

## Note

# Development of *Metridia pacifica* (Crustacea: Copepoda) reared at different temperatures in the laboratory

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Received 18 February 2002; accepted 26 April 2002

*Metridia pacifica* is one of the most abundant calanoid copepods in the epipelagic zone of the subarctic Pacific Ocean and its marginal seas (Minoda 1971; Batchelder 1985; Hirakawa & Imamura 1993). *M. pacifica* is a primary herbivore (Hattori 1989), and its grazing pressure has been known to be high enough to consume a significant part of the low primary production during fall-winter in the eastern subarctic Pacific (Batchelder 1986). However, information about stage-to-stage developmental rates of this copepod is currently lacking, largely because of difficulties in resolving discrete cohorts in seasonal field samples (Batchelder 1985; Hirakawa & Imamura 1993). As an alternative, laboratory-rearing has been demonstrated to be a powerful approach to gain information about the developmental patterns and generation lengths of many planktonic copepods (see Mauchline 1998 for review). To date, only a limited attempt at laboratory-rearing of *M. pacifica* has been made on its early naupliar stages (Pinchuk & Paul 1998). This study is the first report on successful rearing of *M. pacifica* from eggs to adults under controlled laboratory conditions.

Live specimens of *Metridia pacifica* were collected at a station (42°00'N, 141°30'N; 500 m-depth) off Cape Esan, southern Hokkaido, on April 2001 by vertical hauls with an 80-cm ring net (0.33 mm mesh) from near the bottom to the surface. Upon the retrieval of the net, the contents in the cod-end were diluted in 20-litre plastic containers filled with surface seawater and transported to a land laboratory within 4–5 h. At the same station, seawater was collected from 100 m depth with Niskin bottles, filtered through GF/F filters, and well oxygenated for use in the following experiments.

At the land laboratory, adult female *Metridia pacifica* were sorted out from the other zooplankton and placed individually in 100-ml glass containers filled with filtered seawater, and the containers were placed in 3, 5 and 8°C incubators in the dark. These temperatures represent typical conditions *M. pacifica*

encounters in the western subarctic Pacific (2 to <15°C, Padmavati & Ikeda unpublished data). As food, a mixture of laboratory phytoplankton cultures such as *Heterocapsa triquetra* (Dinophyceae), *Chaetoceros gracilis* (Bacillariophyceae), *Pavlova* sp., *Isochrysis* sp. (Haptophyceae) and *Synechococcus* sp. (Cyanophyceae) were provided at a final concentration of  $3.5 \times 10^6$  cells ml<sup>-1</sup> (or 1.7 µgC ml<sup>-1</sup>). Batches of 11–35 eggs laid by individual females were observed daily for hatched nauplii, and hatching success of eggs were recorded for each batch. Batches of 11–33 newly-hatched nauplii thus obtained were placed in 10 ml glass vials, and maintained at the same temperature as that of the eggs they originated from. When the first feeding stage (naupliar stage 3, cf. Pinchuk & Paul 1998) occurred, the mixture of phytoplankton cultures was given (final concentration: 1.3 µgC ml<sup>-1</sup>). The concentration of the mixed algal diets was in excess, compared with concentrations that *M. pacifica* encounter in the field (<0.5 µgC ml<sup>-1</sup> in the Oyashio region, western subarctic Pacific, Kasai et al. 2001).

*Metridia pacifica* has six naupliar stages (N1 to N6) and six copepodite stages (C1 to C6) with C6 being the adult. Development of naupliar and copepodite stages was monitored daily by collecting cast molts. However, as quantitative recovery of naupliar molts was difficult we therefore estimated total developmental time from N1 through N6, instead of the developmental time of each naupliar stage. From C1, specimens were placed individually into 10 ml glass vials, and they were transferred into new vials filled with new seawater and a fresh preparation of food once a week. Copepodite development time from one stage to the next was calculated based on the records of molting dates. Mean developmental times of N1 through N6 and each copepodite stage were computed by assuming normal distribution of the data. While a gamma distribution model has been suggested to be superior to a normal distribution model to analyze development time (cf. Klein Breteler et al. 1994), our preliminary calculations indicated that the generation length, as a sum of developmental times of each stage based on the normal distribution model, yielded only <6 d longer than that based on the gamma distribution

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model. The prosome length of copepodite molts was measured under a dissecting microscope to the nearest 0.01 mm.

Eggs of *Metridia pacifica* were spherical in shape with a mean diameter of 145  $\mu\text{m}$  ( $\pm 9.7$ ,  $N=18$ ). Egg hatching success was 93% ( $\pm 11$ ,  $N=9$ ) at 3°C, 87% ( $\pm 3$ ,  $N=6$ ) at 5°C, and 89% ( $\pm 4$ ,  $N=4$ ) at 8°C. The effect of temperature on egg hatchability was not significant (one-way ANOVA,  $p>0.05$ ). Successful survival from N1 through C1 was 43, 50 and 53% at 3, 5 and 8°C, respectively. Of the 48 C1 specimens raised at 3°C, 2 reached C6 (both females). The number of specimens that reached C6 was 4 (2 females, 2 males) out of 52 C1 specimens at 5°C, and 7 (5 females, 2 males) out of 28 C1 specimens at 8°C (Table 1). The highest mortality was observed during the molt from C2 to C3 in the 3 and 5°C experiments, and during the C5 to C6 molt in the 8°C experiment.

At the three temperatures, both egg hatching time and naupliar developmental time (N1 to N6) shortened with increases in temperature, ranging from 2–3 d and 29–46 d, respectively (Table 1). Across the three temperatures, copepodite development times increased with the progress of stages with the exception of C2 (less than that of C1). The higher temperature resulted in shorter development times for each copepodite stage; overall ranges were 10–15 d for C1, 9–15 d for C2, 13–20 d for C3, 17–32 d for C4 and 32–55 d for C5. The generation length, as the sum of developmental times from eggs to C5, was 182 d for females grown at 3°C (no male data), 167 d and 161 d for females and males, respectively, at 5°C, and 112 d and 123 d for females and males, respectively, at 8°C (Table 1).

Taking into account the temperature regime, our results for egg hatching time are in good agreement with previous reports for *Metridia pacifica* (Pinchuk & Paul 1998) (Fig. 1). While Pinchuk & Paul (1998) were not successful in raising *M. pacifica* beyond N4, they estimated the entire naupliar development time (N1–N6) to be 23–30 d at 3–9°C, assuming a constant relationship between naupliar body length and developmental time. Compared with our results, their estimated naupliar development time is similar to that (30 d) of nauplii raised at 8°C, but too short compared with those raised at 5°C (45 d) and 3°C (46 d) (cf. Table 1). This suggests that the relationship between naupliar body length and their development time may not be constant.

According to Landry (1983) and Klein Breteler et al. (1994), the developmental pattern for marine calanoid cope-

pods (mostly neritic species) has such general features as; a relatively shorter pre-feeding naupliar stage, a longer first feeding stage, C5 stage longer than any previous stages in females, and earlier maturation of males than females. As a result, egg, naupliar and copepodite stage durations account for 4–9%, 30–44% and 48–62%, respectively, of the generation length. Since we were unable to estimate the stage duration of each naupliar stage of *Metridia pacifica*, the first two generalizations could not be tested in this study. The observed longest C5 developmental time for *M. pacifica* (Table 1) is consistent with the generalization. Earlier maturation of males was seen for specimens raised at 5°C, but this was not the case for those raised at 8°C. Perhaps, the number of specimens that reached C6 in this study were too few to test the generalization. The relative proportions of the duration of egg, naupliar and copepodite stages in the generation time of *M. pacifica* is 1–2, 24–28 and 71–74% (3, 5 and 8°C data pooled), respectively, showing a greater partition of generation time in copepodite stages than for the copepod data that led to the generalization. Because of the lack of precise information about the developmental patterns of many oceanic copepods, it is premature to judge whether the developmental pattern of *M. pacifica* is unique or not. From the viewpoint of life history traits, relatively smaller partitions of egg and naupliar stages in the generation time of *M. pacifica* may be advantageous to reduce premature mortality. Evidence shows possible high predation mortality of *M. pacifica* nauplii by the larvae of walleye pollock (*Theragra chalcogramma*) in the coastal waters of southwestern Hokkaido (Nakatani 1995) and the southeastern Bering Sea (Hillgruber et al. 1995; Pinchuk & Paul 1998).

For the *Metridia pacifica* population in the eastern subarctic Pacific, Batchelder (1985) estimated the generation length of *M. pacifica* to be 3–4 months. For the southern Japan Sea population, Hirakawa & Imamura (1993) noted that N1 developed to C5 in 2–3 months, then C5 underwent aestivation in the cool deep-layer. In order to compare the present results with these estimates, cumulative development time data for eggs to N1 (egg hatching time), C1 (naupliar development time), C5 and C6 (generation time; female and male data pooled) were fitted to the Bělehrádek function;  $D=a(T-\alpha)^{-2.05}$ , where  $D$  is developmental time,  $T$  is temperature, and  $a$  and  $\alpha$  are constants (cf. McLaren et al. 1969) (Fig. 1). For the temperature regimes of the study sites of Batchelder's (6–13°C; median 9.5°C) and Hirakawa & Imamura's (ca. 10°C), our calculation yielded 107 d

**Table 1.** Development times of eggs, total naupliar stages, and each copepodite stage of *Metridia pacifica* reared in the laboratory at three different temperatures. Mean  $\pm$  SD with the number of specimens in parentheses.

Temperature (°C)	Eggs	N1–N6	C1	C2	C3	C4	C5	Eggs–C6
3	2.7 $\pm$ 0.5 (118)	46.3 $\pm$ 5.9 (51)	14.5 $\pm$ 2.6 (48)	14.5 $\pm$ 3.5 (27)	20.4 $\pm$ 5.8 (9)	31.7 $\pm$ 2.3 (4)	♀ 51.5 $\pm$ 4.5 (2)	181.6
5	2.2 $\pm$ 0.5 (105)	45.1 $\pm$ 5.8 (56)	14.2 $\pm$ 3.2 (52)	11.9 $\pm$ 3.5 (41)	16.2 $\pm$ 4.8 (17)	22.5 $\pm$ 5.7 (7)	♀ 55.0 $\pm$ 1.0 (2) ♂ 49.0 $\pm$ 0.0 (2)	167.1 161.1
8	1.6 $\pm$ 0.5 (53)	29.8 $\pm$ 8.1 (28)	9.6 $\pm$ 2.5 (28)	8.8 $\pm$ 4.2 (19)	13.3 $\pm$ 9.5 (14)	17.1 $\pm$ 3.8 (11)	♀ 32.0 $\pm$ 4.5 (5) ♂ 43.0 $\pm$ 1.0 (2)	112.2 123.2

(or 3.6 months) at 9.5°C as compared with 3–4 months for the former, and 70 d (or 2.3 months) at 10°C as compared with 2–3 months for the latter. Thus, the predicted development times from the Bělehrádek equation in Fig. 1 fall well within the ranges derived from field population analyses by Batchelder (1985) and Hirakawa & Imamura (1993).

Under conditions of sufficient food supply, the prosome length of copepods are known to be largely a function of temperature, and the higher the temperature the smaller the length (Mauchline 1998, and literature therein). For the copepodite stages of *Metridia pacifica* raised at 3, 5 and 8°C in this study, a reduction in the prosome length with increasing temperature was seen for C1, C2 and C6 females, but not for C3, C4, C5 and C6 males (Table 2). These inconsistent results across copepodite stages may imply that the temperature range (3–8°C) of this study was not wide enough to induce significant effects of temperature on prosome lengths. The prosome length data for each copepodite stage raised at the three temperatures in this study were pooled and compared with those for wild specimens collected from the Oyashio region, western subarctic Pacific (Fig. 2). The difference between laboratory-raised and wild specimens was not significant for most stages, excepting C5 females and C6 females in which laboratory-raised specimens were significantly smaller than wild specimens (U-test,  $p < 0.01$ ). Considering that the late copepodite stages of *M. pacifica* are known to undertake diel vertical migration (cf. Batchelder 1985; Hirakawa & Imamura 1993), and their continuous swimming behavior is closely associated with their feeding patterns (Wong 1988), it is conceivable that the volume of the containers (10 ml) in which they were raised was too small to induce their normal feeding activities. Ozaki & Ikeda (1998) raised the oceanic copepod *Paraeuchaeta elongata* from eggs to adults in the laboratory and observed also a reduction in body length of late copepodite stages as compared with wild specimens. Since the perception of prey by *P. elongata* is mediated by mechanical or tactile receptors, Ozaki & Ikeda (1998) considered the suppressed feeding activity of the copepod within the limited space of the containers (200–500 ml) to be a possible cause. Because of the difficulties in mimicking natural environmental conditions for zooplankton in laboratory experiments, extrapolation of laboratory results to field

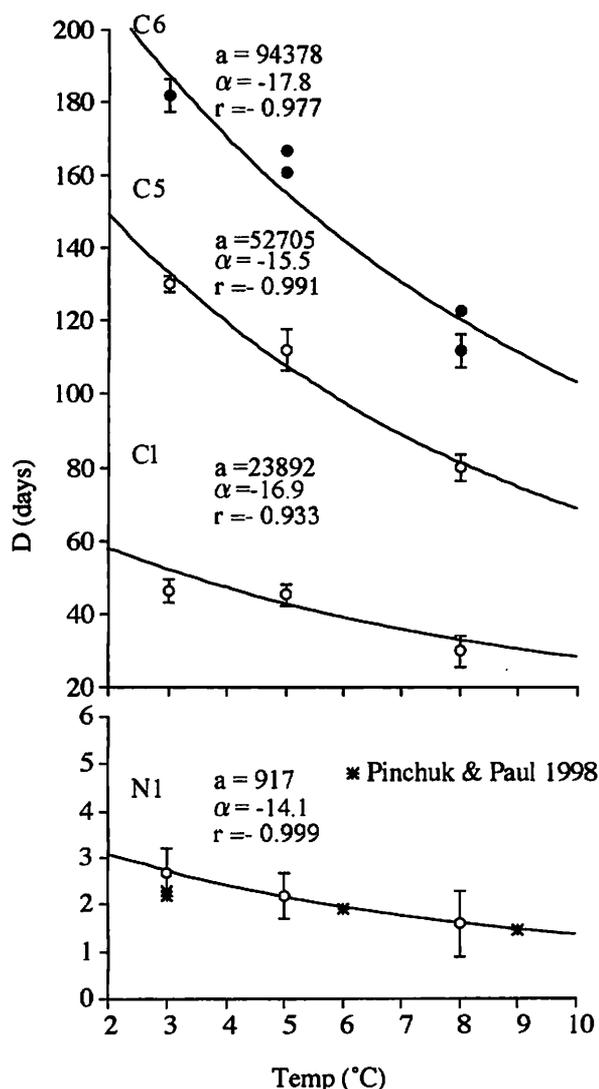


Fig. 1. The relationship between cumulative developmental times (D, days) from eggs to N1 (=egg hatching time), C1 (=naupliar developmental time), C5 and C6 (=generation length) of *Metridia pacifica* and temperature (T, °C) fitted to the Bělehrádek equation  $D = a(T - \alpha)^{-2.05}$  (cf. McLaren et al. 1969). Vertical bar denotes  $\pm 1SD$ . For N1, the data of Pinchuk & Paul (1998) were superimposed for comparison.

Table 2. Prosome length of copepodite stages raised at three temperatures in the laboratory. Mean  $\pm$  SD with the number of specimens in parentheses. NS: not significant.

Stage	Temperature (°C)			Difference F-test ( $p$ )	Grand Mean $\pm$ SD
	3	5	8		
C1	0.41 $\pm$ 0.02 (34)	0.38 $\pm$ 0.03 (37)	0.39 $\pm$ 0.02 (22)	<0.001	0.40 $\pm$ 0.03 (93)
C2	0.59 $\pm$ 0.03 (21)	0.56 $\pm$ 0.03 (35)	0.54 $\pm$ 0.02 (22)	<0.001	0.56 $\pm$ 0.03 (78)
C3	0.79 $\pm$ 0.04 (12)	0.77 $\pm$ 0.04 (16)	0.76 $\pm$ 0.03 (16)	0.081 NS	0.77 $\pm$ 0.44 (44)
C4	1.10 $\pm$ 0.05 (7)	0.98 $\pm$ 0.06 (11)	1.02 $\pm$ 0.07 (14)	0.128 NS	0.99 $\pm$ 0.18 (32)
C5F	1.34 $\pm$ 0.03 (4)	1.27 $\pm$ 0.05 (6)	1.30 $\pm$ 0.04 (11)	0.831 NS	1.23 $\pm$ 0.27 (21)
C5M	1.30 (1)	1.24 $\pm$ 0.04 (3)	1.26 $\pm$ 0.06 (5)	0.285 NS	1.26 $\pm$ 0.03 (2)
C6F	1.74 $\pm$ 0.01 (2)	1.70 $\pm$ 0.02 (2)	1.65 $\pm$ 0.02 (4)	0.017	1.68 $\pm$ 0.04 (8)
C6M	—	1.43 $\pm$ 0.03 (2)	1.23 $\pm$ 0.12 (3)	0.179 NS	1.31 $\pm$ 0.14 (5)

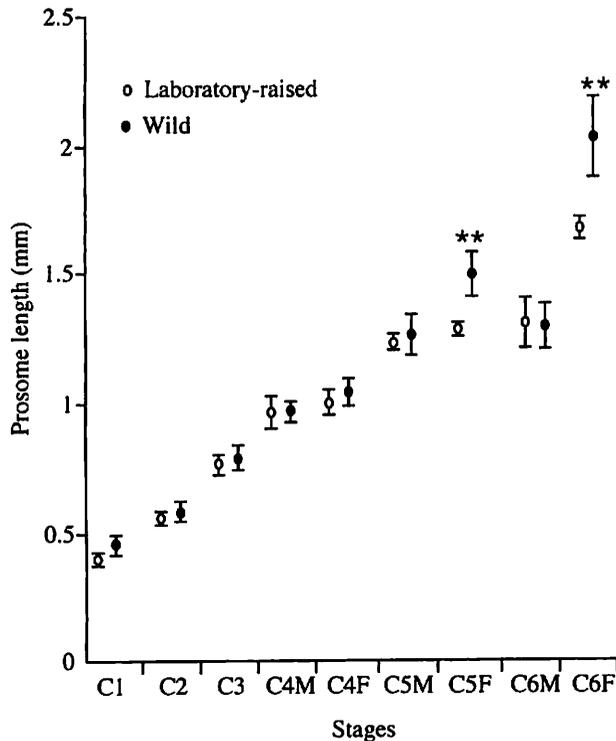


Fig. 2. The prosome length of each copepodite stage of *Metridia pacifica* raised in the laboratory (Laboratory-raised) and from the western subarctic North Pacific (Wild). \*\*:  $p < 0.01$

populations requires caution (cf. Paffenhöfer & Harris 1979). Despite this inherent drawback, the present results show clearly that laboratory rearing is a powerful approach to fill gaps in our knowledge about developmental patterns and generation lengths of various oceanic zooplankton.

### Acknowledgments

We are grateful to the members of the Plankton Biology Group of the Marine Biodiversity Laboratory for their help in the course of this study.

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