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Presumption of Food of Eel Leptocephalus: A Review

Abstract

Stomach and intestinal contents of eel leptocephalus are largely composed of amorphous substance. What kind of food do they eat, and how do they digest and absorb the nutrient from the food to make their unique form filled with amorphous substance in the body?

Before the commencement of artificial feeding, forced administration of protein solution into the stomach of elvers resulted in a rapid increase of the protein in the blood plasma. However, the protein transport into the blood decreased with the development of digestive ability. The results, on the contrary, indicate that eel larva before metamorphosis, i.e., leptocephalus, has less digestive ability.

The stomach and intestinal contents in the larval Japanese eel *Anguilla japonica* and bucktooth conger *Gonathophis nystromi* were analyzed by western blotting technique using antibodies against several species of gelatinous plankton. Stomach contents of the two species showed positive reaction against some species of cnidarians and pelagic tunicates.

From these findings and other observations, we presumed that suspended organic matter, so-called marine snow, derived from gelatinous planktons are the natural foods of leptocephali.

Key words: Leptocephalus, Marine snow, Western blotting

It is of interest to find the natural foods of eel larvae, i.e., leptocephali, since they spend exceptionally long period until metamorphosis to juvenile with their specific form completely different from that of adults. This is also expected from the researchers of seed production in eel; we are able to obtain eggs and hatch out larvae from artificially matured females, but have not yet been able to rear the larvae up to elvers. What kind of food do they eat, and how do they digest and absorb the nutrient from the food to make their unique form filled with amorphous substance in the body? To answer these questions, we paid attention to the digestive ability of elver eel just after the metamorphosis from leptocephali[(1)]. In addition, we examined the stomach and intestinal contents of the leptocephali using antibodies against several species of gelatinous plankton.

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Digestive Ability of Elver Eel

It is well known that protein directly intubated into the gut of fish appears intact and/or bio-active macromolecules in the blood of fish including eel[2]. In agastric species, such as goldfish and carp, a noticeable amount of protein transport occurred when the protein was administered via oral route[3-6]. The intensity of protein transport from the gut may differ at different developmental stages even in identical species. In fact, the protein uptake by enteric epithelium observed in larvae disappeared accompanied with the development of digestive tract[7-9]. These findings suggest that uptake and transport of food protein into blood occur more intensively in larval stage. Until now, analysis using leptocephali has not been carried out, since live leptocephali are hardly obtained. To estimate the digestive ability of leptocephali, we analyzed the changes in the amount of protein transport in the elver of the Japanese eel *Anguilla japonica* in relation to their development.

IgY extracted from hen's egg yolk was administered via oral route to elver eels at a dose of 2.0ug/0.1g body weights at 0, 12, 25, and 42 days after the initiation of feeding of protein-rich feed mixture. With the lapse of time after the administration, 8 or 9 individuals were sampled, and blood was taken into a heparinized micro hematocrit tube from caudal vasculature by cutting their tail. Blood plasma was separated by centrifugation and the plasma IgY concentration was measured by enzyme Eminase using the antibody against IgY. The digestive tract with some other organs was removed and fixed with Bouin's fluid for histological observation.

The first experiment, performed before the commencement of artificial feeding (mean BW 0.130 g) resulted in a rapid increase of plasma IgY to the maximum of 2.03ug/ml at 6 hours after the administration. However, the IgY transport to the blood decreased significantly at the experiments performed at 12 days later (0.175 g), and was almost negligible at 25 days (0.424 g), and at 42 days (1.100 g). Throughout this period, the stomach grew remarkably in size, in the extent of foldings and in the number of stomach glands, with the rapid development of the body size, approximately eight times in BW.

These observations clearly show that a part of orally delivered proteins comes into blood circulation as macromolecules in the newly caught elvers. However, the results also show that this transport was diminished soon after the commencement of feeding protein-rich feed mixture, accompanied with the development of stomach. The results, on the contrary, indicate that eel larva before metamorphosis, i.e., leptocephalus, has less digestive ability. Leptocephali take up food protein with less digestion in the alimentary canal, and/or they feed materials already processed, such as marine detritus.

Analysis of Stomach and Gut Contents in Leptocephali

There are several observations suggestive of food for eel leptocephalus larvae. Otake et al.[10] reported that stomach and intestinal contents of eel leptocephali are largely composed of amorphous materials, although fecal pellets of copepods were found. Mochioka et al.[11] considered that the discarded house of appendicularians and fecal pellets are important as food, by analyses of several species of eel leptocephali. Tsukamoto (unpublished) identified specific groups of bacteria in the gut of leptocephali similar to that in the marine snow. In addition to these field survey, Mochioka and Iwama[12] observed the larvae of pike conger *Murdenesox cinereus* feed minced squid in an aquarium. Tanaka *et al.* (unpublished) also succeeded to rear the eel larvae obtained from artificially matured mothers for about 250 days by feeding with paste containing shark egg extract. All these observations indicate that the possible natural food of leptocephalus is the marine snow, which is made up of dead plankton.

Eel leptocephalus larvae may have limited digestive ability with a simple straight intestine, and the body is filled with gelatinous substances as already discussed. We therefore hypothesized that leptocephali eat, absorb and utilize substances derived from gelatinous plankton. We made immunological analysis of the stomach and intestinal contents in the larval of the Japanese eel and the bucktooth conger, *Gonathophis nystromi,* using antibodies against several species of gelatinous plankton.

Antisera were made against three species of cnidarians, *Achira caerulescens* (AE), *Arolla wyvillei* (AT), *Periphylla periphylla* (PE), and four species of pelagic tunicates, *Pyrosoma atlanticum* (PA), *Pyrostremma agassizi* (PJ), *Thetys vagina* (TV), *Thalia democratica* (TD). Rabbits were injected with the extracts of the planktons. From the antiserum, IgG was purified and labeled with biotin.

Leptocephalous larvae of eel and conger were collected in the Pacific Ocean southeast of Taiwan on the KT96-19 cruise of *Tansei-maru* of the Ocean Research Institute, the University of Tokyo, in October 1996. Digestive tract was removed under a dissecting microscope, and stored at -80°C until use.

Extracts of alimentary canal samples of leptocephali were analyzed with SDS-PAGE and western blotting technique. Proteins were blotted onto a blotting membrane, which was further treated with the biotinylated antibodies. The proteins labeled with the antibodies were then visualized by the avidin-biotin peroxidase technique.

Western blotting analysis of the alimentary canal of the Japanese eel showed several positively stained bands with the antibody against AE, in some individuals. In conger, positive reactions were obtained with the antibody against PE and PA. These reactions were reduced by addition of the respective antigen to the reaction medium of the antibody, indicating the reactions are antigen specific. But the positive bands obtained with each antigen were not at the same positions to the protein bands of the respective animal antigen.

The results obtained here proposed the candidates of natural food of leptocephali AE in eel, and PE and PA in conger eel. But the specificity of each antibody is not necessarily high, since the antibodies cross-react with other species in their own taxonomic group. This indicates that these fish feed some species of animals in the groups of AE, PE and PA. From these findings and other observations such as epithelial structure of intestine, we think that suspended organic matter, so-called marine snow, derived from gelatinous planktons are the natural foods of leptocephali.

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