to a randomization distribution created by randomly interchanging every data set (metamorphic size and the corresponding date) between the 2 populations.