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# Growth of *Metridia pacifica* (Copepoda: Calanoida) nauplii in the laboratory

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**Abstract:** This paper presents data on naupliar stages of *Metridia pacifica* Brodsky reared in the laboratory. Eggs were obtained from females collected in the south-eastern Bering Sea and the Gulf of Alaska. The feeding stages were fed a surplus of food so development was regulated primarily by temperature. The stage durations for nauplii stages I–VI (NI–VI) were estimated for animals reared at 3, 6 and 9°C. Intermolt periods at 3°C were 49–120 h for the stages from egg to N IV and 33–69 and 27–74 h at 6 and 9°C, respectively. Growth, expressed as length, was linear. Growth from NI to the end of NVI took 23–30 d in the laboratory and rates were similar to those previously estimated from field studies in the Bering Sea.

Key words: Metridia, Bering Sea, Gulf of Alaska, copepod, nauplii

## Introduction

The copepod *Metridia pacifica* Brodsky, 1950 is common in the oceanic waters of the subarctic Pacific and Bering Sea (Batchelder 1985, 1986; Coyle et al. 1996). In the southeastern Bering Sea, its nauplii are one of the most important prey of larval walleye pollock *Theragra chalcogramma* Pallas (Hillgruber et al. 1995), one of the region's most abundant fish (Wespestad 1993). However, knowledge of the recruitment biology of *M. pacifica* is limited (Batchelder & Miller 1989). Data on developmental biology, hatching time, and growth rates of different stages of *M. pacifica* will improve the understanding of temporal variations in feeding success of the larval walleye pollock (Paul et al. 1996). This paper presents information on naupliar development and growth at different temperatures for *M. pacifica*.

# **Materials and Methods**

Nauplii of *M. pacifica* from the Aleutian Basin in the southeastern Bering Sea were collected in April of 1992 and 1993 (Paul et al. 1996) and preserved for morphometric analyses. In this study the first to sixth naupliar stages are termed NI–NVI, respectively. Live nauplii (NV to NVI) for growth experiments were caught in the Gulf of Alaska near the town of Seward in March 1997 using a 0.5-m-ring plankton net with 140- $\mu$ m mesh. Nauplii were also reared from eggs produced by live captives. Living females were collected in both the south-



Fig. 1. Map showing collection sites for *Metridia pacifica*.

eastern Bering Sea in early May 1995, 1996 and in Resurrection Bay near Seward in the Gulf of Alaska in September 1995 and February 1997 using the same ring net (Fig. 1).

The females were placed in 500-ml beakers with one female per beaker. The females were held with excess amounts of a mixture of autotrophic flagellates (*Isochrysis galbana* and *Tetraselmis suecica*) and diatoms (*Chaetoceros calcitrans*). The phytoplankton was replaced weekly so that the food was never more than one week old. The phytoplankton was kept at 35,500-50,000 cells ml<sup>-1</sup> (cell counts determined with microscope). *Artemia* sp. that had been reared for two weeks with the above flagellates were added to the mixture as an animal food for egg-producing females, because *Metridia* is considered to be omnivorous (Haq 1967). Examination of the beakers showed that the *Artemia* were being eaten. The number of new eggs and newly hatched nauplii were recorded daily through observations under a dissection microscope. Periodically (1–7 d) the females were transferred to other beakers with fresh filtered seawater and food to remove eggs or nauplii.

All work was done in a constant-temperature room set at  $3^{\circ}C(\pm 0.5)$ , and incubators set at  $6^{\circ}C(\pm 0.5)$  and  $9^{\circ}C(\pm 0.6)$  under continuously low light, 2.5–3.5 lux maximum. A temperature of  $3^{\circ}C$  was selected because it is the average value observed for the upper 90 m of the water column over the Bering Sea collection site during spring (Hillgruber et al. 1995). A temperature of  $6^{\circ}C$  is typical for early spring in the Gulf of Alaska, while  $9^{\circ}C$  would represent a late summer or fall temperature in the upper 100 m (Geletin 1975; A. J. Paul, unpublished data).

For developmental experiments, a known number of eggs were placed in a 50-ml beaker. After hatching, the nauplii were pipetted into 100-ml beakers for all subsequent developmental observations. There were 26 experiments, each using a single clutch of eggs from different females. Ten 3°C experiments were started with eggs from Bering Sea females collected in May 1995 (4 females) and May 1996 (5 females) and Gulf of Alaska females collected in February 1997 (1 female). Four 6°C and 12 9°C experiments were started with eggs of 16 females caught in February 1997. Additionally, 3 experiments were started with NI–II that hatched in the fall of 1995 and 4 experiments during May 1996.

The time intervals between observations for eggs and nauplii varied with stage and temper-

ature and were based on continual assessments of developmental rate. The time between stage verifications ranged from every 6 h for 9°C to 48 h for the latest stages at 3°C. On the census day, individuals were enumerated by developmental stage and placed into new beakers filled with fresh seawater. Since both preliminary experiments and published data (Peterson 1986) showed that high concentrations of phytoplankton increased the mortality of early stage nauplii, they were avoided for NI and NII non-feeding stages. After NIII, when nauplii were feeding, phytoplankton were kept at ca 16,500–20,000 cells  $ml^{-1}$ .

Since all reared nauplii died before molting from NIV to NV, 28 NV specimens were caught near Seward during February 1997, placed individually into 100-ml beakers, and kept at 3°C to obtain stage duration and growth information for NV and NVI. A census of the nauplii was made at 3-d intervals. Anesthetics were not used.

*T*-tests were used to compare body size between the Bering Sea and Gulf of Alaska specimens and between the lengths of field-caught and laboratory-reared nauplii. Differences were investigated at a significance level of p < 0.05. The total length of a nauplius was measured from the anterior to the posterior end excluding the caudal setae or spines. Body width was measured for the widest part of the naupliar shield. Development time was determined as the time from the egg laying until molting into a specific stage, while the intermolt period was taken as the time between molts. The size of *M. pacifica* and other members of the genus were compared. For this comparison the lengths were from 8 NI reared in the laboratory; 11 cultured NII; 62 NIII from the Bering Sea and 17 cultured specimens; 67 NIV from the field and 8 cultured individuals; 60 NV from the field and 43 Bering Sea NVI.

## **Results**

#### Development

Estimates of the duration of the eggs and NI–III at different temperatures appear in Table 1. The developmental rate (stage duration vs time) from NI to NIV at 3°C was a linear function with y=0.012x+0.458,  $r^2=0.98$ . However, the development seemed to be faster in the non-

Stage	Hours (Mean/SD/Range)			
	3°C	3°C	6°C	9°C
	Bering Sea	Gulf of Alaska	Gulf of Alaska	Gulf of Alaska
Egg	53/12/24-72	56/0/56-56	46/5/43-53	35/6/27-42
	(n=89)	(n=9)	(n=48)	(n = 130)
NI	49/3/48-60	52/0/52-52	33/2/32-36	27/4/24-32
	(n=14)*	(n=9)	(n=48)	(n=75)*
N II	90/10/72-96	70/0/70-70	60/6/51-68	48/7/38-59
	(n=33)	(n=9)	(n=48)	(n=90)
N III		120/0/120-120	69/1/68-70	74/4/72-83
		(n=8)	(n=33)	(n=24)
N VI	_	180/0/180-180		
		(n=3)		

**Table 1.** Duration of egg and naupliar stages of *Metridia pacifica* reared in the laboratory at 3°C, 6°C and 9°C.

\*The exact time of molting of some NI was missed so their further development was monitored from stage NII.



Fig. 2. The ratio of body length divided by body width for the naupliar stages of *Metridia pacifica* (vertical bars=SD).

feeding stages NI–II than in those of NIII–IV (Table 1). Development times for each stage at 6°C and 9°C were faster than for those at 3°C, and the overall developmental patterns were also linear with y=0.018x+0.416,  $r^2 \ge 0.97$ , and y=0.019x+0.560,  $r^2 \ge 0.97$  respectively. The ratio of body length divided by body width increased considerably from stage NIII through NIV (Fig. 2). The guts of the feeding stage nauplii were full of green material. The fidelity to linear developmental patterns seen in the non-feeding and feeding stages suggests that development in the feeding stages was not markedly impaired by food availability.

## Growth

When the stage specific measurements of body length of both laboratory reared specimens and those that had naturally developed in the sea were combined, the changes in length were



**Fig. 3.** Comparison of average naupliar body length changes in different *Metridia* species (vertical bars=SD).



**Fig. 4.** A. The changes in body length ( $\mu$ m) of *Metridia pacifica* naupliar stages NI-IV reared at 3°C, 6°C and 9°C (vertical bars=SD). **B.** The growth, expressed as change in length with time, of naupliar stages NI-IV reared at 3°C, 6°C and 9°C. Length values are logarithmically transformed (horizontal bars=SD).

generally linear (Fig. 3). The body length measurements of NIII and NIV field-caught specimens did not differ significantly from those of laboratory reared specimens (p>0.05). These results suggest that laboratory conditions did not negatively alter the relationship between stage and size.

Temperature did not seem to affect stage specific body length (Fig. 4A). The changes in body length with time from stage NI through NIV seemed to take a longer period to grow from NIII into NIV with a larger increase in body length. However, logarithmically transformed data was approximated by a linear function (Fig. 4B).

## Discussion

We were unable to rear *Metridia pacifica* from eggs past NIV but are unsure why they died. This species undergoes strong diel vertical migrations (Vinogradov 1968; Batchelder 1985; Hattori 1989). Nauplii can be found at 500-m depth (Batchelder 1985), while they are most numerous in the upper 100-m layer (Batchelder 1985). Perhaps, being unable to move vertically in the 500-ml beakers caused stress that resulted in the deaths of older nauplii. Little is known about the diets of *Metridia* naupliar stages. It could be that some bacteria or heterotrophic protozoans are important food for the nauplii (Roff et al. 1995) and our diet did not contain all the elements necessary for the nauplii to survive. Alternatively, the phytoplankton we fed the later stages may have had inhibitory compounds that were detrimental to the nauplii (Poulet et al. 1994, 1995).

In marine copepods growth rate may change with stage (Paffenhöfer & Harris 1976; Klein-Breteler et al. 1982; Kimoto et al. 1986; Peterson 1986). The pattern of early development of *M. pacifica* obtained in this study largely conforms to previously described patterns (Landry 1983, Peterson 1986). It features the relatively short duration of the pre-feeding NI–II and longer duration thereafter. Landry (1983) suggested that the NI–II are short because they do not feed and that NIII is longer because it needs to recover the weight lost during the non-feeding stages. However, the overall developmental pattern from nauplii to adults, with the possible exception of copepodid stage V for some species, is generally approximated by a linear function (Miller et al. 1977; Landry 1983; Peterson 1986; Sabatini & Kiorboe 1994) like that which we observed for M. pacifica.

The stage vs length measurements for *M. pacifica* show the same general development patterns seen in *M. lucens* (Gibbons 1938; Ogilvie 1953) (Fig. 3). It has been suggested that despite some differences in thermally related specific growth rates "an exponential model provides the best general approximation of the growth of marine copepods over the entire life cycle from egg to adult" (Huntley & Lopez 1992: p. 207). Our linear approximation of logarithmically transformed length growth data for most of the stages of *M. pacifica* seems to be consistent with this assumption.

This project was part of a larger study that was examining how the timing of copepod reproduction might impact the feeding success of different cohorts of walleye pollock larvae. Walleye pollock larvae eat primarily NIV–NVI *Metridia* nauplii and seldom eat the younger stages (Hillgruber et al. 1995). Length growth approximations (Fig. 4B) can be used to estimate the duration of various phases of *Metridia* life history. Based on our growth rate studies at 3°C it takes about 12 d for *M. pacifica* to go from egg to NIV, while at 6°C it takes only 8 d.

At 3°C it would take about 40 d to grow from egg to adult, if the development rates remain constant for the older nauplii and copepodids. The generation time for adult *M. pacifica* of 2.4- to 3.6-mm length at 3°C would be 56–65 d from the length growth model (Fig. 4B). Vidal & Smith (1986), using cohort analysis, estimated *M. pacifica* generation time in the southeastern Bering Sea in spring at 50–55 d assuming the copepodid phase took 30–35 d. Our results show that the naupliar phase takes 23–30 d and thus the laboratory growth rate measurements appear to approximate *M. pacifica* growth in vivo as estimated by Vidal & Smith (1986). However, the Bering Sea is a food rich region and in other parts of the open subarctic Pacific food may be more limiting and generation times significantly greater (Batchelder 1985). Further study, using lower food concentrations, would improve our understanding of the interrelationship between food, temperature and the growth of *M. pacifica* nauplii.

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