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## The effect of temperature on maturation threshold body-length in *Daphnia magna*

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**Abstract** Aquatic invertebrates are usually larger at maturity when water temperatures are lower. For the freshwater cladoceran *Daphnia*, it has been suggested that a threshold size must be attained to initiate maturation, which results two instars later in the deposition of eggs into the brood chamber. This threshold size is believed to be independent of food. Here, we examine the effect of temperature on maturation threshold body-length in *Daphnia magna*. Daphnids were raised from birth to maturity under three constant-temperature regimes (12°C, 16°C, 22°C), and two food-level conditions. Animals were measured daily, and a body-length based maturation threshold determined for each individual. We demonstrate that mean maturation threshold length is negatively correlated with ambient water temperature. Further, daphnids with a larger threshold length tended to be larger at maturity. A maturation threshold linked to body length suggests that reduced variation in size at maturity is adaptive, even at the cost of additional variation in instar number or age at maturity.

**Key words** *Daphnia magna* · Temperature · Food supply · Maturation · Threshold · Body length

### Introduction

Phenotypic plasticity of life-history traits help organisms to allocate resources optimally in response to varying en-

vironmental conditions. In particular, plasticity in the life-history traits associated with maturation is of great importance. Maturation is a critical point in the life cycle of an organism because it marks the achievement of reproduction and is therefore crucial for fitness (Bernardo 1993).

In the freshwater cladoceran *Daphnia*, maturity is reached when the first clutch of eggs is laid into the brood pouch. Ovary development however, is initiated in the preadolescent instar, two instars before maturity (Zaffagnini 1987). It has been suggested that a maturation threshold body-length must be crossed before the onset of ovary development (and therefore egg production) can occur (Ebert 1992, 1994). This threshold is defined as the minimal length a daphnid must attain before it invests resources into reproduction, and is estimated as the mid-point length between the pre-preadolescent and the preadolescent instars. Animals crossing the threshold in an early instar will produce eggs sooner than animals crossing it in a later instar. Recent studies indicate that the maturation threshold body-length can vary across different populations and clones of the same species of *Daphnia* (Ebert 1991, 1994), but is independent of food concentration (Ebert 1992; Perrin 1989; see also Enseink et al. 1995).

The experiment described here was designed to examine the effect of temperature on the maturation threshold body-length in *Daphnia magna*. A decline in body size at maturity with increasing temperature is well-documented in zooplankton (e.g. Culver 1980; Perrin 1988; Yampolsky and Scheiner 1996). Our working hypothesis therefore was that the maturation threshold body-length should also decline with increasing temperature.

### Materials and methods

The *Daphnia* were a German clone which had been under continuous laboratory culture for at least 5 years. Experimental animals and stock cultures were maintained in artificial pond water (AD-aM medium, Klüttgen et al. 1994) and fed log-phase *Chlamydomonas reinhardtii* (strain S1D2, Gross et al. 1988) grown in a che-

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mostat. Food was prepared by concentrating the algae and resuspending them to the required concentration in ADaM medium. Lighting was 16 h:8 h light:dark soft white fluorescent light at a mean intensity of  $3.6 \text{ W m}^{-2}$ .

Individual daphnids were kept in separate plastic beakers containing 150 ml of algal suspension (either  $10,000 \text{ cells ml}^{-1}$ , or  $150,000 \text{ cells ml}^{-1}$ ) at one of three incubator temperatures ( $12^\circ\text{C}$ ,  $16^\circ\text{C}$ ,  $22^\circ\text{C}$ ). It was necessary to have different food levels within each temperature treatment in order to increase the range of growth rates and lengths at birth of the experimental animals. Animals were transferred to new beakers and medium twice weekly, and algae provided daily under the assumption that over the previous 24 h period food levels were entirely depleted. In reality this meant that there was a slight build-up of food between medium changes, particularly at  $12^\circ\text{C}$ , although at all treatment levels the relative difference between "high" and "low" food levels was maintained. Since we were looking at life history until maturity (when *D. magna* is still relatively small), it is unlikely that using 150 ml of medium produced a container effect.

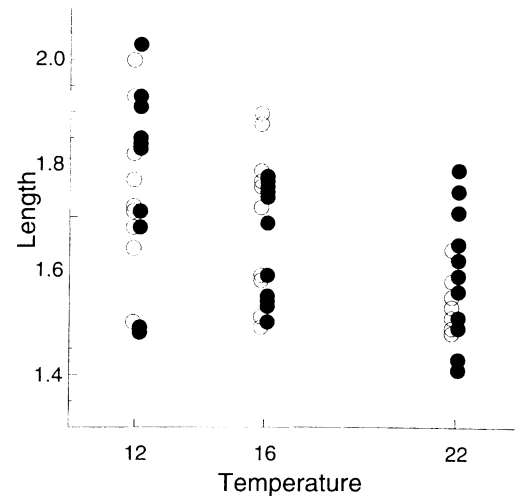
Fifth clutch newborn from females that had been raised under the above constant conditions for two generations were used for measurements (i.e. these newborn were the granddaughters of the animals used to start the experimental line). The number of replicates at each treatment level was 12. However, one animal died under the low-food conditions at  $12^\circ\text{C}$  and so the data set consists of measurements for 71 individuals. Animals were measured within 8 h of birth and then every 24 h until release of their first eggs into the brood pouch. Length measurements were made from the top of the eye to the base of the tail spine using a microscope ocular micrometer, and the number of instars each daphnid passed through up until maturity were counted.

To calculate maturation threshold lengths the mid-point length between the pre-preadolescent and preadolescent instar was determined for each daphnid. All length data were natural log-transformed for analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Juvenile growth rates and juvenile increment were also calculated and are defined as increase in length (mm) per day, and mean percentage increase in length per instar respectively (both values up to and including the adolescent instar).

## Results and discussion

Figure 1 is a plot of the estimated maturation threshold body-lengths for each daphnid, and indicates that, within each temperature group, threshold lengths differed little between food levels, but that there was an overall negative relationship with temperature. ANOVA confirmed these trends, showing that temperature was a highly significant factor influencing mean threshold lengths (Table 1). As temperature increased threshold lengths declined from a mean of 1.76 mm (SD = 0.15,  $n = 23$ ) at  $12^\circ\text{C}$  to a mean of 1.56 mm (SD = 0.13,  $n = 24$ ) at  $22^\circ\text{C}$ , and  $16^\circ\text{C}$  animals were intermediate at 1.68 mm (SD = 0.09,  $n = 24$ ). On the other hand, neither food concentration nor the interaction term (temperature  $\times$  food) were significant in the model (Table 1).

Lengths at birth of the experimental animals varied from 0.825 to 1.100 mm. However, there was no significant relationship between length at birth and maturation threshold length (ANCOVA: beta value for covariate length at birth = 0.17,  $P < 0.177$ ). Similarly, juvenile growth rates varied from 0.126 to  $0.388 \text{ mm day}^{-1}$  (increasing with temperature and food); and juvenile increments varied from 23 to 35% instar $^{-1}$  (increasing with food but decreasing with increasing temperature). Again,



**Fig. 1** Plot of the estimated maturation threshold body lengths (mm) for each daphnid at each temperature ( $^\circ\text{C}$ ) and food level (open circles low food, filled circles high food)

**Table 1** Analysis of variance for maturation threshold body-length

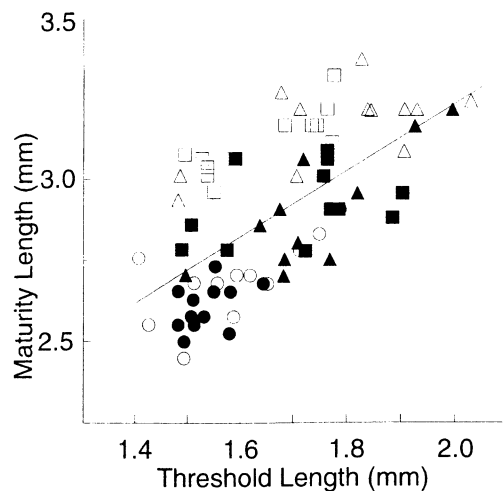
Effect	df	MS	F	P
Temperature	2	0.0791	13.88	0.0001
Food	1	0.0006	0.11	0.7536
Temperature $\times$ Food	2	0.0090	1.58	0.2135
Error	65	0.0057		

maturation threshold length was not significantly affected by either growth rate (ANCOVA: beta value for covariate juvenile growth rate = 0.21,  $P < 0.0885$ ) or increment (ANCOVA: beta value for covariate juvenile increment =  $-0.06$ ,  $P < 0.6598$ ).

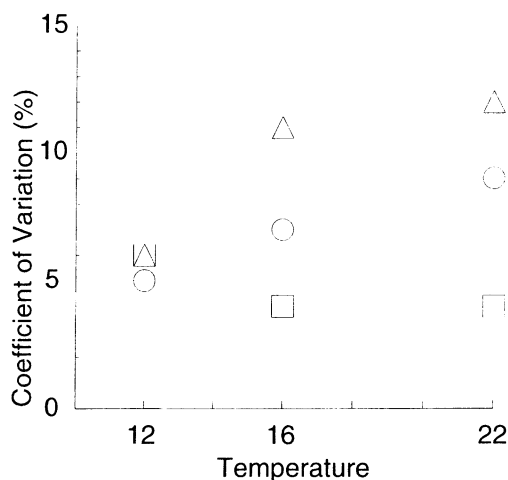
Animals with a greater maturation threshold length tended to have a greater length at maturity (ordinary least-squares regression:  $r = 0.65$ ,  $n = 71$ ,  $P < 0.001$ ; Fig. 2). This relationship held across all treatment levels, so only the overall regression line is shown in Fig. 2.

We observed variability in age, instar number, and size at maturity both within and across temperature treatments. If maturation is size based then stabilising selective pressure within a given environment should dictate an optimal size at maturity and reduce variation in this trait. Indeed, life-history evolution in *Daphnia* is believed to be shaped by size efficiency and size selective predation (Brooks and Dodson 1965; Lynch 1977; Bengtsson 1986; Taylor and Gabriel 1992; Tollrian 1995). A maturation threshold body-length, as compared to a case where age or instar number is the critical parameter, would allow an individual to minimise deviations from optimal size at maturity (Stearns and Kawecki 1994), even though this might bear costs from more variation in age at maturity or instar number at maturity.

If the maturation threshold body-length in *Daphnia* serves to reduce variation in body-length at maturity, then we should find that the coefficient of variation (CV)



**Fig. 2** Relationship between maturation threshold body length (mm) and body-length at maturity (mm). The line is the overall regression line ( $y = 1.03x + 1.18$ ,  $r = 0.65$ ,  $n = 71$ ,  $P < 0.001$ ) (Circles 22°C, squares 16°C, triangles 12°C; full symbols high food, open symbols low food)



**Fig. 3** Coefficients of variation for three life-history traits across temperature treatments (°C) (squares length at maturity, circles instar at maturity, triangles age at maturity)

for body-length at maturity is always low, whereas for age and instar number at maturity it might be low or high depending on the environmental variance. In other words, size at maturity should be more buffered against variation in growth and development than age or instar number.

Our results conform to the above prediction reasonably well. The CVs for body-length at maturity were relatively low, while for instar number, and particularly for age at maturity, the CVs were relatively higher (Fig 3). This result holds even when we consider that age at maturity necessarily involved a degree of measurement error because animals were only examined every 24 h. An individual might have produced its first clutch of eggs at any time during the 24 h period prior to measurements being taken. Using the experimental means and variances

we simulated an age-at-maturity data set and calculated CVs based on true ages and on age recorded on a 24 h time interval (including the uncertainty of time when born). Differences between CV values were never greater than 1%, and thus error in measuring age at maturity probably had an insignificant effect in determining the overall pattern of CVs in the real data.

The threshold body-length works to reduce size variability in the maturation phenotype. It appears that size at maturity is the key fitness component in *D. magna* rather than instar number or age. Being the right size at maturity helps to optimise production of offspring and may also reduce adult mortality due to size selective predation. Depending on size at birth and on environmental conditions the number of juvenile instars is adjusted to regulate length at maturity (Green 1956; Ebert 1991). Within a given growth condition lengths at maturity fall within a given range independent of the number of instars. This is because moult increments in *Daphnia* appear to be inflexible (i.e. each stepwise increase in body-length is of the same magnitude) and therefore once the body-length threshold is crossed animals reach maturity (two instars later) at similar sizes regardless of the number of instars taken to reach the threshold. However, each additional instar takes time and animals with a greater number of instars are older at maturity. Length at maturity is thus regulated at the expense of age at maturity.

In this study we have demonstrated that the maturation threshold body-length can vary across different ambient water temperatures in a phenotypically plastic manner. As temperature increases over the range 12°C to 22°C, threshold body-lengths decrease. Further, the threshold length appears to be a determinant of actual length at maturity in *D. magna*, with both variables being positively correlated. Why should the threshold length decrease with increasing temperature? The answer is difficult to ascertain, but two sets of explanations exist. Firstly, temperature may be the ultimate selective factor because it directly influences physiological rates. Body size is constrained at elevated temperatures because the metabolism of larger animals is disproportionately high, leading to energy deficits for growth and moulting (e.g. Gophen 1976; Lehman 1988; Lynch 1989). Secondly, temperature may act proximately, other temperature related variables being the ultimate factors. For example, higher levels of predation during the warmer summer season may favour smaller body sizes (Culver 1980).

Our results are for a single clone of *Daphnia*. Future work should include the testing of a greater number of clones, as well as different species of cladoceran and different environmental variables (for example predator mediated cues), if the maturation criterion is to be fully understood.

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