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ABSTRACT

To infer diet and feeding mode of zoea of *Nihonotrypaea harmandi* (Bouvier, 1901), fine functional morphology of the mouthparts and composition of undigested remnants of ingested prey organisms contained in the gut were investigated. A mesh-like structure was observed for
the coxal and basal endites of the maxilla, which would be suitable for filter feeding. The mandible edge is equipped with many fine processes and resembles the mandible edge of herbivorous copepod. Abrasion of the incisor processes, which must be caused by frequent mastication of planktonic diatoms, was recognized within a molting interval. Furthermore, diatom frustules and their crushed fragments were frequently observed in the gut contents. These observations indicate that zoea of *N. harmandi* may regularly feed on phytoplankton, especially diatoms.

**KEY WORDS:** gut contents, mouthpart morphology, *Nihonotrypaea harmandi*, planktonic diatoms, zoeal stage

**DOI:**

**INTRODUCTION**

The callianassid shrimp, *Nihonotrypaea harmandi* (Bouvier, 1901) (Decapoda: Axiidea), commonly occurs on intertidal sandflats in Japanese waters, dwelling in a 30-60 cm deep burrow (Tamaki and Ueno, 1998; Kubo et al., 2006). Through its intense bioturbation of sediments, considerable effects are exerted on both sediment properties and benthic
community structure of an intertidal sandflat located on the northwestern corner of Amakusa-Shimoshima Island in western Kyushu, southern Japan (Flach and Tamaki, 2001; Fig. 1, Tomioka sandflat). The life cycle of *N. harmandi* includes planktonic larvae, which consist of six zoeal and one decapodid stages; five stages were recorded for zoea in Konishi et al. (1999) and Tamaki et al. (2010), but a recent study has detected six (A. Tamaki, personal observation). The nursery grounds for larvae released from the Tomioka sandflat is situated in nearby inner shelf waters of the coastal ocean (Amakusa-Nada), and the mean position of zoeae in the water column is about 40 m with a temperature of 21°C (Tamaki et al., 2010, 2013). When reared at this temperature, it took about a median of 30 days for zoea I to reach decapodid (Tamaki et al., 2013).

In the larval rearing experiments including that of Konishi et al. (1999), cultured rotifers (*Brachionus rotundiformis*) and newly-hatched nauplii of *Artemia* sp. have been used as the main foods, and they were given in very high abundances. These conditions were similar to the larval rearing of other callianassid species (Aste and Retamal, 1984; Konishi et al., 1990; Thessalou-Legaki, 1990; Miyabe et al., 1998; Strasser and Felder, 2000). By contrast, food items of field-caught specimens remain to be investigated. Although rotifers and/or *Artemia* nauplii are also most commonly used for rearing of other decapod crustacean larvae, a variety of possible wild food items are listed, including phytoplankton (Jones et al., 1997; Anger,
2001). Taking into account a rather slow vertical migration speed of zoeae of *N. harmandi* at a maximum of 0.18-0.30 cm s\(^{-1}\) in the water column (Tamaki et al., 2010), there arises some doubt as to whether these stages are actually capable of raptorial feeding by pursuit of sparsely distributed zooplankton. Alternatively, it may be possible for those larvae to carry out filter-feeding for relatively motionless phytoplankton available in the ambient water in higher densities. One way to infer feeding habits of decapod crustacean larvae under natural conditions may be to examine the functional morphology of their feeding appendages in relation to gut contents. However, few inferences based on digestive enzyme activities (e.g. Jones et al., 1997) have not concomitantly been backed by gut content and functional morphology analyses. By comparison, substantial data sets regarding feeding habit and mouthpart functional morphology are available for planktonic copepods (Schnack, 1989).

The objective of the present study was to examine gut contents and detailed anatomical structure for some selected mouthparts in zoeae of *N. harmandi* collected from Amakusa-Nada. Special attention was paid to the possibility of filter feeding for planktonic diatoms for comparison to findings on herbivorous and carnivorous planktonic copepods. Some notes were made regarding a few descriptions on callianassid larvae and scarce information on other decapod crustacean larvae that were studied on both mouthpart morphologies and feeding habits based on digestive enzyme activities.
MATERIALS AND METHODS

Zoeae of *N. harmandi* were collected every month from June to August, 2011 and from June to September, 2012 on cruises of the T/V Kakuyo-maru, Nagasaki University. A plankton net (1.3-m opening diameter, 4.5-m length, and 330-µm mesh size) was towed at 1.5 knots for 5 min. horizontally at 20-m depth at Stn. A (32º 32.0' N, 129º 57.2' E) in an inner shelf area (Amakusa-Nada), western Kyushu, Japan (Fig. 1). Collected samples were immediately fixed with neutralized formalin solution (5% final concentration) and brought to the laboratory.

We sorted and dissected zoeae under a stereo-microscope (Nikon SMZ 1500). Feeding-related parts such as the maxilla, maxillule, mandible, and paragnath (= labium) were individually mounted with Apathy’s Mounting Media (Wako 010-13811) between a slide glass and a cover slip and observed under biological microscopes (Olympus IX71 and Nikon E600) with bright field and the differential interference contrast (DIC) observation methods. For observing ingested prey plankton in the gut, larvae with gut contents recognized under a stereo-microscope were selected, dehydrated with ethanol (30, 50, 75, 95, and 100%, each for 5 min.) and mounted with Mountmedia (Wako 139-06682) between a slide glass and a cover slip. Undigested plankton remnants in the gut were observed through the body tissue under a biological microscope with DIC observation methods. The identification for zoea I to zoea V was based on Konishi et al. (1999) and that for zoea V and zoea VI on the distinctly longer
pleopods in the latter, suggesting the molt between the two. Diatoms in the gut were
taxonomically classified in accord with methods of Hasle and Syvertsen (1997).

RESULTS AND DISCUSSION

Maxilla and Maxillule

[Fig. 2]

The maxilla is composed of a coxal endite, a basial endite, an endopod, and a scaphognathite
in all zoeal stages (Fig. 2A). The coxal and basial endites have luxuriantly sprouting setae
with thick shaft bases (4-8 \(\mu\)m in diameter). From the setal shafts, many sharp setules are
spreading at 10-20 \(\mu\)m intervals. These shafts and setules overlap each other and form a
mesh-like structure (Fig. 2B) on the inside of both maxillae. Such a mesh-like structure is
generally observed on the maxilla of herbivorous copepods, e.g. *Calanus finmarchicus*
(Gunnerus, 1770) (see Marshall and Orr, 1956) and *Acartia clausi* Giesbrecht, 1889 (see
Conover, 1956; Gauld, 1966), and it is considered to be a sieving device for collecting prey
organisms entrained in a stream of water past their bodies (Marshall, 1973). From this
structural similarity, it is inferred that zoea of *N. harmandi* might possess an herbivorous habit,
sieving ambient phytoplankton with the mesh-like structure on the maxilla.
Plumose setae extend from the scaphognathite of the maxilla, arrayed in a plane with the setule intervals at 1-2 μm (Fig. 2C). An opening between the setules is finer than that of the mesh-like structure on the coxal and basial endites of the maxilla. Such an ultra-fine opening would not be suitable for sieving plankton, with water not easily passing through them (Tangen, 1978). Due to this insufficient function in filtering, the plumose setae on the maxilla might be used as a wall-like device regulating a water stream rather than a sieving device collecting prey particles.

Some zoeae observed in this study had elongate pennate diatoms tightly bundled between both mandibles (Fig. 3). This position is within the reach of setae extending from the maxillules, which are the only appendages extending between the maxillae and mandibles. Furthermore, maxillule setae of the zoea have stiff serrated appearances particularly in those extending from the coxal and basal endites (Fig. 4). This structure resembles the grasping setae typically observed for the maxilla of carnivorous copepods, e.g. *Labidocera aestiva* Wheeler, 1899 and *Tortanus discaudatus* (Thompson I.C. and Scott A. in Herdman, Thompson, and Scott, 1897) (see Anraku and Omori, 1963; Marshall, 1973). From this morphological similarity, it is inferred that the maxillule of zoea of *N. harmandi* might have a grasping ability and work for bundling elongate diatoms as well as sending them to the mandible.
The mandible is equipped with incisor and molar processes in all zoeal stages; the former processes are arrayed in a row on the anterior part of the mandible edge, while the latter processes are situated on the area almost fringed by the mandible edge (Fig. 5). The incisor processes of zoea I were 4-23 µm in distance between each successive pair and 3-14 µm in height, and those of zoea VI was 8-36 µm in distance and 6-20 µm in height. On the other hand, the molar processes of zoea I were 7-13 µm in distance and 2-11 µm in height, and those of zoea VI were 5-15 µm in distance and 5-16 µm in height. To infer the feeding mode from the edge structure on the mandible blade for planktonic copepods, Itoh (1970) proposed the edge index ($I_e$):

$$I_e = \sum_i (w_i / W \cdot h_i / H \cdot 10^4) / N,$$

where $w_i$ is the distance between each successive pair of processes, $W$ is the total width of the mandible blade, $h_i$ is the height of each process, $H$ is the total height of the cutting edge, and $N$ is the total number of processes on the mandible blade. Based on $I_e$ values, the feeding mode of planktonic copepods was classified into three categories ($I_e \leq 500$ for herbivores, $500 < I_e \leq 900$ for omnivores, and $I_e > 900$ for carnivores). When this system is applied to the zoeal stage of *N. harmandi*, the $I_e$ values of zoea I and zoea VI are 319-390 (mean = 359, SD
= 25, \( N = 5 \) and 199-238 (mean = 217, \( SD = 19, N = 5 \)), respectively. These values are within
the range of herbivory for copepods, suggesting that the mandible blade of zoea of \( N. \) harmandi equipped with many fine processes is suitable for grazing phytoplankton.

[Fig. 5 & 6]

When apolysis was observed on the mandible, hypodermal incisor processes undergoing
development for the next stage were always sharper than the epidermal ones (Fig. 6A). In the
case of zoea VI, incisor processes in the post-molting stage, which can be confirmed by the
undeveloped, rudimentary palp on the mandible, were sharper than those in the pre-molting
stage (Fig. 6B, C). Each molting interval in the zoeal stages was reported to be 3-4 days
(Konishi et al., 1999), and the incisor processes would perhaps become dulled within such a
short period. This abrasion is likely caused primarily by frequent mastication of planktonic
diatoms, which have hard siliceous frustules.

Gut Contents

Diatom frustules or their crushed pieces contained in the gut, along with other unidentified
remnants, were observed through the zoeal body tissues. Of the zoeae with gut contents
recognized under a stereo-microscope, 97.5% possessed diatom frustules. Although detailed
morphology of diatom frustules was not always clearly observed, some were classified into
order or suborder ranks such as Rhizosoleniineae in zoea VI, Coscinodiscineae in zoea VI, Biddulphineae in zoea I-VI, and Pennales in zoea I-VI (Fig. 7). These observations suggest that larvae of *N. harmandi* actually fed on planktonic diatoms in all zoeal stages.

Most diatom frustules and their crushed pieces contained in the gut were limited in their sizes. Each frustule or piece was shorter than 25 µm in width and height, while the length was not limited. Smaller or slender diatoms could be ingested without being fragmented (Fig. 7E); however, larger or wider diatoms would be masticated before ingestion (Fig. 7A-D).

The paragnath (= labium), which is positioned behind the maxillule, has a bilobed shape in all zoeal stages (Fig. 8). Between the lobes, slender pennate diatoms were sometimes tightly held (Fig. 9A, C-H), and these diatoms were arrayed in a row extending into the gut (Fig. 9B). Although such a bilobed paragnath is widely reported in other crustaceans, e.g. adult females of copepod *Heterorhabdus spinifrons* (Claus, 1863) (see Nishida and Ohtsuka, 1996) and the phyllosoma stage of decapod *Panulirus japonicus* (von Siebold, 1824) (see Konishi, 2007), its functional role has not been elucidated as yet. The bilobed shape observed in this study would be suitable for holding and swallowing pennate diatoms, guiding slender prey organisms to enter the gut.
The morphologies of the mouthparts for zoea of *N. harmandi* given in the first two sub-sections above are basically similar to those described for the other callianassid species with 4 to 6 zoeal stages during which planktotrophic development underwent (or was expected to undergo) for the median durations of over 11 days to reach the decapodid stage. These species include *Callichirus seilacheri* (Bott, 1955) as *Callianassa garthi* (see Aste and Retamal, 1983), *Callichirus major* (Say, 1818) (see Strasser and Felder, 1999), *Callichirus islagrande* (Schmitt, 1935) (see Strasser and Felder, 2000), *Neotrypaea uncinata* (H. Milne Edwards, 1837) as *Callianassa uncinata* (see Aste and Retamal, 1984), *Nihonotrypaea petalura* (Stimpson, 1860) as *Callianassa petalura* (see Konishi et al., 1990), and *Nihonotrypaea japonica* (Ortmann, 1891) as *Callianassa japonica* (see Miyabe et al., 1998). To our knowledge, their wild diets are unknown.

The mouthpart morphologies for zoea of *N. harmandi* are also similar to those of protozoae of a penaeid, *Penaeus monodon Fabricius*, 1798 (see Motoh, 1979), which is recognized as an herbivore based on its higher trypsin activity (Jones et al., 1997). The setal densities (= number of setae / length from base of coxa to top of basis) of protozoae I-III of this species are 54-170 setae $\text{mm}^{-1}$ according to the line drawings of Motoh (1979, figs. 8-10) and almost comparable with those of zoeae I and VI of *N. harmandi* (140-170 setae $\text{mm}^{-1}$).
The edge index of protozoea III of *P. monodon* is estimated around 75 according to the line drawing of Motoh (1979: fig. 10), and this value is within the range of herbivory for copepods as the values of *N. harmandi* are. By contrast, zoeae of carideans, *Palaemon elegans* Rathke, 1837 and *Macrobrachium rosenbergii* (de Man, 1879), which are recognized as carnivores based on their lower trypsin activities (Jones et al., 1997), possess different morphologies. Although available information is limited, setal densities of zoeae IV, VI, and VII of *P. elegans* (79-110 setae mm\(^{-1}\) according to the line drawings of Tsurnamal, 1963: figs. 9, 16, 27) are smaller than that of *N. harmandi*. The edge indices of zoeae VII and VIII of *P. elegans* (510-580 according to the line drawings of Tsurnamal, 1963, figs. 25, 38) and of zoeae I-III of *M. rosenbergii* (1300-2400 according to the line drawings of Ling, 1969, figs. 3, 4) are greater than those of *N. harmandi* and beyond the range in herbivorous copepods.

CONCLUSIONS

Zoea of *N. harmandi* has a mesh-like structure on the inside of both maxillae and many fine processes on the mandible edge. Abrasion of the incisor processes was recognized within a molting interval. Furthermore, diatom frustules and their crushed fragments were frequently observed in the gut contents. These observations indicate that zoea of *N. harmandi* would regularly feed on phytoplankton, especially diatoms.
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Fig. 1. Sampling station for *Nihonotrypaea harmandi* larvae. Stn. A (32°32.0' N, 129°57.2' E) is located in Amakusa-Nada, western Kyushu, southern Japan.

Fig. 2. A, Maxilla of zoea VI composed of coxa (C), basis (B), endopod (E), and scaphognathite (S); B, Setal shafts and sharp setules extending from the coxal and basial endites; C, Plumose setae extending from the scaphognathite.

Fig. 3. A, Pennate diatoms (in box) bundled between both mandibles (Md); B, Enlarged microphotograph of pennate diatoms. Mx1, maxillule.

Fig. 4. A, Maxillule of zoea VI composed of coxa (C), basis (B), and endopod (E); B, Stiff serrated setae extending from the coxal and basal endites.

Fig. 5. Incisor processes (IP) and molar processes (MP) on the mandible of zoea VI. $W$, total width of the mandible blade; $wi$, distance between each successive pair of processes; $H$, total height of the cutting edge; $hi$, height of each process.
Fig. 6. A, Apolysis structure in the mandible of zoea V. Hypodermal incisor processes (IP) and molar processes (MP) are undergoing development for the next, zoea-VI stage; B, Sharp incisor processes (arrows) in the post-molting stage of zoea VI; C, Dulled incisor processes (arrows) in the pre-molting stage of zoea VI.

Fig. 7. Diatom frustule fragments in the gut contents of zoea VI. A, Rhizosolenineae (arrow); B, Coscinodiscineae (arrows); C, D, Biddulphineae (arrows: the same specimen at different focal depth); E, Pennales (arrow).

Fig. 8. A, Schematic mouth structure in zoea of *Nihonotrypaea harmandi* (ventral view). Right maxillule is omitted. Paragnath (= labium) is positioned behind the maxillule; B, Bilobed paragnath of zoea VI. Md, mandible; Mx1, maxillule; P, paragnath.

Fig. 9. A, Pennate diatoms ingested by zoea II; B, Enlarged microphotograph of the box area in A. Bundles of pennate diatom frustules (arrows) in an array from the oral region (lower part) to the digestive canal (upper part). Three microphotographs taken at different focal depths are stacked; C-H, Pennate diatoms tightly held between the two lobes of paragnath. Three photographs (C, E, G) taken at different focal depths, with three line drawings (D, F, H) for the traces of the respective photographs. E, eye; Md, mandible; P, paragnath; RL, right
lobe of the paragnath; LL, left lobe of the paragnath.
Fig. 1
Fig. 8