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Early Development of Euphausiid *Thysanoessa inspinata* and *T. longipes* Observed in the Laboratory

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Abstract

This paper presents the first data on the early larval morphology of euphausiid *Thysanoessa inspinata* and *T. longipes*. Female animals were captured in the Oyashio region during May 2006 and investigated through a 31 day period in a laboratory experiment. Between 76–142 eggs were found to be released from *Thysanoessa inspinata* during each spawning (hatching success=81–99%) and 136 eggs (hatching success=65%) were released from the *T. longipes* females. The morphology of *T. inspinata* and *T. longipes* during all stages as eggs, Nauplius I, II and Metanauplius larvae were similar, but distinguished significantly by size (*T. inspinata* < *T. longipes*).

Key words: Early development, Euphausiacea, *Thysanoessa inspinata, Thysanoessa longipes*

Introduction

In the Oyashio region, *Euphausia pacifica* is the most dominant euphausiid, followed by *Thysanoessa inspinata* and *T. longipes* (Kim et al., 2009). In contrast to a large body of information available about biology and ecology of *E. pacifica*, little information is available on the two *Thysanoessa* species, *T. inspinata* and *T. longipes*, which are morphologically similar, but adults can be distinguished by the absence (*T. inspinata*) or presence (*T. longipes*) of abdominal spines (Nemoto, 1963).

Boden (1950) and Suh et al. (1993) analyzed field samples and described detail morphological characteristics of all larval stages of *E. pacifica* in the southern California waters and Yellow Sea, respectively. Endo and Komaki (1979) gave diagnostic features for calyptopis I to juvenile of *T. longipes* based on the preserved samples collected in the Japan Sea. To date, the morphological characteristics of eggs and naupliar stage of *T. longipes* are not reported. No information is presently available for the morphology of larval stages (except nauplius II) of *T. inspinata* (cf. Gõmez-Gutiếrrez, 2003).

The aim of this study is to describe morphological characteristics of the early developmental stages (eggs, nauplius and metanauplius) of *T. inspinata* and *T. longipes*, collected in the Oyashio region, through a laboratory experiment.

Materials and methods

Euphausiids were collected with Bongo nets (70 cm mouth diameter, 330 μm mesh size) hauled vertically from 200 m depth to the surface aboard the R/V '*Tansei Maru*' at Site H (41°30′N, 145°50′E) in the Oyashio region, western subarctic Pacific during 24–25 May 2006. A selection from the catch with apparently undamaged females *T. inspinata* and *T. longipes* with spermatophores were performed immediately after the nets were retrieved. The animals were transferred individually into 1-L glass bottles filled with surface seawater. To prevent spawned eggs from predation by the females, a 2-mm mesh netting was fixed 2–3 cm above the bottom of each bottle (cf. Ross and Quetin, 1983; Harrington and Ikeda, 1986). Glass bottles were placed in a dark plastic bag that was immersed into a water tank on the deck at which the surface seawater (3°C) was overflowing.

Glass bottles were inspected daily for spawned eggs. When eggs were observed, they were transferred individually into wells of multi-well plates filled with chilled seawater with a pasteur pipette. The eggs were examined daily for hatching and seawater in the wells was changed every 3–7 days. After hatching, the development of nauplii was followed up to the metanauplius

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stage (end of non-feeding stage). During the development, several specimens of nauplii were preserved in 2% borax buffered formalin for detailed observations of morphological characteristics. Naupliar appendages were removed under a dissecting microscope and mounted on slides. Preparations were sealed with transparent nail varnish. Each appendage was drawn under a light microscope equipped with a camera lucida (Olympus BH-2). The total length of nauplii was measured from the anterior tip of carapace to the midpoint of the telson spines (Hirota et al., 1984) with an aid of eye-piece micrometer. Nauplius stage I, nauplius stage II and metanauplius are hereafter abbreviated as NI, NII and MN.

Results

Laboratory raising

The eggs of *T. inspinata* and *T. longipes* were slightly heavier than seawater, and settled on the bottom of the bottles. The brood size (the number of a batch of eggs released at one spawning event) was 76-142 eggs per female for *T. inspinata*, and 136 eggs per female for *T. longipes* (Table 1). Egg hatching success was 81.0-98.7% for *T. inspinata* and 64.7 % for *T. longipes* (Table 1). Mortality of both euphausiids during nauplius stages was low, but increased from metanauplius to calyptopis I stage from which feeding appendages developed (Fig. 1). The laboratory raising ended after 31 days when all metanauplius specimens had deceased (Fig. 1). The development from one stage to the next stage was largely synchronized. The hatching time, developmental time of NI and NII was 3.0, 4.7 and 7.8 days, respectively, for *T. inspinata* and 3.0, 4.5 and 8.5 days, for *T. longipes* (Table 2, Fig. 1).

Table 1. Brood size (number of eggs in one brood) and hatching success of *Thysanoessa* spp. observed at 3°C.

Experimental no.	Species	Brood size	Hatching success (%)
1	*T. inspainta*	112	85.7
2	*T. inspinata*	76	98.7
3	*T. inspinata*	142	81.0
4	*T. longipes*	136	64.7

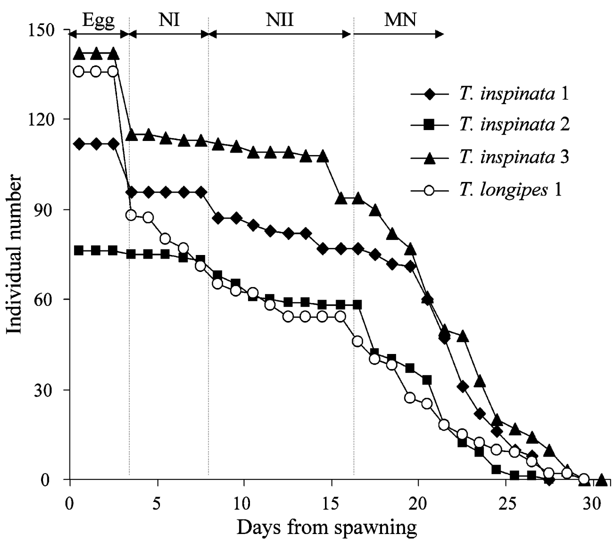

Fig. 1. Temporal changes in survival number of *Thysanoessa inspinata* (1-3) and *T. longipes* (1) raised in the laboratory. Approximate duration of eggs, NI, NIIand MN are shown with the horizontal bars.

Descriptions of eggs, nauplius stage I and II, and metanauplius

No apparent morphological differences were observed between *T. inspinata* and *T. longipes*, drawing figures are presented only for *T. inspinata* in the present study.

Egg (Fig. 2A)

Eggs were sphere in shape (Fig. 2A), and its diameter ranged from 0.33-0.38 mm (mean : 0.36 mm) for *T. inspinata*, and 0.35-0.44 mm (mean : 0.39 mm) for *T.*

Table 2. The developmental times (days) from eggs to metanauplius stage of *Thysanoessa inspinata* and *T. longipes* observed at 3°C. Values are mean±1SD.

	T. inspinata	*T. longipes*
Egg	3.0±0.0	3.0±0.0
Nauplius I	4.7±0.3	4.5±0.5
Nauplius II	7.8±0.6	8.5±0.5

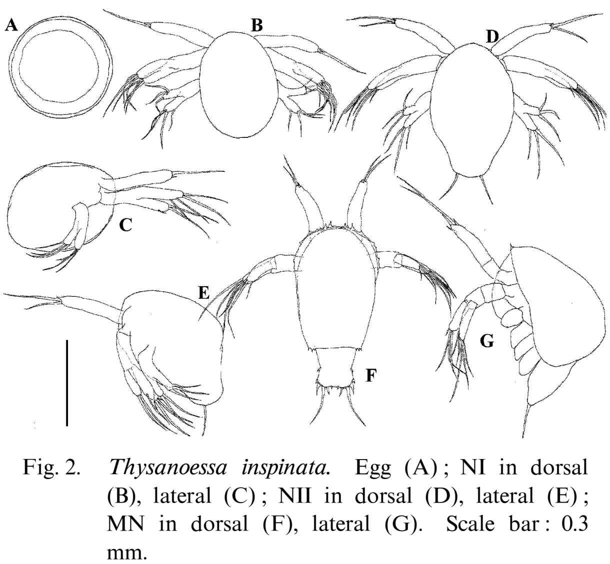

Fig. 2. *Thysanoessa inspinata.* Egg (A) ; NI in dorsal (B), lateral (C) ; NII in dorsal (D), lateral (E) ; MN in dorsal (F), lateral (G). Scale bar : 0.3 mm.

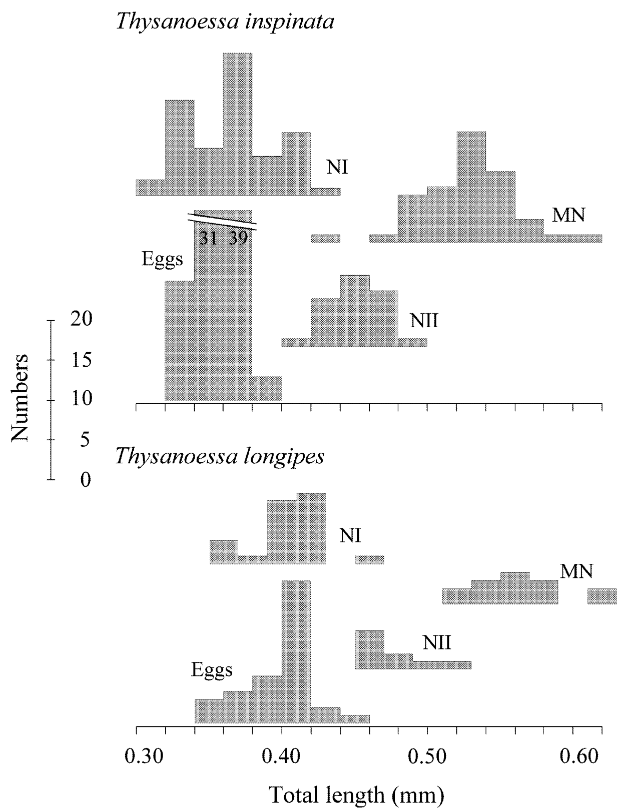

Fig. 3. *Thysanoessa inspinata* and *T. longipes.* Length-frequency of eggs, nauplius I (NI) and II (NII), and metanauplius (MN) at Site H in the Oyashio region during May 2006.

longipes (Fig. 3). Perivitelline space of eggs relatively small.

Nauplius I (Fig. 2B, C ; 4A, D, G)

Total length ranged from 0.30–0.42 mm (mean : 0.35 mm) for *T. inspinata* and 0.35–0.45 mm (mean : 0.39 mm) for *T. longipes* (Fig. 3). Dorsal and lateral views of carapace smooth (Fig. 2A, B). Lateral view hood-shaped. Body egg-shaped from the dorsal view, with three pairs of appendages. Antennule uniramous, with one terminal long seta, two terminal spines, and one subterminal seta (Fig. 4A). Antenna biramous ; endopod with two setae and one small seta ; exopod with four setae (Fig. 4D). Mandible biramous ; exopod and endopod unsegmented, with three setae (Fig. 4G).

Nauplius II (Figs. 2D, E ; 4B, E, H)

Total length ranged from 0.41–0.48 mm (mean : 0.45 mm) for *T. inspinata* and 0.45–0.51 mm (mean : 0.47 mm) for *T. longipes* (Fig. 3). Dorsal and lateral views elliptical (Fig. 2D, E). Body with one pair of spines on posterior margin. Antennule with two terminal long setae, one terminal spine, and one subterminal seta (Fig. 4B). Antenna biramous ; endopod with two setae and one subterminal seta ; exopod four setae and one small seta (Fig. 4E). Mandible setation and segmentation unchanged from NI, while size larger (Fig. 4H).

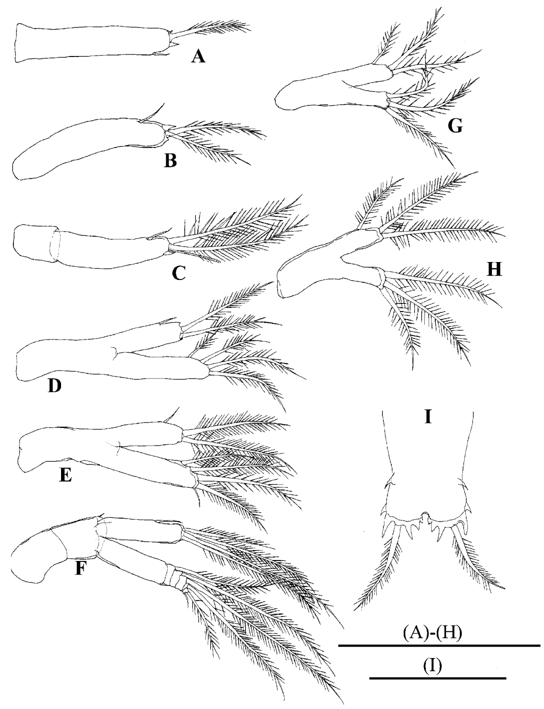

Fig. 4. *Thysanoessa inspinata.* Antennule of NI (A), NII (B) and MN (C). Antenna of NI (D), NII (E) and MN (F). Mandible of NI (G) and NII (H). Telson of MN (I). Scale bars : 0.1 mm.

Metanauplius (Figs. 2F, G ; 4C, F, I)

Total length ranged from 0.43–0.61 mm (mean : 0.52 mm) for *T. inspinata* and 0.51–0.60 mm (mean : 0.55 mm) for *T. longipes* (Fig. 3). Carapace fringed with spines on anterior margin and first half of the lateral margin and on both sides of posterior margin (Fig. 2F, G). Anterio-median margin slightly indented. Antennule with one aesthete, two long setae, one terminal spine, and one subterminal seta (Fig. 4C). Antenna biramous ; endopod with four setae, one subterminal seta ; exopod, six-segmented with six setae distally (Fig. 4F). Telson with five pairs of spines on posterior margin, one pair of spines on post-lateral margin (Fig. 4I).

Discussion

This paper presents the first description of the early larval morphology of *T. inspinata* and *T. longipes* from the Oyashio region. The brood size of *T. inspinata* (76–142 eggs, Table 1) and *T. longipes* (136 eggs) observed in this study falls well within the broad range of these previous results on the other euphausiids.

Hatching success of eggs has been reported to be 67–

Table 3. Morphological comparison on nauplius II stage of *Thysanoessa inspinata* reported by Gõmez-Gutiẽrrez (2003) and this study.

Position	Gõmez-Gutiẽrrez (2003)	This study
Posterior margin	Two broad spines and four small spine	Only two broad spines
Antennule	Two long setae and one subterminal seta	Two long setae and one terminal spine, and one subterminal seta
Antenna	Endopod : two setae and one small spine, and one subterminal seta	Endopod : two setae and one subterminal seta
	Exopod : five setae	Exopod : four setae and one small seta (five setae)
Mandible	Endopod : two setae and one terminal spine	Endopod : three setae
	Exopod : three setae and one terminal spine	Exopod : three setae

Table 4. Comparison on egg diameter and total lengths of nauplius I, II and metanauplius of several euphausiids dominated in the various locations. Values are mean±1SD (mm), and their size range and measured number are shown in the parentheses. *Nauplius I and II were combined.

Species	Area	Eggs	Nauplius I	Nauplius II	Metanauplius	References
Thysanoessa inspinata	Site H	0.36±0.01	0.35±0.02	0.45±0.02	0.52±0.03	This study
	(laboratory)	(0.33-0.38, 88)	(0.30-0.42, 52)	(0.41-0.48, 24)	(0.43-0.61, 43)	
Thysanoessa longipes	Site H	0.39±0.02	0.39±0.02	0.47±0.02	0.55±0.03	This study
	(laboratory)	(0.35-0.44, 34)	(0.35-0.45, 22)	(0.45-0.51, 9)	(0.51-0.60, 14)	
Euphausia pacifica	Toyama Bay	-	0.45±0.02	0.46±0.02	0.48±0.03	Kim and Ikeda
	(field)		(0.43-0.48, 6)	(0.42-0.50, 35)	(0.42-0.52, 54)	(unpublished)
Euphausia pacifica	Yellow Sea	0.58±0.03	0.38±0.03	0.52±0.01	0.58±0.05	Suh et al. (1993)
	(field)	(0.50-0.68, 326)	(0.35-0.45, 13)	(0.50-0.53, 7)	(0.45-0.70, 239)	
Euphausia nana	Suruga Bay	0.47±0.04	0.44±0.02	0.44±0.02	0.46±0.01	Hirota et al. (1984)
	Sagami Bay	(0.40-0.54, 281)	(0.42-0.48, 6)	(0.40-0.47, 34)	(0.43-0.48, 14)	
	(field)					
Euphausia superba	Scotia Sea	0.57±0.01	0.70±0.02	0.78±0.04	0.91±0.02	Ikeda (1984)
	(laboratory)	(10)	(5)	(4)	(5)	
Euphausia crystallorophias	Prydz Bay	0.60±0.02	0.58±0.02*	0.58±0.02*	0.69±0.05	Ikeda (1986)
	(laboratory)	(6)	(6)	(6)	(7)	
Thysanoessa spinifera	Barkley Sound	0.41±0.003	0.46±0.005	0.49±0.004	0.54±0.003	Summers
	(field)	(185)	(142)	(196)	(161)	(1993)
	Barkley Sound	0.42	0.49	0.54	0.56	Summers (1993)
	(laboratory)	(10)	(0.38-0.56)	(0.38-0.54)	(0.49-0.59)	

69% for *E. superba* (Harrington and Ikeda, 1986), 96-99% for *E. pacifica* within their normal habitat temperature (0-20°C) (Iguchi and Ikeda, 1994), over 90% for *T. inermis* and *E. pacifica* under 5-12°C (Pinchuk and Hopcroft, 2006), 50-94% for *E. pacifica* off the coast of Newport of their normal habitat (10°C) (Feinberg et al., 2006). The present results on *T. inspinata* (81-99%, Table 2-1) and *T. longipes* (65%) overlap the broad range of previous studies on the other euphausiids.

According to Ikeda (1986), eggs of *E. crystallorophias* and *E. superba* are spherical in shape, but perivitelline space of the former is much wider than that of the latter. Such the species-specific differences in the perivitelline space were not observed for *T. inspinata* and *T. longipes* in this study (Fig. 2A).

While the general morphology of naupliar stages of euphausiid species are more or less similar to each other and often difficult to separate into species, the total length, dorsal and lateral view, the shape of the posterior margin of the body, and the setation pattern of the antennule, antenna and mandible are known as useful species-specific characteristics of nauplius (cf. Mauchline and Fisher, 1969). In the present study, the morphology of NI, NII and MN of *T. inspinata* and *T. longipes* were similar to each other, and no morphological differences were recognized, excepting greater sizes of *T. longipes* (Fig. 3). It is observed that the formation of the abdominal spines (Fig. 2) and appendages (Fig. 4) from NI to NII were gradual with no obvious molting, as was reported on *E. superba* by Marschall and Hirche (1984). The molts were only found from NII to MN for both *T. inspinata* and *T.*

longipes. While delayed hatching as NII or MN has been reported in *Thysanoessa* spp. (Gõmez-Gutiérrez, 2002), this was never observed for *T. inspinata* and *T. longipes* in this study.

It is noted that the morphology of NII *T. inspinata* given by Gõmez-Gutiérrez (2003) is somewhat different from the results in this study in several points, including the number of spines in the posterior margin, the terminal margin spine of antennule, the terminal margin spine of endopod of antenna, and the number of setation of endopod of mandible (Table 3). The reason for the differences between the two studies is currently unknown.

Metanauplii of some euphausiids such as *E. crystallorophias*, *E. triacantha* and *E. frigida* have characteristic long setae on the frontal margin of carapace, but such the setae are lacking in *E. superba* and *T. macrura* (Mauchline and Fisher, 1969). Frontal and posterior margin of metanauplii of *T. inspinata* and *T. longipes* raised in this study was fringed with short spines (Fig. 2F). However, metanauplii of *E. pacifica* only fringed with spines on frontal margin (Suh et al., 1993).

Based on the summarized data in Table 4, we examined the correlation between mean egg diameter and mean adult size (median *TL*), and found no correlation between them ($p=0.305$). Size of eggs and nauplii of *T. inspinata* and *T. longipes* in this study was slightly smaller than those of others euphausiids. For *E. pacifica*, the nauplii derived from the Yellow Sea population (Suh et al., 1993) were larger than those from Toyama Bay population (Kim and Ikeda, unpublished). Regional variation in nauplii size with same species may related with the changes in environmental conditions (temperature, food, etc.). According to Summers (1993), eggs, nauplii and metanauplii of *T. spinifera* reared in the laboratory were larger than those of wild populations (Table 4). Thus, the size of eggs and early larvae of euphausiids appears to be variable not only habitats but also conditions under which they were developed (laboratory raised or wild collected).

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