



Research

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A low trophic position of Japanese eel larvae indicates feeding on marine snow

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What eel larvae feed on in the surface layer of the ocean has remained mysterious. Gut contents and bulk nitrogen stable isotope studies suggested that these unusual larvae, called leptocephali, feed at a low level in the oceanic food web, whereas other types of evidence have suggested that small zooplankton are eaten. In this study, we determined the nitrogen isotopic composition of amino acids of both natural larvae and laboratory-reared larvae of the Japanese eel to estimate the trophic position (TP) of leptocephali. We observed a mean TP of 2.4 for natural leptocephali, which is consistent with feeding on particulate organic matter (POM) such as marine snow and discarded appendicularian houses containing bacteria, protozoans and other biological materials. The nitrogen isotope enrichment values of the reared larvae confirm that the primary food source of natural larvae is consistent only with POM. This shows that leptocephali feed on readily available particulate material originating from various sources closely linked to ocean primary production and that leptocephali are a previously unrecognized part of oceanic POM cycling.

1. Introduction

Leptocephali are a remarkable type of fish larvae because of their extreme lateral body compression and transparency, unique physiology, and growth to much larger sizes than most fish larvae [1]. They are the larvae of the ancient and diverse fishes of the Elopomorpha that include freshwater eels, marine eels and their relatives, which live in most environments of the ocean [2]. Despite the diverse habitats of adult elopomorphs, their larvae live in the euphotic zone where oceanic primary production occurs.

The food source of leptocephali was completely unknown until recently when particulate organic matter (POM) was documented in their intestines [3–5]. These observations suggested that marine snow or discarded appendicularian (filter feeding tunicates) houses were the main food of leptocephali, and these types of POM are consistently present in the surface layer of the world's oceans [6]. However, ciliates have been observed in their intestines [7] and DNA barcoding analyses detected sequences of a wide range of marine animals in the gut contents of small European eel leptocephali [8].

Bulk nitrogen isotope ratios ($\delta^{15}\text{N}$) of both freshwater [9] and marine eel [3] leptocephali have indicated that they feed at a low level in the oceanic food web. However, the bulk $\delta^{15}\text{N}$ method requires the isotopic composition of either the primary producers or nutrients (e.g. nitrate) be examined to precisely estimate the trophic position (TP) of consumers [10]. Moreover, the magnitude of ^{15}N enrichment can vary among organisms [11], leading to further uncertainty about the estimated TP.

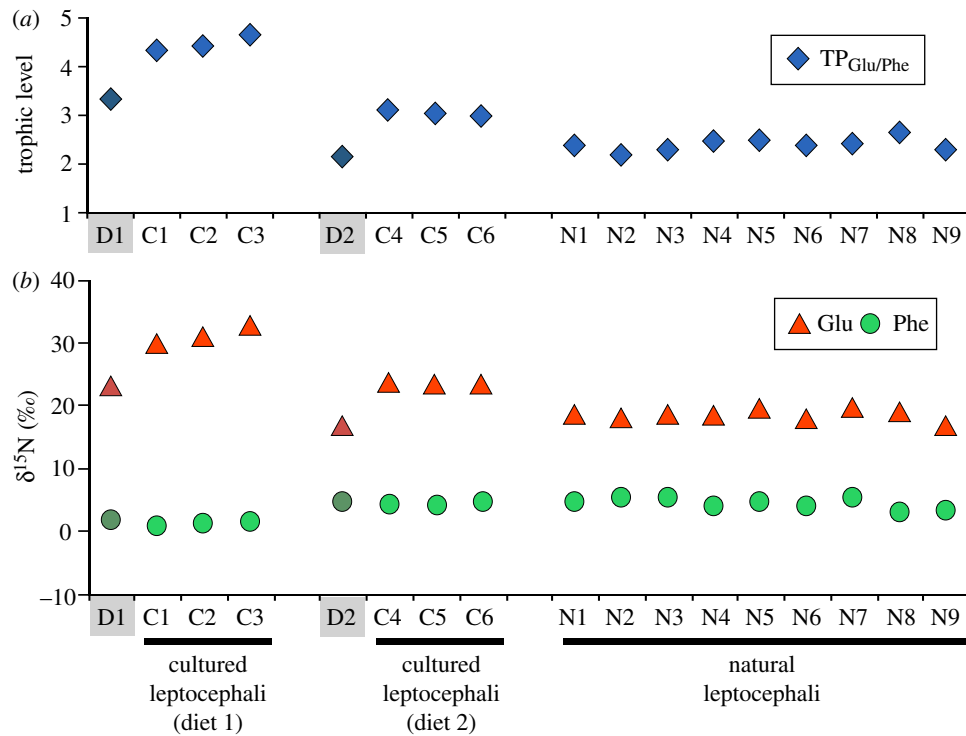


Figure 1. The nitrogen isotopic composition of phenylalanine ($\delta^{15}N_{Phe}$, reflecting the isotopic ratio of the food source) and glutamic acid ($\delta^{15}N_{Glu}$, reflecting the isotopic ratio after enrichment) of natural Japanese eel, *Anguilla japonica*, leptocephali (specimens N1–N9) and the diets (D1, D2) fed to the artificially cultured Japanese eel leptocephali (specimens C1–C6) (b) and the trophic positions (TP) of each specimen or diet (a) calculated from their $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ values.

In this study, we applied a new technique of amino acid $\delta^{15}N$ analysis [12–15] to solve this problem. We used both natural larvae and two groups of artificially spawned and reared larvae of the Japanese eel, *Anguilla japonica*, which is a freshwater eel in East Asia. This anguillid eel spawns offshore in the western North Pacific, with its leptocephali feeding and growing as they are transported back to East Asia by ocean currents [16,17], and its larvae have been reared in the laboratory in recent years [18].

Our objective was to estimate the TP of natural leptocephali within the oceanic food web. We used $\delta^{15}N$ values of glutamic acid and phenylalanine, which are useful for determining the TP of marine organisms [19], to estimate the TP of natural leptocephali and cultured larvae to validate the TP of the food source of natural leptocephali.

2. Material and methods

(a) Specimens analysed

Japanese eel leptocephali were collected, using plankton nets in the western North Pacific and preserved in formalin [16] ($n = 9$, 15–18 mm). Two groups of artificially spawned and reared Japanese eel leptocephali were fed different artificial diets four times each day, which contained spiny dogfish (*Squalus acanthias*) egg yolks, Antarctic krill extract, peptides and vitamin mixtures. The two diets had different trophic signatures that probably resulted from different peptides or krill extracts (see the electronic supplementary material). Larvae were reared to 50 days old ($n = 3$, 13–16 mm) for diet 1, and 87 days ($n = 3$, approx. 20 mm) for diet 2 before analysis.

(b) Isotopic analysis and trophic position estimate

Natural and laboratory-reared leptocephali (see the electronic supplementary material, figure S1) were analysed for their nitrogen isotopic composition of phenylalanine ($\delta^{15}N_{Phe}$) and glutamic

acid ($\delta^{15}N_{Glu}$) to estimate their TP. Samples were prepared and analysed according to previously described procedures [13]. Samples were hydrolysed, hydrophobic constituents such as lipids were removed and the amino acids were extracted. Nitrogen isotopic composition of the two amino acids was determined by gas chromatography/combustion/isotope-ratio mass spectrometry. Analyses of amino acids such as $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ have been determined to be possible even using samples preserved for many years in formalin [20], as was the case in this study.

The TP of each specimen was calculated, using the previously established formula [19]: $TP = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4)/7.6 + 1$. Only $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ were used in the analysis, because this combination of amino acids appears to provide the most precise estimate of the TP of marine organisms [19,21]. For comparison, a few specimens of other marine organisms were also analysed, and some values from the literature were included (see the electronic supplementary material, table S1).

3. Results

The natural Japanese eel leptocephali showed similar $\delta^{15}N_{Phe}$ (3.1–5.5‰, mean \pm s.d.: 4.5 ± 0.9 ‰) and $\delta^{15}N_{Glu}$ (16.6–19.5‰, 18.4 ± 0.9 ‰) values (figure 1). The reared leptocephali showed values related to each artificial diet. The $\delta^{15}N_{Phe}$ of those fed diet 1 (0.9–1.4‰, 1.2 ± 0.3 ‰) was about the same as that of their diet (1.8), whereas their $\delta^{15}N_{Glu}$ (29.6–32.8‰, 31.1 ± 1.6 ‰) was much higher than the $\delta^{15}N_{Glu}$ of diet 1 (23.0‰). Similarly, the $\delta^{15}N_{Phe}$ values of the larvae fed diet 2 (4.3–4.8‰, 4.5 ± 0.2 ‰) were about the same as their diet (4.7‰), and their $\delta^{15}N_{Glu}$ values (23.1–23.8‰, 23.4 ± 0.3 ‰) were similarly higher than that of their diet (16.9‰).

The calculated TP of the larvae and diets showed that the natural leptocephali were at a lower level than the cultured larvae. The TPs of the natural larvae (2.2–2.6, 2.4 ± 0.1) were all within the second trophic level, but the cultured larvae were either at TPs of 4.3–4.7 (4.5 ± 0.2) for larvae

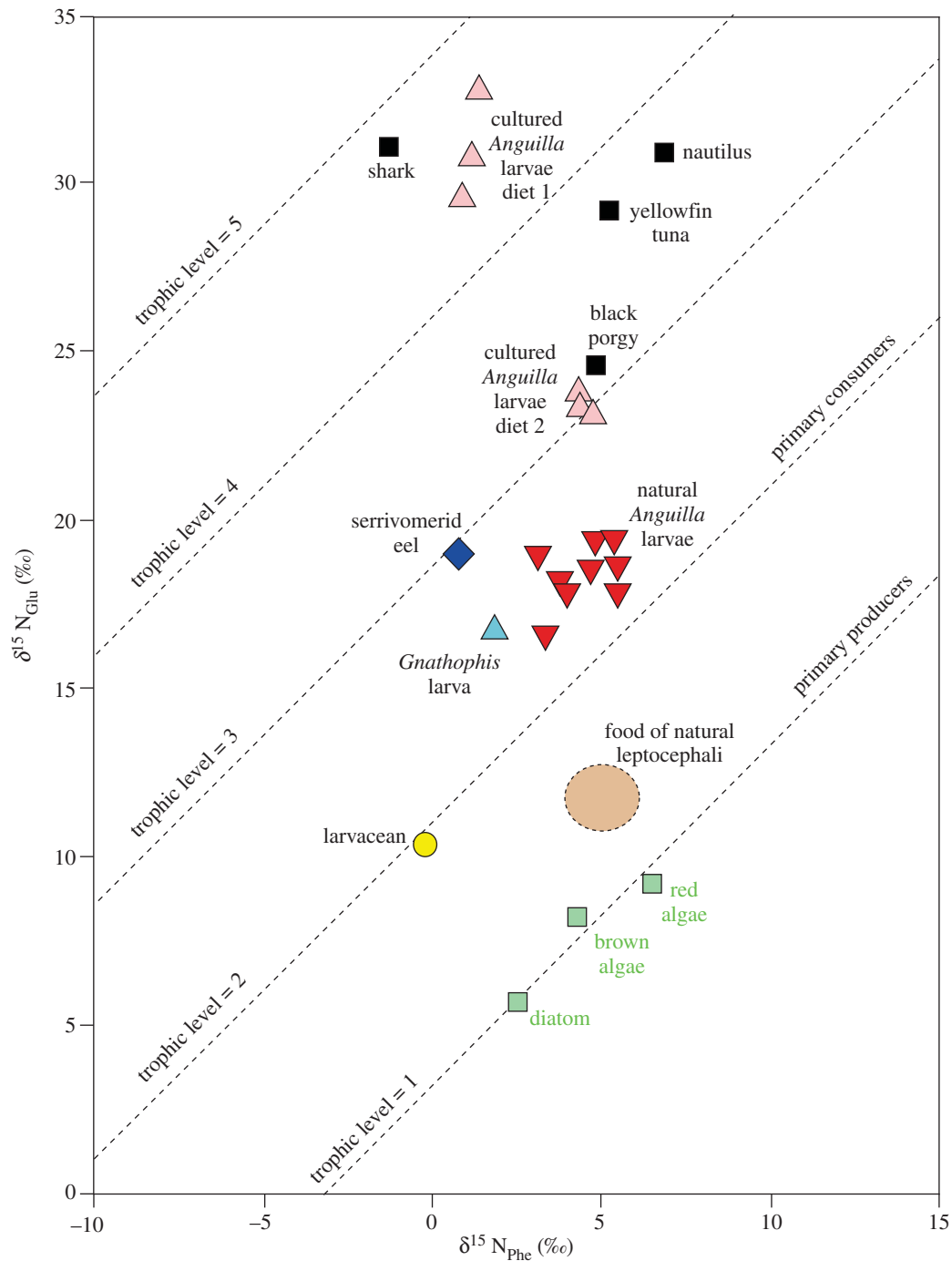


Figure 2. Plot of nitrogen isotopic compositions of phenylalanine ($\delta^{15}\text{N}_{\text{Phe}}$) and glutamic acid ($\delta^{15}\text{N}_{\text{Glu}}$) of the specimens of this study, the estimated TP of the food of natural leptocephali, and some specimens from previous studies in relation to the trophic levels (diagonal dotted lines) that are determined by the values of $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{15}\text{N}_{\text{Glu}}$. (See the electronic supplementary material for details about specimens.)

fed diet 1, or 3.0–3.1 (3.0 ± 0.1) for larvae fed diet 2 (figure 1). They were at higher positions because of their high-TP diets. The amount of enrichment of $\delta^{15}\text{N}_{\text{Glu}}$ determined from the leptocephali fed artificial diets was about 1 trophic level (1.2 and 0.9 trophic levels for diet 1 and diet 2). This confirms that the food source of the natural leptocephali would have been at a TP of about 1.4, which is between primary producers and primary consumers (figure 2).

The TP values of other organisms included another species of leptocephalus (Congridae: *Gnathophis*, TP = 2.5), a pelagic filter feeder (appendicularian, TP = 1.9), a juvenile mesopelagic eel of the Serrivomeridae (TP = 2.9), and various predatory fishes (black porgy, *Acanthopagrus schlegeli*, yellowfin tuna, *Thunnus albacares*, shark, *Squalus* sp.) and a nautilus,

Nautilus pompilius, that had higher TPs (figure 2). The primary producers of red algae, *Gelidium japonicum*, brown algae, *Sargassum filicinum* and a diatom, *Rhizosolenia* sp., all had low TPs of 0.9–1.1.

4. Discussion

The use of $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{15}\text{N}_{\text{Glu}}$ values enabled the low TP of leptocephali to be validated for the first time, because the natural Japanese eel leptocephali had a TP of 2.4 and the *Gnathophis* leptocephalus had a TP of 2.5. The verification of ^{15}N enrichment by 1 trophic step for the two groups of reared larvae indicated the food source of these natural leptocephali was

between trophic levels 1 (primary producers) and 2 (primary consumers). This is consistent with the TP range of POM [14], and also shows that the diet of these leptocephali did not include much material originating from predatory zooplankton such as crustaceans and gelatinous zooplankton (see the electronic supplementary material, figure S2). Although the TP of each individual natural leptocephalus examined in this study was consistent with feeding on POM, the sample sizes were small, so future studies using more larvae of different sizes and species are needed to better understand the TP of leptocephali in the ocean areas where they are found.

A feeding ecology based on consuming marine snow can explain the observations of the DNA barcoding study of leptocephalus food contents [8] and of ciliates being consumed [7] because marine snow comprises detritus-like materials from all types of marine organisms that aggregate together, and thus these species or their tissue fragments could be present in the material ingested by leptocephali. Direct observations of the food contents of various species of leptocephali have consistently shown material that appears to be amorphous marine snow or discarded appendicularian houses and zooplankton faecal pellets [3–5] (see the electronic supplementary material, figure S3). The houses of appendicularians are frequently discarded after their filters clog with particles that could originate from various organisms [6]. Therefore, marine snow and appendicularian houses contain micro-organisms such as bacteria and

protozoans and colonizing animals such as ciliates, copepodid stages, and anthozoan, mollusc and polychaete larvae [6,22]. The aggregation of organismal materials in POM probably explains the presence of the DNA sequences detected in small European eel larvae [8], although leptocephali may also sometimes directly consume small organisms.

Marine snow also includes exudates from bacteria and phytoplankton that provide the 'glue' to facilitate other particles to stick together [6]. New analytical techniques are showing more clearly that these exudates release polysaccharides and simple sugars into the ocean [23] that can aggregate into marine snow and these carbohydrates may also be an important food source for leptocephali, in addition to nutrition obtained from digestion of the micro-organisms aggregated in marine snow.

There is no evidence of differing feeding ecologies among species of leptocephali [1,3–5], so their previously unrecognized feeding on particulate material at the tropical and subtropical latitudes where they live throughout the world's oceans has implications for increasing understanding of the ocean carbon cycle [1], especially, because the presence of additional direct consumers of POM has been hypothesized to be able solve some inconsistencies in particle export studies [24]. The widespread presence and abundance of leptocephali that are feeding on particulate material suggests they should be evaluated for their importance in POM cycling and global carbon transport in the ocean.

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