Distinguishing between Stage I and Stage II nauplii of the copepods *Metridia* and *Pseudocalanus* from the Bering Sea

Deborah L.W. Siefert

*Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration, 7600 Sand Point Way NE, BIN C15700, Seattle, WA 98115-0700, USA*

Abstract. Stage I nauplii of *Metridia pacifica*, Stage I and II nauplii of *Metridia lucens*, and the equivalent stages of *Pseudocalanus mimus* were reared from eggs to find morphological characters for separating the genera at these stages. Differences in naupliar body shape and caudal armature permit the differentiation at Stage I and II *Metridia* from *Pseudocalanus* in southeastern Bering Sea plankton.

Introduction

*Pseudocalanus* spp. often dominate the zooplankton in temperate–boreal neritic (<200 m) seas of the Northern hemisphere (Corkett and McLaren, 1978; Frost, 1989), while *Metridia* spp. of temperate–boreal waters of the Northern hemisphere are considered both oceanic (>200 m) species and coastal species (Brodsky, 1950; Thorpe, 1980; Osgood and Frost, 1994). These genera may cohabit and co-dominate the coastal boreal plankton over continental shelves, although their origins differ.

To determine the role of these copepods in the ecosystem, it is necessary to understand their life history and identify all developmental stages. For example, many fish taxa, including commercial species, prey upon all developmental stages of *Metridia* and *Pseudocalanus* (Kamba, 1977; Antipova *et al.*, 1985; Montelone and Peterson, 1986; Grover, 1990; Kendall and Nakatani, 1992; Takatsu *et al.*, 1995). The developmental stage of the prey changes with the ontogeny of the fish. Food availability for fish larvae is believed to be one of the key factors controlling larval growth and survival, and subsequently affecting recruitment. Therefore, recent studies have stressed that it is essential to identify copepod nauplii (including *Pseudocalanus* and *Metridia*) to the lowest possible taxon in order to define properly the foraging environment for larvae of walleye pollock in the southeastern Bering Sea, Alaska (Hillgruber *et al.*, 1995), walleye pollock and flathead flounder of Funka Bay, Japan (Nakatani, 1995), and Pacific cod of Mutzu Bay, Japan (Takatsu *et al.*, 1995).

Each life history stage of a copepod species has a different morphological form, but species descriptions are only based on the characteristics of the adult male and female. Descriptions of diagnostic characters for the other five copepodite and six naupliar stages of copepod species are uncommon. Concise descriptions of *Metridia* and *Pseudocalanus* early stage nauplii are limited, although a few reviews provide good information on their characteristic morphology. The
characters illustrated and tabulated by Ogilvie (1953) for *Metridia lucens* and *Pseudocalanus minutus* are especially useful for staging *Metridia* and *Pseudocalanus* nauplii. However, it is unclear whether these specimens were reared or collected from the plankton, and only illustrations of antennules and caudal armature are provided. To be certain of the species identity, nauplii must be reared from known parentage and a description of general body shape is useful for their identification. The recent work of Pinchuk (1997) and the rarely cited work of Klein Breteler (1982) with laboratory-reared nauplii from *Metridia* and *Pseudocalanus* adults, respectively, exemplify the distinct characters Ogilvie used for staging.

There are two descriptive accounts for early nauplii of *Metridia* before Ogilvie (1953): (i) Gibbons (1938) briefly compares *M. lucens* and *Calanus* sp. nauplii collected from the plankton of Scottish waters and (ii) Miller et al. (1984) suggest that Campbell (1934) mistakenly describes *Metridia pacifica* nauplii collected in the North Pacific as *Calanus tonsus*. Campbell carefully illustrates each naupliar stage and their appendages, and provides a written description. Since Ogilvie's work, literature describing *Metridia* spp. naupliar morphology was lacking until the recent work of Pinchuk (1997) in which the six naupliar stages of *M. pacifica* reared from adults collected in the southeast Bering Sea are illustrated and described.

Corkett and McLaren (1978) review accounts of *Pseudocalanus* naupliar morphology in the literature, and Sazhina (1985) provides a compilation of illustrations of *Pseudocalanus* nauplii from the literature and additional illustrations of all six *Pseudocalanus elongatus* naupliar stages from the Black Sea. These reviews include Corkett (1968) and Oberg (1906) who illustrate a *P. elongatus* Stage I nauplius from the waters off Scotland and *Pseudocalanus* sp. Stage II–IV nauplii from the Baltic Sea, respectively. The work of Ogilvie (1953), described above, and of Faber (1966), who includes *P. minutus* in a key based on caudal armature for copepod nauplii of Narragansett Bay, are also listed by Corkett and McLaren (1978). Klein Breteler (1982) includes photographs of all developmental stages of *Pseudocalanus* reared in continuous cultures started from the North Sea adults and comments on stage-specific characters that are discernible at low magnification.

Even with the literature cited above, it is difficult to identify positively *Metridia* and *Pseudocalanus* Stage I and II nauplii from plankton samples, whereas identification of Stage III–VI nauplii is not so difficult. Recent attempts to distinguish between the younger stages of these dominant taxa have been unsuccessful (Nakatani, 1988; Hilgruber et al., 1995; Nakatani, 1995; Takatsu et al., 1995; Paul et al., 1996). This study was undertaken to assist identification of the developmental stages of *Metridia* and *Pseudocalanus* in field samples. The purpose of this paper is to: (i) clarify previously described or illustrated diagnostic naupliar characteristics and (ii) highlight some morphological differences between these genera to aid in distinguishing between them at first naupliar stages, particularly at the lower magnification often used for routine plankton enumeration.
Distinguishing between *Metridia* and *Pseudocalanus* nauplii

**Method**

*Pseudocalanus mimus* Stage I and II nauplii from Shelikof Strait, Alaska, USA, *M.pacifica* Stage I nauplii from the southeast Bering Sea, and *M.lucens* Stage I and II nauplii from Dabob Bay, Washington, USA, all spawned from adult females, were examined for distinctive morphological characters and compared to Stage I and II nauplii from preserved samples collected from the southeast Bering Sea. The systematics of northern Pacific Ocean *Metridia* populations are problematic (Thorpe, 1980; Dagg et al., 1989), hence the Alaska females were identified as *M.pacifica* (*sensu lato*) since they more closely resembled Thorpe's *M.pacifica* (Brodsky) than *M.lucens* (Boeck).

**Specimen collection**

Live *P.mimus* and *M.pacifica* adult females were sorted from 1994 spring plankton collections from Shelikof Strait and the southeast Bering Sea, respectively. A 0.8 m diameter, 200 μm mesh ring net with a large-volume, non-filtering cod end was deployed each time to ~50 m. Individual females were pipetted into 60 ml containers of filtered seawater, covered, placed in a 5°C incubator and examined every 12 h for egg production or hatching. *Metridia* females were removed after spawning and preserved to avoid egg cannibalism. *Pseudocalanus* females, which carry their eggs, remained in the containers until their eggs hatched, then were also preserved. Once hatching occurred, subsamples of nauplii were preserved at 24 h intervals. All specimens were preserved in ~2% buffered formaldehyde:filtered seawater solution. The nauplii spawned by *M.lucens* females (Osgood and Frost, 1994) from Dabob Bay were reared by K.Osgood at the University of Washington in Seattle. Reference slides of adults and reared nauplii stored in 100% glycerin were loaned to the author for examination.

Field specimens of copepod nauplii were collected from the southeast Bering Sea in April 1994. Water samples collected from 10 l Niskin bottles closed at 10 m depth intervals were filtered with a 41 μm mesh sieve, obtaining plankton that were then preserved in an ~2% buffered formaldehyde:filtered seawater solution. These samples were stained with Rose Bengal at the laboratory prior to examination to ease the search for nauplii.

**Laboratory procedures**

Reared and wild nauplii were placed in 50% glycerin:water solution on microscope slides. A few nauplii were cleared with lactic acid and preserved in 100% glycerin for further examination. A dissecting scope at 100–160× magnification and a compound microscope at 200–250× magnification were used to stage all nauplii using antennule characters (Ogilvie, 1953; three distal setae, Stage I; and three distal setae and an aesthetasc, Stage II). Wild nauplii were sorted as Stage I and II *Pseudocalanus* or *Metridia* according to size and the distinguishing characters observed for the reared nauplii described in Results. Line drawings of dorsal and lateral views of individual reared specimens were made from digital images using a compound scope at 250× magnification.
Dorsal and lateral measurements of cephalic (anterior margin to point of flexion and/or dorsal shield demarcation) and total length (anterior margin of cephalic region to dorsal margin of posterior region) (Figure 1) were made using video image analysis (measurement error ±5 μm) with a dissecting scope at ~94x power. The length of the cephalic region and its proportion of the total length, rather than total length as most often cited in the literature, aided the separation of the Alaska *Pseudocalanus* and *Metridia* nauplii (Table I). However, size was not considered a diagnostic character because it may vary with environmental and maternal factors (Melle, 1991).

**Species diagnostic characters**

Nauplii collected from Bering Sea plankton could be offspring of several species of *Metridia* and *Pseudocalanus* (Brodsky, 1950; Frost, 1989). Species-specific diagnostic characters were not identified in this study because specimens were numerically, seasonally and geographically limited. Further, there are few distinct morphological features at these stages (Conover, 1956), and characters noted in past studies, like ventral hairs on the posterior region, relative lengths of caudal setae or spines and lack of or differences in morphology of masticatory spines on the basipod of the second antenna (Johnson, 1935; Conover, 1956; Koga, 1960; Grice, 1969; Lawson and Grice, 1970), are difficult to employ at relatively low magnification.

**Nomenclature**

Terms for naupliar copepod morphology have been used inconsistently in the literature; the following are definitions for the terms used here (Figure 1). The cephalon or cephalic region is the region of the nauplius where its three pairs of

<table>
<thead>
<tr>
<th>Table I. Mean (and SD) (in μm) of total length (TL), cephalon length (CL) and their ratio (CL:TL) of Stage I and II <em>Metridia</em> and <em>Pseudocalanus</em> nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dorsal view</strong></td>
</tr>
<tr>
<td>TL</td>
</tr>
<tr>
<td>NI <em>Metridia</em></td>
</tr>
<tr>
<td>Reared Bering Sea (n = 10)</td>
</tr>
<tr>
<td>Reared Dabob Bay (n = 8)</td>
</tr>
<tr>
<td>Plankton Bering Sea (n = 10)</td>
</tr>
<tr>
<td>NI <em>Pseudocalanus</em></td>
</tr>
<tr>
<td>Reared Shelikof St. (n = 9)</td>
</tr>
<tr>
<td>Plankton Bering Sea (n = 8)</td>
</tr>
<tr>
<td>NI <em>Metridia</em></td>
</tr>
<tr>
<td>Reared Dabob Bay (n = 2)</td>
</tr>
<tr>
<td>Plankton Bering Sea (n = 10)</td>
</tr>
<tr>
<td>NI <em>Pseudocalanus</em></td>
</tr>
<tr>
<td>Reared Shelikof St. (n = 10)</td>
</tr>
<tr>
<td>Plankton Bering Sea (n = 9)</td>
</tr>
</tbody>
</table>
Distinguishing between *Metridia* and *Pseudocalanus* nauplii

![Diagram showing dorsal and lateral aspects of early stage copepod nauplii indicating measurements and terminology used for this study.](https://academic.oup.com/plankt/article-abstract/20/6/1061/1430898)

**Fig. 1.** Dorsal and lateral aspects of early stage copepod nauplii indicating measurements and terminology used for this study.

Appendages are attached, and where the labrum and labium are located (Figure 1A). It is anterior to the point of flexion and includes the region anterior to the dorsal shield demarcation (Figure 1C), if present. Behind the point of flexion or posterior to the dorsal shield demarcation is the posterior region (Figure 1B). The dorsal shield referred to above is a shield or carapace that forms over the anterior region beyond the point of flexion in later naupliar Stages IV–VI. On the terminal end of the posterior region is a complement of spines and/or setae, referred to as caudal armature. For some nauplii, it is difficult to determine whether the caudal armature is actually composed of setae or slender spines. Here, seta is used for a slender hair-like process (about as thick as the terminal setae on the antennule), whereas spine is used for a process that is heavier in appearance, and thicker at the base tapering to a sharp point.

**Results**

Stage I and II nauplii of *Metridia* and *Pseudocalanus* can be distinguished from each other at low magnification by their general body shape and caudal armature (Figure 2). Differences in these morphological characters were consistently found in both reared and wild nauplii.

**Stage I nauplii**

*Metridia I—dorsal view.* The general body shape consists of a circular cephalic region and a slightly tapered terminally rounded posterior region, giving the organism a skull-shaped appearance. The anterior margin of the cephalic region
is generally smooth. The caudal armature consists of a pair of centrally located long slender setae set somewhat apart, then projecting posterolaterally and curving slightly inward from the base.

*Metridia* I—*lateral view*. The cephalic region, including the labrum and labium, is globose and the posterior region is flexed ventrally and terminally rounded. Dorsally, at the point of flexion, the profile is generally smoothly rounded, although, on occasion, there is demarcation where the posterior margin of the dorsal shield will form, just behind the point of flexion. The ventral point of flexion where the labium meets the posterior region is angular. The pair of setae
Distinguishing between *Metridia* and *Pseudocalanus* nauplii

forming the caudal armature point up at a slight angle to the posterior region and are slightly curved towards their tip.

*Pseudocalanus I—dorsal view.* The general body shape consists of an elliptically shaped cephalic region with a slightly tapered terminally rounded posterior region; the cephalic region dominates the dorsal view. On the anterior margin of the cephalon is a prominent centrally located process. The caudal armature consists of a pair of minute, terminally located, thick spines, often barely visible.

*Pseudocalanus I—lateral view.* The labrum protrudes ventrally, accenting the oblong shape of the cephalic region, while the terminally rounded posterior region is flexed ventrally. Part way down the anterior margin of the cephalon is the rounded process seen in the dorsal view. The cephalic region is dorsally flattened to the point of flexion, behind which there is a slight ridge where the posterior margin of the shield will form. The posterior region meets the labium at the ventral point of flexion, forming a concave profile. The small spines of the caudal armature are midway along the terminal margin of the posterior region.

*Stage II nauplii*

*Metridia II—dorsal view.* The general body shape is pyriform (anterior wider, posterior narrower), consisting of a circular cephalic region and a tapering posterior region. The anterior margin of the cephalic region is generally smooth. The terminal margin of the posterior region is slightly cleft where the caudal armature is attached. The caudal armature consists of a pair of long, straight setae that project posteriorly.

*Metridia II—lateral view.* The cephalic region, including the labrum and labium, is globose. The slightly tapering posterior region is flexed ventrally and bluntly pointed terminally where the caudal setae are attached. The setae are attached dorsally on the point and project at an angle posteriorly. There is a demarcation, dorsally, just posterior of the point of flexion, where the posterior margin of the shield will form. The ventral point of flexion where the labium meets the posterior region is angular.

*Pseudocalanus II—dorsal view.* An elliptically shaped cephalic region dominates the view. The relatively minor posterior region is terminally rounded. Midway along the anterior margin of the cephalon is a rounded process. The caudal armature consists of two moderate sized spines that project posteriorly.

*Pseudocalanus II—lateral view.* The cephalic region is oblong in profile and the angular posterior region is flexed ventrally. The rounded process lies part way down the anterior margin of the cephalon. Ventrally, the labrum is pronounced and extends past the labium in profile. The cephalic region is dorsally flattened to the point of flexion, behind which there is a ridge where the posterior margin of the shield will form. At the ventral point of flexion, the posterior region meets
D.L.W. Siefert

the labium forming a concave profile. The caudal armature, a pair of moderate-sized spines, is located terminally on the tip of the posterior region and points posteriorly.

Differences between and within genera

Both the dorsal and lateral views of Stage I and II nauplii are useful for distinguishing between the genera *Metridia* and *Pseudocalanus*. Table II provides a list of morphological characters for separating *Metridia* and *Pseudocalanus* Stage I and II nauplii from each other in the dorsal and lateral views.

Sometimes, staging *Metridia* or *Pseudocalanus* as Stage I and II nauplii is more difficult than distinguishing between the genera. Although these nauplii are typically staged by counting the terminal setae on the antennule, these setae can be broken and/or difficult to see. Alternatively, a few characters observed in the lateral view can be used to determine the naupliar stage of each genus (Table III).

Discussion

Other studies

The distinguishing characteristics for *Metridia* and *Pseudocalanus* described above were compared to descriptions and illustrations in the literature for species of these genera. Consistent similarities and a few erratic differences were found.

*Metridia* spp. Specimens of *Metridia* Stage I and II nauplii were compared to illustrations by Campbell (1934), Ogilvie (1953) and Pinchuk (1997). Gibbons (1938)

### Table II. Character differences for distinguishing between *Metridia* and *Pseudocalanus* Stage I and II nauplii

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Metridia</em></th>
<th><em>Pseudocalanus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dorsal view</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape of cephalic region</td>
<td>Circular</td>
<td>Elliptical</td>
</tr>
<tr>
<td>Prominent process on anterior margin of cephalon</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Caudal armature</td>
<td>Long slender setae, pointing laterally to a small degree (especially in Stage I)</td>
<td>Thick spines, short (Stage I) or moderate (Stage II) in length, pointing posteriorly</td>
</tr>
<tr>
<td>Posterior region</td>
<td>Posterior margin slightly cleft where caudal setae attach (Stage II only)</td>
<td>No cleft on posterior margin (Stage II only)</td>
</tr>
<tr>
<td>Dorsal shield demarcation</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ventral point of flexion</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Lateral view</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape of cephalic region</td>
<td>Circular</td>
<td>Elliptical</td>
</tr>
<tr>
<td>Prominent process on anterior margin of cephalon</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Caudal armature</td>
<td>Long slender setae that are tipped dorsally (slightly curved in Stage I and strongly angled in Stage II)</td>
<td>Thick spines, short (Stage I) or moderate (Stage II) in length, projecting posteriorly</td>
</tr>
<tr>
<td>Posterior region</td>
<td>Tapers to a blunt point (Stage II only)</td>
<td>Angular in appearance (Stage II only)</td>
</tr>
<tr>
<td>Dorsal shield demarcation</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ventral point of flexion</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1068
Table III. Morphological characters useful for staging Stages I and II nauplii within each genus with posterior region and caudal armature seen in the lateral view

<table>
<thead>
<tr>
<th></th>
<th>Metridia</th>
<th>Pseudocalanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Terminal setae (aesthetasc) on antennule</td>
<td>3</td>
<td>3(1)</td>
</tr>
<tr>
<td>Posterior region</td>
<td>Terminally rounded</td>
<td>Gradually tapering terminally to a blunt point</td>
</tr>
<tr>
<td>Caudal armature</td>
<td>Pair of setae, slightly curved dorsally</td>
<td>Pair of straight setae, projecting dorsally</td>
</tr>
</tbody>
</table>

Did not include illustrations for these stages. The *Calanus tonsus* Stage I and II illustrated by Campbell, later suggested to be *M. pacifica* (Miller, 1984), and the *M. pacifica* illustrated and described by Pinchuk, are very similar in shape to the *Metridia* observed during this study. Ogilvie did not illustrate the entire nauplius, therefore shape comparisons were not made. All authors illustrate caudal armature similar in form and proportion to caudal armature seen in this study; only Pinchuk illustrated the setae projecting in the manner observed here and only Ogilvie showed the cleft at the terminal end of the posterior region of Stage II nauplii.

The differences mentioned above could be due to the aspect from which the nauplii were drawn or to damage to the specimens. One difference observed that cannot be explained is the presence of a pair of minute hairs between the two caudal setae in Ogilvie’s illustration of the *M. lucens* Stage II nauplius. These hairs were not seen on the specimens examined during this study or included in the other authors’ illustrations. Finally, *Metridia* nauplii from the Bering Sea and Dabob Bay were smaller than those measured by Campbell, but closer in size to the measurements of Ogilvie, Gibbons and Pinchuk [see Table I and Pinchuk (1997)].

*Pseudocalanus* spp. Alaska *Pseudocalanus* Stage I and II nauplii were compared to those illustrated by Oberg (1906), Ogilvie (1953), Corkett (1968) and Sazhina (1985), and the photographs in Klein Breteler (1982). All authors, except Ogilvie, illustrated *Pseudocalanus* nauplii with an elliptical/oblong-shaped cephalic region and a process on the anterior margin of the cephalon. Ogilvie did not illustrate the entire nauplius or the anterior region. Sazhina represents the labrum prominently in relationship to the labium as found in Alaska *Pseudocalanus* nauplii, although it is exaggerated in the figures. Photographs of *Pseudocalanus* Stage I and II nauplii in Klein Breteler (1982) show all the characters listed in Table II for distinguishing *Pseudocalanus* from *Metridia* Stage I and II nauplii as seen in Alaska nauplii. The dorsal shield demarcation on the Stage I nauplius was not seen, but it is not always seen on Alaska nauplii either.
D.L.W. Siefert

The relative size and form of caudal armature were variable in the literature, although in all it consists of a single pair of spines. The spines of *P. minutus* illustrated by Ogilvie and those of *Pseudocalanus* sp. in the photographs of Klein Breteler were most similar in appearance to those of the nauplii examined for this study, while Corkett and Sazhina illustrate relatively longer spines on *P. elongatus* nauplii. Oberg’s figure of *Pseudocalanus* sp. has spines that appear to taper less gradually than observed here. Also, small ventral bristles are shown on the posterior region, a character not seen on Alaska specimens. Differences in caudal armature and ornamentation of the posterior region may be due to species or geographical variability. Finally, the size range given for Ogilvie’s specimens, 176–187 μm, is similar to those measured for this study (Table I).

**Application to unidentified specimens.** Nakatani (1988) was unable to distinguish *Metridia* and *Pseudocalanus* Stage I and II nauplii from Funka Bay, Japan, plankton samples, but illustrates nauplii that could be either of these genera. The illustrations of different body parts in Plate I (Nakatani, 1988) are most likely of *Pseudocalanus* Stage I nauplii because of the size and shape of caudal armature and the setation of the antennule. Plate II is probably illustrations of *Metridia* Stage I nauplii because of the rounded shape of the cephalon and the smoothness of its anterior margin, the distal setation of the antennule, and the seta-like caudal armature. Plate III is an illustration of *Pseudocalanus* Stage II nauplii because it shows an elliptically shaped cephalon and the slight process on the anterior margin. Further, the moderate-sized spines of the caudal armature in Plate III are most definitive for *Pseudocalanus* Stage II nauplii.

**Conclusion**

The diagnostic characters described here provide a practical means for the routine identification of *Metridia* and *Pseudocalanus* Stage I and II nauplii from the plankton of the southeastern Bering Sea. These characters are potentially useful for distinguishing *Metridia* and *Pseudocalanus* nauplii from other regions. Additional information that may now be attained about the early life history of these genera should contribute to the understanding of their role in various ecosystems.

**Acknowledgements**

I appreciate the advice given by J. Napp when preparing this manuscript and I would like to thank K. Zecca for her skillful preparation of the figures included here (both at AFSC). G. Heron and B. Frost at the University of Washington were generous to provide the nauplii reared by K. Osgood for my examination, and M. Cohen at AFSC sorted specimens from Bering Sea samples. M. Canino (AFSC), A. J. Paul (University of Alaska) and T. Nakatani (Hokkaido University) were very helpful upon initial review of the manuscript. Thanks to the anonymous reviewer who provided the citation for an additional pertinent reference. This is contribution FOCI-0282 to Fisheries Oceanography Coordinated Investigations, NOAA.
References


*Received on June 2, 1996; accepted on December 21, 1997*