

Phytoplankton as a principal diet for callianassid shrimp larvae in coastal waters, estimated from laboratory rearing and stable isotope analysis

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Marine Ecology Progress Series 592: 141–158 (2018)

Supplemental Materials and Methods

1.1. Setup for ghost shrimp larval rearing in the laboratory

Surface water was collected from a coastal ocean area in Nagasaki Prefectural Institute of Fisheries (32.809°N, 129.771°E). The laboratory room temperature for rearing experiments was kept at 23°C using air-conditioner. Each rearing tank was soaked with freshwater in a bath made with 60-mm thick polystyrene foam. The freshwater temperature in each bath was controlled by a thermoregulator with a cooling coil (REI-SEA AZ, IWAKI) and by a pump for water circulation (REI-SEA RSD-20A, IWAKI). The black sheets on the top of tanks served for preventing larvae from being panicked under light.

1.2. Determination of embryonic states of *Nihonotrypaea harmandi*

The embryonic-state-(1) was determined by noting 2 morphological features by eye: no dorsal ova (red or orange in color) visible through the transparent female dorsum, indicating her full oviposition immediately before the sampling occasions (cf. Somiya & Tamaki 2017), and full red (orange) color in the embryos, suggesting no or little consumption of yolk in the preceding development. The embryonic-state-(2) was determined by embryos' largest eyes and transparent bodies due to a minimum amount of remaining yolks.

1.3. Diatom sample processing for stable isotope analyses

For stable isotope analyses of the diatoms (*Chaetoceros gracilis*) fed to the larvae of *Nihonotrypaea harmandi*, a few ml of liquid diatoms was transferred to a pre-combusted (450°C, 3 h) glass fiber filter (GF/F: 0.7 µm pore size, 25 mm Ø, Whatman), and gently washed with 1 ml distilled water. The filter samples were dried with a vacuum freeze dryer (DRT140FB, JFE Advantech) and fumigated with 12 N HCl for 1 d to remove inorganic carbon (i.e. HCO₃⁻ and CaCO₃). A one third- or half-portion of the filter was then wrapped with an acetone-rinsed tin capsule after the remaining HCl and humidity had been removed by vacuum desiccation in the presence of NaOH.

1.4. Amino acid extraction and purification for zoeal samples

Dried samples of zoea VI (2 sets of about 20 ind., ca. 4 mg dry weight) collected at Stn A in August 2012 were hydrolyzed in 12 mol l⁻¹ HCl at 110°C for 12 h. The hydrolysates were filtrated through a nanosep tube (GHP, 0.45 mm, PALL), washed with n-hexane/ dichloromethane (3:2, v/v) to remove large particles and hydrophobic constituents (e.g. lipids), respectively, and evaporated to dryness under a N₂ stream (Nitromini NM 910, GL Sciences). After derivatization with thionyl chloride/ 2-propanol (1:4, v/v) at 110°C for 2 h and pivaloyl chloride/ dichloromethane (1:4, v/v) at 110°C for 2 h, and liquid–liquid extraction with 0.5 ml of n-hexane/ dichloromethane (3:2, v/v) and 0.2 ml of distilled water, the Pv/iPr derivatives of amino acids were dissolved in dichloromethane.

LITERATURE CITED

Somiya R, Tamaki A (2017) Unraveling mating behavior for Axiidea (Crustacea: Decapoda): burrow-dwelling callianassid shrimp in intertidal sandflat. *J Exp Mar Biol Ecol* 486: 305–313

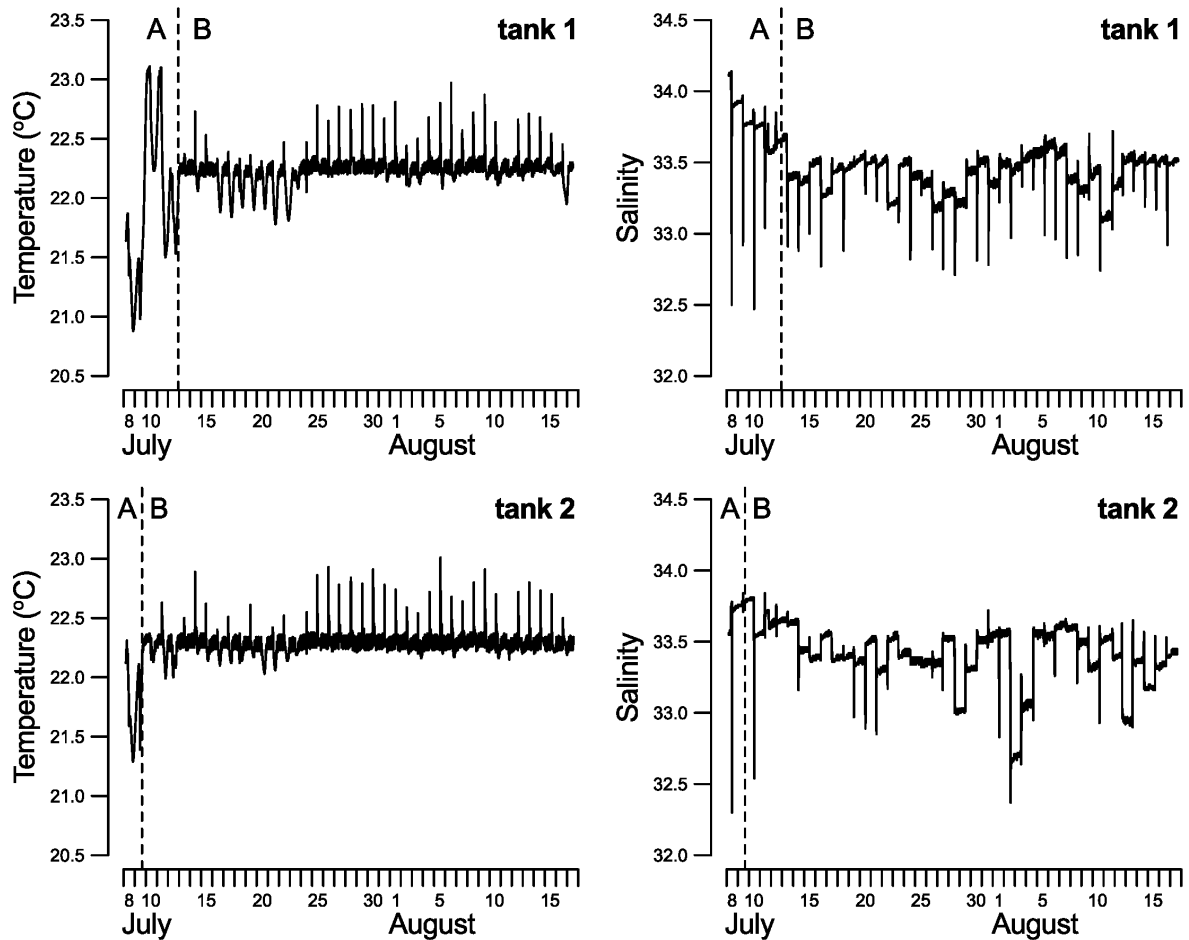


Fig. S1. Time series of seawater temperature and salinity in the 2 rearing tanks for *Nihonotrypaea harmandi* larvae from 8 July to 17 August in 2013. Each daily tick mark shows 00:00 h. In both tanks, the whole period was divided into 2 sub-periods (A, B) based on the ranges of fluctuations. After the short start-up duration in sub-period A, the later regular pulse-like fluctuations were mainly caused by the daily water exchange. The summary values for the water temperature and salinity given in the text were for those through the 2 sub-periods. In rearing tank 1, sub-period A lasted until 22:27 h on 12 July, with its duration occupying 11.8% of the whole rearing period (up to Day 40). In this sub-period, the water temperature ranged from 20.9 to 23.1°C, with mean (\pm SD) of $22.0 \pm 0.6^\circ\text{C}$. The salinity ranged from 32.5 to 34.1, with mean (\pm SD) of 33.8 ± 0.2 . In sub-period B, the water temperature ranged from 21.8 to 23.1°C, with mean (\pm SD) of $22.2 \pm 0.1^\circ\text{C}$. The salinity ranged from 32.7 to 33.7, with mean (\pm SD) of 33.4 ± 0.1 . In any single water-temperature pulse above or below the mean, the outlier values were defined as those either higher than the (mean + SD) or lower than the (mean - SD). The duration with those outlier values was calculated for each pulse in this sub-period: the median duration was 11 min, and the sum of all outlier durations accounted for 12.0% of the whole rearing period. In rearing tank 2, sub-period A lasted until 15:48 h on 9 July, with its duration occupying 3.6% of the whole rearing period. In this sub-period, the water temperature ranged from 21.3 to 22.3°C, with mean (\pm SD) of $21.8 \pm 0.6^\circ\text{C}$. The salinity ranged from 32.3 to 33.8, with mean (\pm SD) of 33.7 ± 0.1 . In sub-period B, the water temperature ranged from 22.0 to 23.0°C, with mean (\pm SD) of $22.3 \pm 0.1^\circ\text{C}$. The salinity ranged from 32.4 to 33.8, with mean (\pm SD) of 33.4 ± 0.2 . In this sub-period, the median of the durations with the outlier water temperatures was 9 min, and the sum of all outlier durations accounted for 14.9% of the whole rearing period.