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# Possible food sources of eel leptocephali*

Tsuguo OTAKE**, Kinya NOGAMI*** and Keigo MARUYAKA***

**Abstract**: The gut contents and ultrastructure of midgut mucosal cells were examined in the leptocephali of *Anguilla japonica*, *Conger myriaster* and *Muraenosox cinereus*. Small detrital particles, less than 20 μm diameter, and fecal pellets, 100–250 μm long, were found in the gut of *C. myriaster* and *M. cinereus*. Detrital particles were found quite commonly and seemed to be a major food item. In *A. japonica* ciliates were found. The gut pigment content in *C. myriaster* leptocephali was low. And the Chlorophyll-*a*/Phaeopigment ratio was low. These facts indicated that leptocephali do not feed directly on phytoplankton. The ultrastructure of the midgut mucosal cells revealed that a lamellar membranous structure was well developed in the basal half of the cytoplasm, suggesting that water and ion were actively ingested by the cells. Dissolved organic matter may be part of the nutrition resource for leptocephali. The nitrogen isotopic composition of *C. myriaster* revealed that leptocephali were at the lowest trophic level in the food web. This also strongly supported the suggestion that leptocephali get their nutrition from detrital and dissolved organic matter.

## 1. Introduction

Larval mortality is a major factor determining recruitment of young fishes to a population. A major cause of larval mortality is starvation (THEILACKER and WATANABE, 1989). Information on the feeding of fish larvae, for example prey selection, feeding behavior, and digestion physiology, has been accumulated so as to examine the mechanism of larval mortality (see review in HUNTER, 1981; GOVONI *et al.*, 1986). However, little is known of the feeding of the eel leptocephalus during their long oceanic migration. In particular, nutrition is enigmatic since no food has been found in gut of any leptocephali (MOSER, 1981). In the present study gut contents, gut pigment content, and ultrastructure of gut mucosal epithelium were examined in the leptocephali of the anguilliformes, *Anguilla japonica*, *Conger myriaster* and *Muraenosox cinereus*. Isotopic distribution in animals was found recently to be closely related to dietary isotopic composition. The nitrogen heavy isotope ($^{15}N$) is regularly enriched by 3–4‰ per trophic level (MINAGAWA and WADA, 1984; FRY, 1988). We also examined nitrogen isotopic composition to estimate leptocephalus trophic level in food web and aid in discussion of possible food sources.

## 2. Materials and methods

*Gut contents*

Three leptocephali of *Anguilla japonica* (40.5–43.4 mm TL) collected in the western North Pacific during a 1986 research cruise of R/V Hakuho Maru (KH-86-4) of Ocean Research Institute, University of Tokyo (KAJIHARA, 1988), and 216 specimens of *Conger myriaster* and 77 of *Muraenosox cinereus* leptocephali collected in Harimanada, Seto Inland Sea (Japan) in 1989, were used for examination of gut contents. The specimens of *A. japonica* were collected by oblique hauls of an IKPT net (mesh aperture: 0.5mm) from 500m depth to the surface (KAJIHARA, 1988). *C. myriaster* and *M. cinereus* leptocephali were collected by bag net or bull trawl for sand eel fisheries. The specimens were preserved in 10% neutralized formalin or 2 % paraformaldehyde-2 % glutaraldehyde mixture in 0.1M cacodylate buffer (pH7.4). Measuremants

* Received November 30, 1990
** Ocean Research Institute, University of Tokyo 1-15-1, Minamidai, Nakano-ku, Tokyo, 164 Japan
*** Japan Sea Farming Association, Tamano Station, 5-21-1, Tikkou, Tamano-shi, Okayama, 706 Japan

and counting of myomeres followed the methods described by JESPERSON (1942) and CASTLE (1963). Identification followed MOCHIOKA (1988) and TABETA (1988).

After measurements and myomere counts, the specimens were examined for gut contents under a binocular dissecting microscope. Some identifiable gut contents were examined further with a scanning electronmicroscope.

For scanning eletronmicroscopy (SEM) the gut was removed and cut into several pieces. They were post-fixed in 2% $OsO_4$ for 2h, dyhydrated through a graded ethanol series, and dried in a Hitachi HCP-2 critical point dryer. The dried tissues were mounted on a brass disc, cut into halves using a needle and razor, and the inner surface exposed. They were subsequently spattercoated with gold in a Jeol JFC-1100 ion-spatter and observed under an Akashi-25$\alpha$ SEM.

*Gut pigment contents*

Five specimens of *C. myriaster* leptocephali were used for examination of gut pigment content. After total length measurement each gut was removed and dipped in dimethylformamid. Chlorophill-*a* and phaeopigment extracted by dimethylformamid were measured as gut content pigment with a Turner model 111 fluorescent photometer after STRICKLAND and PARSONS (1972).

*Ultrastructure*

For transmission electronmicroscopy (TEM) the midgut of some *A. japonica* and *C. myriaster* leptocephali were fixed in cold 2% paraform aldehyde-2% glutaraldehyde mixture in 0.1M cacodylate buffer (pH7.4) for several days and post-fixed in 2% $OsO_4$ for 2h. The tissues were dyhydrated through a graded ethanol and embedded in Epon 812 resin. Ultra-thin sections were cut by a LKB-ultro-tome (V), double stained with uranyl acetate and lead citrate, and examined by a Jeol JEM-100CX TEM. Some thicker sections were stained with toluidine blue and observed under a light microscope.

*Stable nitrogen isotopic composition*

Stable nitrogen isotopic compositions were measured for *C. myriaster* leptocephali and other animals, such as sand eel (*Ammodytes personatus*), squid (*Loligo* sp.), and jelly fish, collected at the same time in Harimanada in 1989. The isotopic composition of a mixed-species zooplankton sample and particulate organic matter were also measured. The latter was collected by filtering 15 liters of seawater (15m deep) using a precombusted (400 ℃, 5h) silica filter (pore size 0.22 $\mu$m). Zooplankton was sampled by vertical haul of a handtowed plankton net (30 cm mouth diameter, 0.33mm mesh) from bottom (ca. 45m deep) to surface. All samples were dried at 60℃ and ground. The resulting powder was analyzed for $^{15}N/^{14}N$ with a Finnegan MAT-Delta E mass spectrometer after MINAGAWA and WADA (1984). Results are expressed as $\delta$ values, ‰ deviations from a standard (atmospheric nitrogen) by the following equation:

$$\delta\,^{15}N = (R\ \text{sample}/R\ \text{standard}-1) \times 1000$$

where $R = {}^{15}N / {}^{14}N$.

## 3. Results and discussion

*Gut contents*

Many spherical particles, possessing a large number of long, filamentous projections on the surface, were found in the gut of *A. japonica* (Fig.1). Each projection was found to contain microfilaments extending deep into the body, suggesting it to be a cilium (Fig.2). The particles were enveloped by a single membrane and included cell organelles such as a nucleus, vacuoles, and a myeline body. These suggested that the particles were unicellular organisms, possibly ciliates. In *C. myriaster* and *M. cinereus* two types of particles were identified from the gut. One was a small aggregation of fine particles and amorphous mucous material, and was less than 20 $\mu$m diameter (Fig. 3, OTAKE, *et al.*,1992). TEM examination showed each fine particle to lack an obvious structure and suggested them to be detrital particles. This type of particle was found in considerable numbers in the gut of over 80% leptocephali examined (Table 1) and suggests that detrital

Table 1. Gut contents of *C. myriaster* and *M. cinereus* leptocephali collected in Harimanada.

| Species | No. of specimen examined | Total length range (mm) | Number of specimens containing particulate matter (%) | |
|---|---|---|---|---|
| | | | Detrital particle | Fecal pellet |
| *C. myriaster* | 216 | 89.0−127.0 | 172 (79.6) | 16 ( 7.4) |
| *M. cinereus* | 77 | 35.0− 90.2 | 74 (96.1) | 32 (41.6) |

Table 2. Chlorophill-*a* (Chl. *a*) and phaeopigment (Phaeo.) contents and their ratio in the gut of *C. myriaster* leptocephali collected in Harimanada.

| No. of fish examined | Range of total length (mm) | Chl. *a* (ng/ind.) | Phaeo. (ng/ind.) | Chl. *a*/Phaeo. |
|---|---|---|---|---|
| 5 | 103.0−118.0 | 0.27±0.11 | 1.35±0.56 | 0.21±0.07 |

particles are a major food item of leptocephali. Another type of particle, found in 16% gut examined (Table 1), had an oval shape some 100−250 μm long (Fig.4, OTAKE, *et al.*, 1992). Each was covered by a mucous membrane and included many fragments of cocoliths and diatoms (Fig.5,6, OTAKE, *et al.*, 1992), suggesting them to be fecal pellets of herbivourous zooplankton.

*Gut pigment contents*

The total gut pigment content of each *C. myriaster* leptocephalus was 1.62±0.13 (mean ±S. D.) ng $ind^{-1}$ (Table 2). Following Strathman's equation (1967) wherein the Carbon/Chlorophill-*a* ratio is 50, this value corresponds to only about 45 unicellular flagellates (10 μm diameter) or 120 diatom cells (10 μm diameter). The latter figures are thought to be too low for phytoplankton to be considered a major food of leptocephali. Furthermore, the Chlorophyll-*a*/Phaeopigment ratio was also quite low (0.21±0.07) (Table 2), suggesting that the pigment stayed in the gut for a long time or that it originated from detrital matter such as fecal pellets. Leptocephali do not seem to feed directly on phytoplankton.

*Ultrastructure of midgut epithelium*

The midgut epithelial cells of *A. japonica* and *C. myriaster* leptocephali possessed a typical characteristic of absorptive cells such as a developed brush border, and were additionally characterized by two striking cytological features (OTAKE, et al., 1992). Numerous vacuoles were distributed in the upper half of the cytoplasm (Fig. 6), each including

Fig. 1 SEM micrograph of spherical particles in the gut of *A. japonica* leptocephalus. Filamentous structures are seen on the surface of the particles.

Fig. 2 TEM micrograph of a spherical particle showing cytoplasmic projections. Microfilaments (arrow heads) extending into the cytoplasm are seen in the projections.

Fig. 3 SEM micrograph of a small particle in the gut of *C. myriaster* leptocephalus. It comprised fine particles and amorphous matter (OTAKE, *at al.*, 1992).

Fig. 4 Oval particles in the gut of *C. myriaster* leptocephalus (OTAKE, *at al.*, 1992).

Fig. 5 SEM micrograph of diatom fragment found in an oval particle (OTAKE, *et al.*, 1992).

Fig. 6 SEM micrograph of cocolith fragment found in an oval particle (OTAKE, *et al.*, 1992).

Fig. 7 TEM micrograph of the apical portion of the midgut epithelial cell of *C. myriaster* leptocephalus. Numerous vacuoles containing fine particles are present in the cytoplasm. Mv-microvillii.

Fig. 8 TEM micrograph of basal portion of the midgut epithelial cell of *C. myriaster* lepto cephalus. Membranous lamellar structure (Lm) associated with large mitochondria (M) are highly developed.

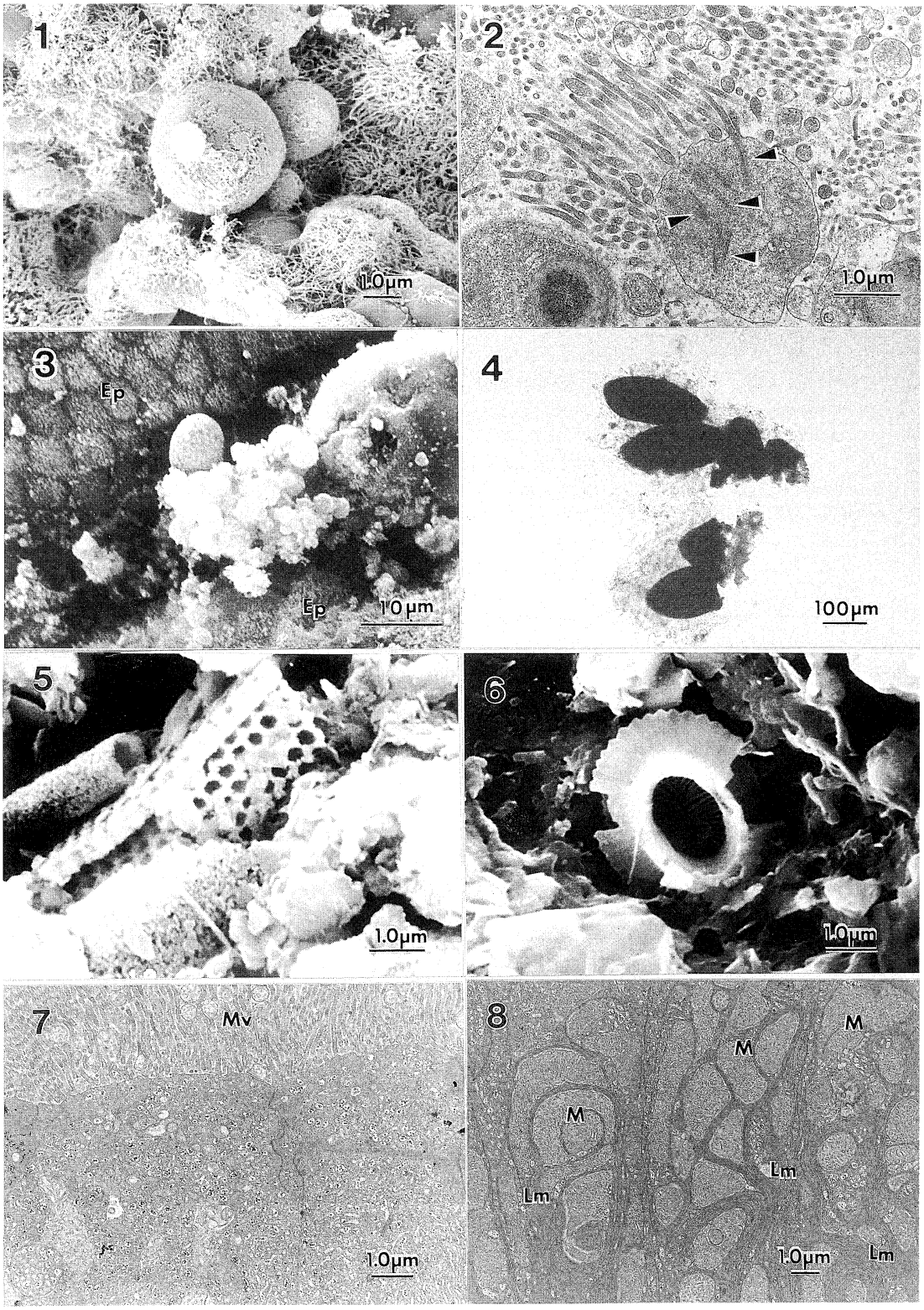
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Table 3. Nitrogen isotopic composition of biota from Harimanada.

| Samples | No. of samples | $\delta^{15}N$ (mean±S. D.) |
|---|---|---|
| Particulate organic matter | 1 | 11.6 |
| Invertebrate: | | |
| Zooplankton | 1 | 11.9 |
| Jellyfish | 2 | 17.5±2.4 |
| Squid (*Loligo sp.*) | 1 | 17.6 |
| Fish: | | |
| Sand eel (*Ammodytes personatus*) | 2 | 17.8±1.8 |
| Leptocephalus (*Conger myriaster*) | 3 | 11.1±0.9 |

fine particles with high electron density. The vacuoles were often found to be in contact with the surface plasmamembrane between microvilli, indicating active phagocytotic ingestion of intact macromolecules. Extremely highly developed membranous lamellar structure closely associated with large mitochondria was found in the basal half of the cytoplasm (Fig.7). A similar structure has been reported in the digestive system of various fishes (OZAKI, 1965; YAMAMOTO, 1966; IWAI, 1968; NOAILLIAC-DEPEYRE and GAS, 1976; STROBAND and DEBETS, 1978) and is thought to be involved in water and ion transport. Furthermore $Na^+-K^+-$ ATP ase activity has been demonstrated on both the lamella membrane and the basal plasmamembrane of midgut epithelial cells in preleptocephali of *M. cinereus* (OTAKE, unpublished). This is direct evidence for the uptake of water and ions by the cells. The highly developed lamellar structure indicates that leptocephali absorb copious seawater in the midgut epithelium. It is known that leptocephali have a high water content and that their ionic composition is in equilibrium with that of seawater (HULET *et al.*, 1972; PFEILER, 1986). PFEILER (1986) reported that bonefish leptocephali contained high levels of essential amino acids which they were presumably unable to synthesize by themselves. Therefore, HULET (1978) and PFEILER (1986) suggested that dissolved organic matter was a plausible source of nutrition for leptocephali. Active seawater ingestion by the gut epithelium of *C. myriaster* leptcephali may support their suggestion.

*Stable nitrogen isotopic composition*

The nitrogen heavy isotopic composition in *C. myriaster* leptocephali was 11.1‰, which is much lower than that found in other herbivorous invertebrates and a planktivorous fish (Table 3, OTAKE, *et al.*, 1992). In addition, the isotopic composition of the leptocephali was rather lower than that of particulate organic matter, which comprised a mixture of phytoplankton, detritus, bacteria, microzooplankton, and small zooplankton. Leptocephali are obviously located at the lowest trophic level in the food web, and accordingly are unlikely to feed on zooplankton and other animals, which occupy higher trophic level. Particulate and dissolved organic materials are plausible nutrition resources for leptocephali.

## 4. Conclusion

Detrital particles, often collectively called "marine snow", are sites of dense community of phytoplanktons, bacteria, and protozoa, and are nutritionally enriched (SILVER *et al.*, 1978; SHANK and TRENT, 1979; CARON *et al.*, 1986). Accordingly, they are considered to be a potential food source for epipelagic and deep sea organisms (WANGERSKY, 1974; GERBER and MARSHALL, 1974; GOTTFRIED and ROMAN, 1984; SHANKS and TRENT, 1980; SILVER and ALLDREDGE, 1981). The results of this study showed that detrital particles are a possible major food item for eel leptocephali, and that dissolved organic matter could also

be part of their nutritional resource.

The activity of digestive enzymes, such as trypsin and kitinase, have been shown to be quite low in the gut of *C. myriaster* leptocephali and *M. cinereus* pre-leptocephalus larvae (OTAKE, unpublished). This suggests that leptocephali cannot fully digest organic matter contained in detrital particles. Decomposition of such organic matter by associated microorganisms, such as bacteria and protozoa, would be important in increasing the nutritional value of detrital particles.

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