The following supplement accompanies the article

Energetic adaptations to larval export within the brackish-water palaemonine shrimp, *Palaemonetes varians*

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Supplement. Experiment 1: The effects of temperature on larval development

Methods

On hatching, actively swimming larvae were separated from 11 females using a plastic pipette, and individually and haphazardly transferred to 100 ml plastic beakers containing ~80 ml (15°C, 32 salinity, 1 µm filtered, UV treated seawater). Larvae were transferred to incubators set at 15, 20, and 25°C and 12:12 h (light:dark); 12 larvae from 8 females were transferred to each temperature. This temperature range reflects the temperature range recorded *in situ* within the environment of adult shrimp during summer months and across 3 years (A. Oliphant unpubl. data). The first instar (zoea 1) of *Palaemonetes varians* is facultative lecithotrophic (A. Oliphant unpubl. data) and as such, the first instar was not fed (following Oliphant et al. 2013). At each temperature, 6 of the 12 larvae per female were not fed (unfed category). The remaining 6 larvae per female were fed from the start of the second instar (fed category). A total of 48 larvae were fed (i.e. 6 larvae from 8 females) and 48 larvae were unfed (i.e. 6 larvae from 8 females). Larvae were monitored daily (am) for mortality and development, assessed by morphological changes and moulting (following Fincham 1979). On moulting to the juvenile stage, individuals were blotted on tissue paper and transferred to pre-weighed tin capsules (6 × 4 mm, Elemental Microanalyser) and frozen at –80°C (using a Kendro Laboratory Products model ULT1386-5-V39 –80°C freezer). Later, samples were freeze dried for 24 h (using a Thermo Scientific Heto PowerDry LL3000 freeze dryer) and weighed for dry weight (DW, µg; using a Sartorius microbalance ME5 mass balance).

Larval development time data were analysed by non-parametric Kruskal-Wallis comparison; juvenile DW data were analysed by General Linear Model (GLM) ANOVA with post-hoc testing using the Sidak method with temperature (°C) and instar (4 vs. 5 instars) as factors.

Results

Effects of starvation on survival

P. varians larvae showed extended periods of survival in the absence of food at all temperatures tested (Fig. S1). Fed larvae moulted regularly whilst for unfed larvae, moulting continued regularly to a point, beyond which moulting became retarded and eventually ceased. At 15°C, the second instar inter-moult period for unfed larvae appeared considerably longer than that for fed larvae (Fig. S1). Whilst fed larvae moulted to the third larval instar after 9.89 ± 0.1 d, unfed larvae moulted to the third instar after 12.3 ± 0.2 d. This corresponded to a considerable increase in mortality with survivorship

decreasing from 48 larvae at 11 d to 33 larvae at 13 d, and subsequently 0 larvae by Day 16 (Fig. S1). At 20 and 25°C, the second instar inter-moult periods for unfed larvae were similar to those for fed larvae (Fig. S1). At both temperatures, most larvae successfully moulted to the third larval instar, and mortality rates increased a few days after this moult. For example, at 20°C, unfed larvae moulted to the third larval instar after 5.4 ± 0.1 d and mortality rates increased considerably from Days 8 to 10. Similarly, at 25°C, unfed larvae moulted to the third larval instar after 4.2 ± 0.4 d and mortality rates increased considerably from Days 7 to 9. Temperature influenced the extent of development of unfed larvae with only 39.6% of larvae moulting to the third larval instar at 15°C; this figure was higher at 20°C (95.8%) and 25°C (97.9%).

Effect of temperature on larval instar number

Temperature influenced the number of instars through which larvae developed (Fig. S2A). With increasing temperature, the proportion of larvae developing through 4 instars increased from 0.33 at 15°C to 0.63 at 25°C, whilst the proportion developing through 5 instars decreased from 0.67 at 15°C to 0.37 at 25°C (Fig. S2A). The dominant development pathway was, therefore, 5 instars at 15°C and 4 instars at both 20 and 25°C (Fig. S2A).

Effect of temperature and larval instar number on development time and juvenile DW

Larval development time through both 4 and 5 instars decreased significantly with increasing temperature ($P \leq 0.001$ in all cases; Fig. S2B). At all temperatures, mean larval development time was not different between development through 5 instars and 4 instars ($p = 0.075$ at 15°C, $p = 0.051$ at 20°C, and $p = 0.058$ at 25°C).

Juvenile DW was highest for larvae developing through 5 instars at all temperatures and for larvae developing through both 4 and 5 instars, juvenile DW increased with increasing temperature (Fig. S2C). Differences in juvenile DW associated with temperature ($F = 24.93$, $p < 0.001$), larval instars number ($F = 79.19$, $p < 0.001$), and the interaction between temperature and larval instars number ($F = 3.53$, $p = 0.032$) were significant. At all temperatures, juvenile DW for larvae developing through 5 instars was greater than that for larvae developing through 4 instars ($T = 4.013$, $p = 0.0015$ at 15°C, $T = 4.085$, $p = 0.0011$ at 20°C, and $T = 7.362$, $p < 0.001$ at 25°C). For larvae developing through 5 instars, juvenile DW was greater at 25°C than at 20°C ($T = 4.662$, $p < 0.001$).

Fig. S1. *Palaemonetes varians*. Survival (initial n = 48) of fed and unfed larvae at 3 temperatures (15, 20, 25°C). Larval instar numbers and inter-moult periods (mean ± SD) for fed and unfed larvae are indicated. All larvae (both fed and unfed groups) were not fed during the first larval instar

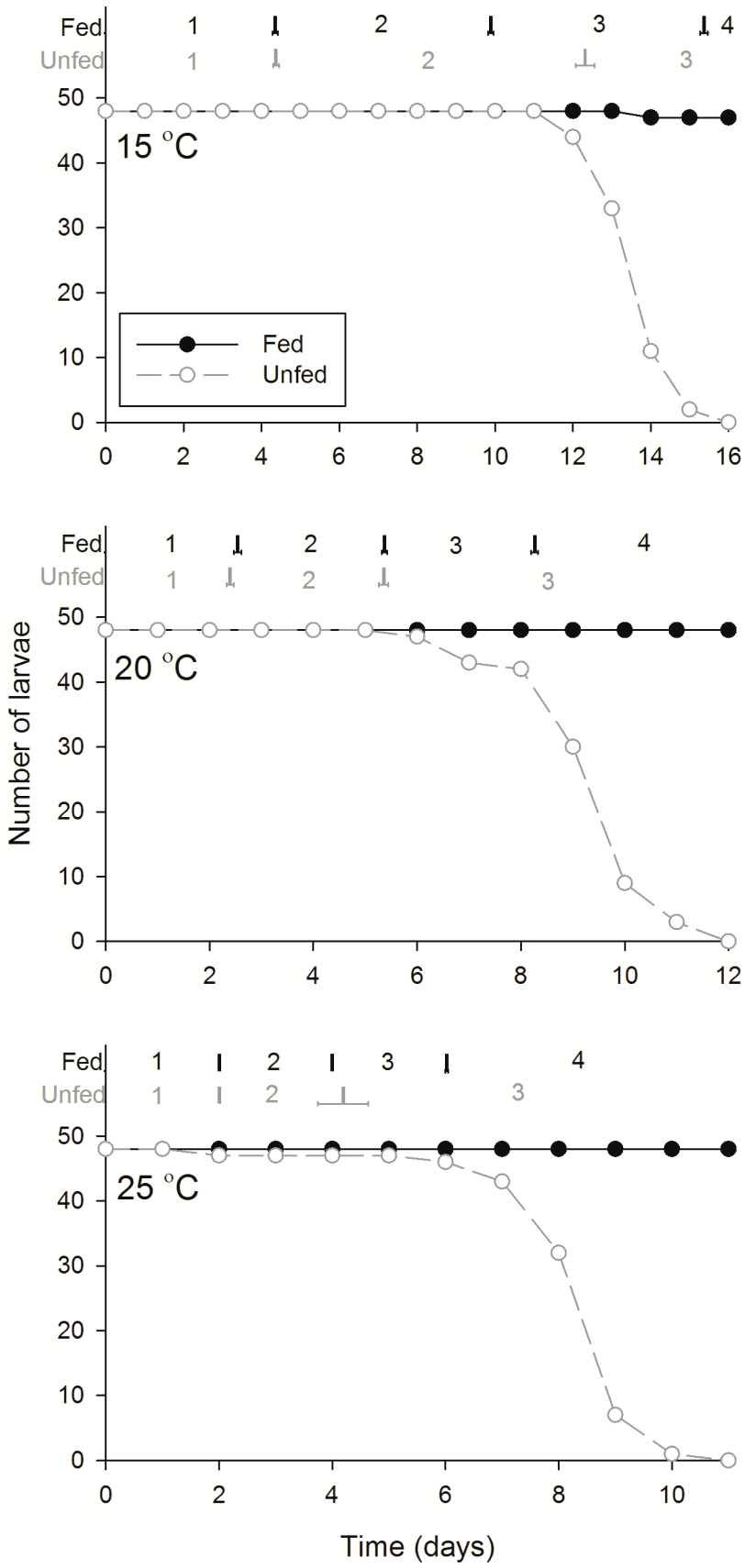

Fig. S2. *Palaemonetes varians* (A) Proportions of larvae developing through 4 and 5 instars at each of 3 temperatures (15, 20, 25°C); (B) development time for larvae developing through 4 and 5 instars at each of 3 temperatures (15, 20, 25°C); (C) juvenile DW for larvae developing through 4 and 5 instars at each of 3 temperatures (15, 20, 25°C). Data are presented as means ± standard deviations. Asterisks indicate significant differences between temperatures (grey, 5 instars; black, 4 instars), ^ indicate differences (within temperatures) between larvae developing through 4 and 5 instars