Distribution and size variability of Japanese eel leptocephali in their Pacific Ocean spawning area using carbon and nitrogen stable isotope ratio analyses

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Research Article

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Abstract

The diets of larval (leptocephali) anguillid and marine eels are poorly understood, despite studies on their gut contents or stable isotope ratios suggesting marine snow particles represent a food source. Concerns for Japanese eel Anguilla japonica stock conservation necessitate an improved knowledge of their larval ecology to better understand the causes of their recent decline in numbers and fluctuating recruitment into East Asia. To understand the distribution of and variation in size of leptocephali in relation to their feeding, we examine carbon and nitrogen stable isotope ratios of larvae from seven research cruises (2002–2013) in the North Equatorial Current spawning area. Preleptocephali (2–3 days old, ~5 mm total length) isotope ratios reflect maternal ratios, but feeding-stage leptocephali (8–56 mm) tend to have higher δ15N values with decrease of latitude typically in areas south of a salinity front. Neither δ15N nor δ13C ratios are clearly related to longitude or larval size < 30 mm, but δ13C values of larvae > 40 mm are lower further downstream in the North Equatorial Current and Subtropical Countercurrent. Differences in δ13C values might be a function of varying spatial baselines in the two currents apart from the spawning area. Although among-year larval isotope ratio differences may reflect temporal baseline variation related to the location of the salinity front, more research with much wider range observations in the spawning season is required because ingested marine snow particles might differ with larval growth and location.

Introduction

Populations of the Japanese eel Anguilla japonica, an important fisheries resource in East Asia, have experienced major declines, raising concerns about stock conservation (Kaifu 2019; Kaifu and Yokouchi 2019). This species is now listed as endangered (Jacoby et al. 2015). Japanese eels spawn in the North Equatorial Current (NEC) to the west of the Mariana Islands about 3000 kilometers from where their juveniles grow in East Asia. Discovery of this spawning area in 1991 (Tsukamoto 1992) led to decades of research trying to understand ecology of the larval (leptocephalus) Japanese eel in the spawning area (Kimura et al. 1994; Tsukamoto et al. 2003, 2011; Shinoda et al. 2011). Research has included studies on the diets and feeding depths of these leptocephali (Miyazaki et al. 2011). Because larval survival might contribute to fluctuations in recruitment success (Miller et al. 2016), understanding all aspects of the life history of this species is essential.

Adult Japanese eels reach their spawning area in the open ocean after migrating long distances from where they grew in East Asia (Japan, China, Taiwan and Korea). A salinity front located around 15°N in the NEC is thought to represent a landmark for termination of spawning migration of the Japanese eel (Kimura et al. 1994, 2001; Kimura and Tsukamoto, 2006). In the far western tropical Pacific, a salinity front of 34.5 is generated at the boundary of southern low-salinity (< 34.2) water diluted by precipitation and northern high-salinity (> 34.8) water caused by excessive evaporation (Delcroix and Henin 1991; Ando and McPhaden 1997; Delcroix 1998; Delcroix and Picaut 1998; Henin et al. 1998).
The main flow of the NEC westward current occurs between about 10°N and 18°N, but the position of the salinity front can vary within and among years, with spawning occurring south of the front (Tsukamoto 1992; Kimura et al. 2001; Kimura and Tsukamoto 2006). In some months during the spawning season when no distinct front exists (even if lower salinity waters are present), spawning still occurs within lower salinity water (Aoyama et al. 2014). In other years when a distinct front forms, spawning appears to be restricted to waters south of the front (Kimura and Tsukamoto 2006; Tsukamoto et al. 2011; Aoyama et al. 2014). Therefore, although larvae can occur at various latitudes that have different upper-layer water masses with different isotope ratios, the location of the salinity front is an important determinant of spawning. (Kimura and Tsukamoto 2006).

Based on studies of eggs, newly hatched preleptocephali, and spawning-condition adult eels, spawning is considered to occur along the western side of the West Mariana Ridge (Chow et al. 2009, 2010; Kurogi et al. 2011; Tsukamoto et al. 2011; Aoyama et al. 2014) around the new moon in late spring and summer (Ishikawa et al. 2001; Tsukamoto et al. 2003, 2011). After spawning, larvae drift westward within the NEC towards where they will recruit as juveniles and grow. Part of the NEC flow turns to the north along the eastern side of the Philippines to then enter the Kuroshio Current (Fig. 1). If Japanese eel larvae reach this NEC bifurcation zone and are transported into the southward flowing Mindanao Current, they will not reach appropriate habitat to settle in East Asia (Kimura et al. 1994, 1999). Therefore, the latitude of the salinity front, which may move southward during El Niño events (Kimura et al. 2001) might play an important role in the success of larval transport towards East Asia. Transport modelling studies have evaluated the effects of El Niño (or other oceanic changes such as the latitude of the bifurcation) on Japanese eel recruitment (Kim et al. 2007; Zenimoto et al. 2009; Hsu et al. 2017; Chang et al. 2018; Hsiung et al. 2018), but no single factor has been definitively linked to fluctuations in recruitment.

Larval survival and eventual recruitment might also be affected by larval feeding success and growth. Leptocephalus larvae are highly transparent, can grow to large sizes (Smith 1989; Miller 2009), and have an unusual physiology and growth strategy (Bishop and Torres 1999; Pfeiler 1999; Bishop et al. 2000; Pfeiler et al. 2002). Their biology has been difficult to fully understand, partly because their diets and feeding behavior are poorly known. ‘Marine snow’ particulate organic matter (POM) within the intestines of leptocephali off coastal Japan, including amorphous material, numerous appendicularian houses and fecal pellets (Otake et al. 1993; Mochioka and Iwamizu 1996), suggests that this represents an important food source. Similar gut contents have been reported elsewhere (Miller et al. 2011, 2019; Tomoda et al. 2018). Gut contents of European eel leptocephali from the Sargasso Sea contained DNA from a wide range of marine taxa and bacteria, with a high proportion of hydrozoa (Riemann et al. 2010; Ayala et al. 2018), with similar results reported for Japanese eel, giant mottled eel (*Anguilla marmorata*) and several other marine eel leptocephali caught in the western NEC in the western North Pacific (Chow et al. 2019). The diversity of organisms contributing to or colonizing marine snow (Alldredge and Silver 1988; Shanks and Walters 1997; Kiørboe 2000) is consistent with the diversity of molecular sequences of taxa from marine snow in the Sargasso Sea, including hydrozoans (Ayala et al. 2018; Lundgreen et al. 2019). Miller et al. (2020) reviewed gut content studies based on a variety of analyses, such as those using DNA, morphology, and stable isotopes.
Carbon and nitrogen stable isotope ratio ($\delta^{15}$N and $\delta^{13}$C) analyses have been used to study the diets of Japanese eels and other species of leptocephali. Similar analyses have been performed on other zooplanktivorous fish larvae (Pepin and Dower 2007; Wells and Rooker 2009). These types of studies provide information on the trophic level and source of food being consumed by a predator (Deniro and Epstein 1978; Minagawa and Wada 1984; Post 2002). $\delta^{15}$N values reflect trophic positions of prey and $\delta^{13}$C values reflect the characteristics of primary producers at the base of the food web for an area (see Layman et al. 2012).

Isotope ratios of Japanese eel larvae and POM in 2002 revealed differences in isotope ratios either side of the salinity front (Kimura and Tsukamoto 2006). Japanese eel leptocephali may also feed on more POM at depths between 5 and 50m, but sometimes deeper in the NEC (Miyazaki et al. 2011). Anguillid leptocephali (including *A. japonica*) were more abundant near the top of the thermocline at 70–100 m at night (Onda et al. 2017).

Stable isotope $\delta^{15}$N and $\delta^{13}$C values of leptocephali, other food web components, and POM have been studied west of the Mascarene Plateau in the western Indian Ocean (Feunteun et al. 2015), where $\delta^{15}$N values indicated leptocephali fed on marine snow, which contributes to the bulk of POM in this area. Isotope ratios of leptocephali in the western South Pacific (Liénart et al. 2016; Ghinter et al. 2020) and Gulf of Mexico/western North Atlantic (Quattarini et al. 2019) also reported geographically variable isotope ratios. POM isotope ratios differed by depth in two Indo-Pacific studies (Feunteun et al. 2015; Ghinter et al. 2020), possibly affecting the isotope ratios of leptocephali in the event species fed at different depths, similar to what Miyazaki et al. (2011) reported for the western North Pacific. Geographical differences could be also caused by differences in baseline primary producer isotopic composition (Waite et al. 2007; Somes et al. 2010; Ghinter et al. 2020). Fatty acid compositions also differed among some taxa (Deibel et al. 2012; Liénart et al. 2016). Accordingly, describing the diets and feeding behavior of leptocephali must take various factors into consideration.

We examine geographic and ontogenetic variation in carbon and nitrogen stable isotope ratios of Japanese eel larvae to identify any change in diet or baseline isotope ratios in different areas of the NEC. Larvae were sourced from collections made during seven research surveys over more than 10 years, from within and downstream of their NEC spawning area. We also perform stable isotope analyses on newly-hatched non-feeding preleptocephali, whose isotope ratios will more likely be affected by maternal influences, to appreciate better understand isotope ratios of leptocephali after they have commenced feeding.

Our research objectives are to geographically evaluate and compare isotopic data collected from previous studies on Japanese eel larvae (Kimura and Tsukamoto 2006; Miyazaki et al. 2011) with our new data. Because previous studies have not focused on spatial (latitude and longitude) differences and temporal (yearly) fluctuation, we describe geographic characteristics for the survey area, including the downstream region of the NEC and Subtropical Countercurrent (STCC) region. We then compare our dietary data with those reported elsewhere to better understand the diets of these leptocephali. These analyses and the
brief overview of the present state of knowledge on this subject will help guide interdisciplinary research on the ecology of anguilliform eel leptocephali.

**Materials And Methods**

Seven research surveys from 2002–2013 by the R/V *Hakuho Maru* (operated by Japan Agency for Marine-Earth Science and Technology) of the Ocean Research Institute (currently, Atmosphere and Ocean Research Institute) of the University of Tokyo, were conducted to study the spawning area of Japanese eels and their larval migration in the NEC. Survey areas, sampling station transect lines, and locations where larvae reported in this study were collected are shown in Fig. 1. However, the sampling locations were not the same year to year. Each survey included sampling for leptocephali (larval stage) and newly hatched preleptocephali (pre-feeding stage), but not all early life history stages or sizes of larvae were collected during each survey.

Depending on the cruise, sampling was conducted with an Isaacs-Kidd Midwater Trawl (IKMT) with a mouth opening of 8.7 m$^2$ or a newly designed 3 m ORI net with a mouth opening of 7.1 m$^2$, both with a 0.5 mm mesh, to depths of about 300 m. Except for 2013 (see Onda et al. 2017), most sampling was conducted just west of about 143°E along the western side of the West Marina Ridge seamount chain (*e.g.*, Tsukamoto et al. 2011) where is a suspected Japanese eel spawning area. In 2013 the survey occurred after the main spawning season, and sampled for larger eel larvae across a wider region west of the spawning area. Sampling occurred along 137°E during all surveys, with several long transects sampled in 2013 (Onda et al. 2017). Because Japanese eels during the spawning season only spawn during the new moon of each month (Ishikawa et al. 2001; Tsukamoto et al. 2003), our non-preleptocephalus larvae mostly collected in transects west of spawning longitudes, spawned at least a month before preleptocephalus catches reported in previous surveys (Tsukamoto 2006; Tsukamoto et al. 2011).

Conductivity, temperature and depth measurements (CTD) were made during surveys, but not always in long transects, or from equally spaced stations. Accordingly, we used modified pre-plotted high-resolution sections of salinity for the Japan Meteorological Agency’s (JMA) 137°E repeat-section obtained from the JMA website (https://www.data.jma.go.jp/gmd/kaiyou/db/mar_env/results/OI/137E_OI_e.html). The JMA 137°E repeat-section provides detailed oceanographic data for summer and winter from 1967 onward (Oka et al., 2018). We used sections from summer for those years in which our surveys were conducted to enable a comparison of hydrographic structure among years (Fig. 2). Because most leptocephali were not collected at the exact same time and longitude of the JMA hydrographic section, direct comparison of leptocephalus isotope ratios and sampling locations with exact locations of the salinity fronts would have some error. However, the JMA 137°E data is a long distance and long term observational data covering whole of the spawning area, and can describe distribution of water mass and
movement of the salinity front. This front occurs at the northern edge of the low salinity water in the upper 100 m where it is associated with a salinity of 34.5 (red lines in Fig. 2).

In addition to newly reported leptocephalus larvae from a 2013 research survey, we report isotope data from larvae collected in two previous studies, a 2002 research survey (Kimura and Tsukamoto 2006) and research surveys from 2004–2009 (Miyazaki et al. 2011). These data are used to determine spatial and temporal variation in Japanese eel larval diet throughout the NEC.

Upon collection, leptocephali were identified, their total length measured, and they were frozen at −80°C. Subsequently, in the Atmosphere and Ocean Research Institute research laboratory at the University of Tokyo, leptocephali were ground to a fine powder using a spatula after drying in an oven at 60°C for 24 h. Carbon and nitrogen stable isotope ratios were then determined using 0.5–1.0 mg of each sample in an elemental analyzer interfaced with a mass spectrometer (without de-lipidization). We express isotope ratios as per mill (‰) deviation according to international standards of Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N\textsubscript{2} for nitrogen, for which \( \delta^{13}C \) or \( \delta^{15}N = (R_{\text{sample}} / R_{\text{standard}} − 1) \times 1000 \) where \( R = ^{13}C / ^{12}C \) or \( ^{15}N / ^{14}N \). Measurement error was within ± 0.25‰ for both \( \delta^{13}C \) and \( \delta^{15}N \) analyses. Differences of larval isotope ratios among years, sizes, and spatially separated groups were tested using ANOVA followed by pairwise Tukey tests if data were normally distributed with equal variances, or Kruskal-Wallis tests followed by Dunn's tests otherwise. Linear regressions were performed on isotope ratio data and were tested for significance using null hypothesis significance tests. Because it was not possible to directly match isotope ratios of leptocephali with precise latitudes of the salinity front for each sample, we separated larvae into basic latitude and longitude groups based on their overall distribution. The detailed separation of the groups were explained in results.

Of 130 pre-leptocephali collected in the 2005 survey (Tsukamoto 2006) we randomly selected 30 for analysis. Because preleptocephali were too small to be individually analyzed for stable isotope ratios, samples were pooled. In 2009 (Tsukamoto et al. 2011) more than 100 pre-leptocephali were collected from the same areas (around 12°30′–13°N and 141–141°30′E, south of the salinity front), from which we randomly selected 50 individuals, which we pooled for analysis into two samples of 25 individuals. Table 1 details the numbers, total lengths, and collection details of leptocephali used in stable isotope analyses, at sites shown in Fig. 1.

**Results**

*Hydrographic structure and larval collections*

JMA hydrographic sections along 137°E indicated similar vertical water mass structures occurred each year during July in the subtropical gyre. Higher salinity water occurred in the upper 400 m in the north, and a more saline core of water (Subtropical Underwater (STUW) (see Schabetsberger et al. 2016) or Pacific Tropical Water) that had been subducted into the thermocline occurred at around 200 m at spawning latitudes within the NEC. The surface layer above the STUW was low in salinity and formed the
salinity fronts at its northern edge. The 34.5 isohaline reached the surface at various latitudes over 7 years of sampling, with the 50 year mean location at 16–17°N (Fig. 2).

During summer of 2002 in the JMA section the main part of the salinity front occurred between about 12–13°N (Kimura and Tsukamoto 2006), and the 34.5 isohaline reached the surface at about 15°N (Fig. 2). The smallest leptocephali were caught at 12°N (8–11 mm total length (TL)) at the front, with larger larvae to 32 mm length caught at higher latitudes (Fig. 3a), albeit within a narrow longitudinal range (Fig. 1b).

In summer of 2004 in the JMA section the salinity front extended further north to about 16°N, with some low-salinity eddies extending even further (Fig. 2). Sampling occurred at a variety of longitudes and latitudes either side of the front in 2004, with 9–21 mm TL larvae caught at 15°N, 10–13 mm TL larvae at 17°N and fewer variably sized larvae, including two of 25–27 mm TL, caught at latitudes from 14.5–16.5°N (Fig. 3b).

Most sampling in 2005 occurred in June and July in the eastern NEC spawning area. Leptocephali were caught from 13–15.5°N at sizes between about 10 and 20 mm TL (Fig. 3c) south of the salinity front, which, in the JMA section, occurred near 18°N (Fig. 2). A similar situation occurred in July and August 2006 when the salinity front was in the north. All but two larvae were caught at two stations at 15°N along 137°E, with others caught further east at 133.5°E (Fig. 1e). All larvae exceeded 15 mm TL (Fig. 3d).

In 2008 the 34.5 isohaline extended north beyond 20°N (Fig. 2), with all but four larvae of 13–18 mm TL being collected at 13.5°N along 137°E on 25–26 June. In summer the front was located at 18°N when the 137°E line was surveyed; two 24 mm TL larvae were caught much further west at latitudes of 11°N and 12°N (Fig. 1f, 3e). In contrast, in 2009 the main front was at 10°N, but low-salinity water occurred further north in the JMA line (Fig. 2). All but three leptocephali were collected further east at 13°N along 140°E, of 9–12 mm TL (Fig. 1g, 3f). A larger 18 mm TL larva was collected along 137°E at 14.5°N.

Sampling in November 2013 occurred outside of the Japanese eel spawning season, and only included standardized widely spaced transects (Onda et al. 2017). Because of the seasonal difference and possible movement of typhoons through the NEC area, no salinity front occurred during this survey (Onda et al. 2017), although a front occurred at 13°N in summer in the JMA section. Collected leptocephali included a 41 mm TL larva retained in northern waters at 16.5°N close to the spawning area at 142°E, a 29 mm TL larva at 14°N, 137°E, and 48, 46, and 26 mm TL larvae from 16.5°N, 15.5°N, and 14°N, respectively (Fig. 1h), and four even larger larvae of 55–56 mm TL caught at a northwestern station (23°N, 129°E) in the STCC (Fig. 1a).

Geographic variation in isotope ratios

When the 154 larvae from all years (except 2013) were combined there was a significant tendency for $\delta^{15}N$ values to be higher in the south, as shown by a linear regression ($P < 0.01$) (Fig. 4a). This trend was partly caused by isotope ratios of larvae collected in 2002, which were spread across a wide latitudinal
range at or near 137°E, with smaller larvae at 12°N in the south having higher $\delta^{15}$N values than many larger larvae; the highest value was from a 32 mm TL larva caught at 16°N (Fig. 5a, b). Larvae were located from and east of 137°E in 2004, and showed a slightly increasing but significant trend in $\delta^{15}$N values from north to south ($P = 0.019$); 19 larvae (9–20 mm TL) from 15°N had widely ranging values (3.0‰ to 6.4‰) (Fig. 5c, d). Small larvae from 17°N had relatively low isotope ratios compared with some larger larvae from 15°N. A more significant decreasing trend in isotope ratios was apparent in 2005, that partly reflected larger northern larvae having lower isotope ratios than many smaller southern larvae ($P < 0.01$) (Fig. 5e, f). Fewer larvae in 2008 showed this same pattern, with two southern larvae from 11°N and 12°N having higher $\delta^{15}$N values than those from 13–15°N (Fig. 4a). Surveys in 2006, 2009, and 2013 did not catch larvae across a sufficiently wide range of latitudes to be comparatively useful (Fig. 3).

There was a significant tendency for $\delta^{15}$N values to be higher in the south (Fig. 4a) and for larvae from 2002 and 2004 to be located over the salinity front (Fig. 2). $\delta^{15}$N values north and south of the salinity front located 14.5°N in 2002 and 16.0°N in 2004 differed significantly ($P < 0.05$), meaning that the salinity front caused difference of the characteristics.

Leptocephalus $\delta^{13}$C values from each survey were not significantly related to the latitude at which they were captured ($P = 0.89$), except that values in 2013 decreased with increasing latitude (Fig. 4b). For example, 2002 larvae were caught from 12–17°N, but larvae of similar $\delta^{13}$C (−20‰ to −21‰) values occurred across that latitudinal range. A wide range of $\delta^{13}$C values occurred in the 19 larvae caught at two stations from 15°N in 2004 (−20.1‰ to −21.8‰), with the lowest values occurring in 9 and 12 mm larvae caught at 139.5°E.

Relationships between larval $\delta^{15}$N values and collection longitude were not significant ($P = 0.012$, but very low $R = 0.20$) (Fig. 4c). Some patterns, such as a narrow range of $\delta^{15}$N at 141°E and 142°E (5.1‰ to 6.4‰) and a wide range at 139.5°E (3.1‰ to 6.1‰) in 2004, were also seen in other years. The lowest $\delta^{13}$C values occurred in 2013 at 131°E and 129°E where the larvae were large in size, but significant relationships were not recognized ($P = 0.78$) (Fig. 4d). The degree of variability in isotope ratios and lack of significant relationships with longitude or latitude for $\delta^{13}$C suggest that several factors might be contributing to geographic patterns.

Variation in isotope ratios depending on larval size

Plots of $\delta^{15}$N and $\delta^{13}$C values from different larval sizes reveal variable isotope values for each size range (Fig. 6). In 2004 both $\delta^{15}$N and $\delta^{13}$C values tended to increase with size ($P < 0.01$), however larvae in 2002 and from 2005–2009 both values were not significantly related to length ($P > 0.01$). Seven large larvae exceeding 40 mm TL in 2013 had $\delta^{15}$N values between 5‰ and 6‰, but $\delta^{13}$C values decreased with increasing length ($P < 0.01$). $\delta^{15}$N and $\delta^{13}$C values of larvae pooled for 2002–2009 were not significantly related to length ($P > 0.01$), suggesting that no obvious relationship existed between leptocephalus size and either $\delta^{15}$N or $\delta^{13}$C, except for $\delta^{13}$C values of larvae exceeding 40 mm TL. Six of
the seven larger larvae were collected downstream of the NEC along the 131°E line and in the STCC. It seems that δ\textsuperscript{13}C values decreased with both leptocephalus total length and distance from the spawning area.

Isotope ratios of ~ 5 mm TL preleptocephali (~ 2–3 days old) likely resemble maternal isotope ratios because feeding had not commenced, and they differed from leptocephali (Fig. 6). Preleptocephalus δ\textsuperscript{15}N values of 11.3‰ to 14.3‰ (2005, n = 30, 11.3‰; 2009 [2 samples, each n = 25], 12.3‰, 14.3‰) were much higher than those of leptocephali (almost all < 8‰). δ\textsuperscript{13}C values of preleptocephali, −21.4‰ to −22.5‰ (2005, n = 30, −21.5‰; 2009 [2 samples, each n = 25], −21.4‰, −22.5‰), overlapped with the lower range of larvae from several years or with the lowest values of the largest 2013 larvae.

**Combinations of isotope ratios**

Due to irregular sampling strategies among surveys and variable patterns in catch of larvae of various sizes at different latitudes and longitudes, the combined δ\textsuperscript{15}N and δ\textsuperscript{13}C values of all larvae were examined (Fig. 7). Among years (Fig. 7a) there were significant differences for δ\textsuperscript{15}N (\(F = 5.144, P < 0.001\), ANOVA) and δ\textsuperscript{13}C values (\(H = 85.037, P < 0.001\), Kruskal-Wallis). In 2002, δ\textsuperscript{15}N values differed from those in 2004 and 2006 (\(P < 0.05\), Tukey tests), and δ\textsuperscript{13}C values differed from those in 2004, 2005, 2008 and 2013 (\(P < 0.05\), Dunn's tests). Results indicate that there was a tendency for higher δ\textsuperscript{13}C and relatively high δ\textsuperscript{15}N values in 2002 (\(n = 18, TL = 18.8 \pm 8.9\) mm (mean ± SD); δ\textsuperscript{15}N = 5.6 ± 0.8‰, δ\textsuperscript{13}C = −20.1 ± 0.2‰). In 2006, δ\textsuperscript{15}N values differed from those in 2002, 2005 and 2013 (\(P < 0.05\), Tukey tests), and δ\textsuperscript{13}C values differed from those in 2005 and 2013 (\(P < 0.05\), Dunn's tests). The 2006 values (\(n = 16, TL = 24.1 \pm 5.7\) mm; δ\textsuperscript{15}N = 4.7 ± 0.6‰, δ\textsuperscript{13}C = −20.6 ± 0.2‰) shifted to lower δ\textsuperscript{15}N values. δ\textsuperscript{13}C values in 2013 (\(n = 9, TL = 46.3 \pm 11.7\) mm; δ\textsuperscript{15}N = 6.5 ± 1.4‰, δ\textsuperscript{13}C = −21.5 ± 0.3‰) shifted to lower values which differed from those in 2004 (\(n = 34, TL = 15.7 \pm 3.7\) mm; δ\textsuperscript{15}N = 5.3 ± 0.8‰, δ\textsuperscript{13}C = −20.6 ± 0.4‰, \(P < 0.05\), Dunn's tests) and 2009 (\(n = 12, TL = 11.5 \pm 2.4\) mm; δ\textsuperscript{15}N = 5.4 ± 0.4‰, δ\textsuperscript{13}C = −20.6 ± 0.2‰, \(P < 0.05\), Dunn's tests), including in 2002 and 2006. δ\textsuperscript{13}C values in 2005 (\(n = 61, TL = 15.2 \pm 1.3\) mm; δ\textsuperscript{15}N = 6.0 ± 0.7‰, δ\textsuperscript{13}C = −21.1 ± 0.2‰) differed from those in 2004 and 2009 including in 2002 and 2006 (\(P < 0.05\), Dunn's tests). These results suggest that 2008 values (\(n = 13, TL = 18.1 \pm 4.5\) mm; δ\textsuperscript{15}N = 5.3 ± 0.8‰, δ\textsuperscript{13}C = −20.9 ± 0.2‰) overlapped considerably with another year because there were no significant differences without δ\textsuperscript{13}C values in 2002.

Larvae from different years separated into three TL size groups (small: 8–19 mm [\(n = 125, 14.5 \pm 2.4\) mm]; medium: 20–29 mm [\(n = 26, 24.4 \pm 3.3\) mm]; large: 30–56 mm [\(n = 12, 43.8 \pm 10.8\) mm]) completely overlapped ranges of δ\textsuperscript{15}N values and partially overlapped δ\textsuperscript{13}C values (Fig. 7b). There were higher proportions of lower δ\textsuperscript{13}C values in small sized larvae (δ\textsuperscript{15}N = 5.4 ± 0.8‰, δ\textsuperscript{13}C = −20.8 ± 0.4‰) compared with medium sized larvae (δ\textsuperscript{15}N = 5.5 ± 1.2‰, δ\textsuperscript{13}C = −20.5 ± 0.4‰), but large sized larvae (δ\textsuperscript{15}N = 5.4 ± 0.8 ‰, δ\textsuperscript{13}C = −21.4 ± 1.0‰) included individuals with the lowest δ\textsuperscript{13}C values (leptocephali from 2013). δ\textsuperscript{15}N values did not significantly differ (\(F = 0.193, P = 0.82\), ANOVA), but δ\textsuperscript{13}C
values did ($H = 11.968, P < 0.003, \text{Kruskal-Wallis}$), with medium sized larvae differing from both small and large sized larvae ($P < 0.05, \text{Dunn's test}$).

All larvae were separated by latitudinal groups (south: $11–13.5^\circ$N [$n = 73, \text{TL} = 14.6 \pm 3.3 \text{mm}$]; north: $14–17^\circ$N [$n = 86, \text{TL} = 19.6 \pm 7.8 \text{mm}$]; northwest: $23^\circ$N [$n = 4, \text{TL} = 56.2 \pm 0.8 \text{mm}$]) based on separating the larvae into two approximately equal groups of numbers of samples and latitudinal distance. In addition, since the latitude separating northern and southern groups is similar to the 40 year average location of the salinity front (Kimura et al., 2001), the separation would be appropriate (Fig. 7c). $\Delta^{13}$C values heavily overlapped (north = $-20.7 \pm 0.4 \%\text{o}$, south = $-20.8 \pm 0.4 \%\text{o}$) except for larvae in 2013 from the far northwestern area which had lower $\Delta^{13}$C values. $\Delta^{15}$N values also overlapped (north = $5.1 \pm 0.1 \%\text{o}$, south = $5.9 \pm 0.8 \%\text{o}$), but there were more low values among larvae collected in the north, with most of the highest values being found in larvae caught in the south. North and south larvae $\Delta^{15}$N values differed significantly ($P < 0.001, \text{t-test}$), as did $\Delta^{13}$C values ($P < 0.03, \text{t-test}$).

Patterns in isotope ratios separated into three longitudinal groups (west: $129–133.5^\circ$E [$n = 14, \text{TL} = 36.1 \pm 4.3 \text{mm}$]; central: $137–138.25^\circ$E [$n = 50, \text{TL} = 19.1 \pm 7.3 \text{mm}$]; east: $139–142^\circ$E [$n = 99, \text{TL} = 15.3 \pm 3.8 \text{mm}$]) (Fig. 7d) that generally reflected patterns based on size (Fig. 7b). Longitudinal groups were classified roughly into: 1) a region close to the spawning area, 2) a downstream region west of the spawning area where spawning does not occur, and 3) a region further downstream that was close to the bifurcation of the NEC. In general, eastern larvae ($\Delta^{15}$N = $5.5 \pm 0.8 \%\text{o}$, $\Delta^{13}$C = $-20.8 \pm 0.4 \%\text{o}$) corresponded to the small larval group, central larvae corresponded to the medium larval group ($\Delta^{15}$N = $5.2 \pm 0.9 \%\text{o}$, $\Delta^{13}$C = $-20.6 \pm 0.5 \%\text{o}$), and western larvae corresponded to the large larval group ($\Delta^{15}$N = $5.9 \pm 1.1 \%\text{o}$, $\Delta^{13}$C = $-21.1 \pm 0.9 \%\text{o}$), although some larger larvae were retained in the east, or smaller larvae were caught further west than most similarly sized individuals. $\Delta^{15}$N values differed significantly ($F = 3.594, P < 0.03, \text{ANOVA}$), but only west and central larvae differed ($P = 0.03, \text{Tukey test}$). Differences in $\Delta^{13}$C values were stronger ($H = 12.815, P < 0.002, \text{Kruskal-Wallis}$), and central larvae differed from both west and east larvae ($P < 0.05, \text{Dunn's test}$). Combined N-C isotope ratios showed the majority of small larvae comprised individuals from southern and eastern areas (Fig. 7b, c, d).

### Discussion

**Geographic variation in leptocephalus isotope ratios**

Our analyses of Japanese eel leptocephali collected from various latitudes and longitudes, but mostly in the NEC, over seven surveys in different years provide a unique opportunity to compare their isotope ratios. Larvae were collected within spawning areas along the western side of the West Mariana Ridge seamount chain, downstream in the NEC, and in the STCC region to the northwest. $\Delta^{15}$N values show significant relationships with latitude, with higher values to the south where larvae also tended to be smaller. This relationship was significant for all larvae pooled together, and for larvae from 2002, 2004 and 2005 in particular. Longitude and larval size showed no clear relationships with $\Delta^{15}$N values,
although larger larvae collected in the western region in November 2013 (several months later than when other larvae were collected) had lower $\delta^{13}C$ values (distinctly lower for the STCC region). Since $\delta^{15}N$ values reflect trophic level and $\delta^{13}C$ values are related to carbon sources within food webs (Layman et al. 2012), these results suggest that differences exist in the isotope ratios of marine snow consumed by larvae as they grow during their westward transport in the NEC and STCC regions. However, different baseline isotope ratios might exist in regions such as the STCC, and larvae may feed at different depths as they grow larger.

The higher $\delta^{15}N$ values of leptocephali in more southern latitudes may be related to several factors. For example, the salinity front that determines the latitude at which spawning of Japanese eels occurs (Tsukamoto 1991; Kimura et al. 2001; Kimura and Tsukamoto 2006; Aoyama et al. 2014) might separate surface layer water masses with different baseline isotope ratios. However, since the front shifts to different latitudes at different times within and between years, this probably introduces variability into the overall analysis of isotope ratios.

The hydrographic sections (Fig. 2) suggest that the basic oceanographic structure of this region is relatively stable. The unbroken westward flow of the NEC typically extends to about 16–17°N (Kaneko et al. 1998; Oka et al. 2018), so water masses north of the salinity front are likely influenced by eastward countercurrent flows, including the STCC (Qiu and Chen 2010). The location of the salinity front may be an important because $\delta^{15}N$ baseline values and those of POM can vary geographically (Waite et al. 2007; Somes et al. 2010), influencing the isotope ratios of species such as tuna which feed at different latitudes in each region (Estrada et al., 2005; Ménard et al. 2007; Lorrain et al. 2015), or at larger scales in different ocean regions such as for squid (Takai et al. 2000). Similarly, different $\delta^{15}N$ values of leptocephali, POM and other food web components reported for the western South Pacific at higher and lower latitudes correspond to different current systems, including the South Equatorial Current (Liénart et al. 2016; Ghinter et al. 2020).

Within our study area, isotope ratio analyses of different size classes of zooplankton and POM in the western North Pacific (120–135°E) were also found to vary according to latitude and corresponding current systems (Yang et al. 2017). $\delta^{15}N$ values in the NEC at mid-latitudes, and in the northern Equatorial Countercurrent at the lowest latitudes, were consistently higher than isotope ratios within the STCC to the north (Yang et al. 2017). Results of Yang et al. (2017) are similar to the lower $\delta^{15}N$ values of leptocephali in our northern survey areas.

Yang et al. (2017) suggested that a greater abundance of nitrogen-fixing cyanobacteria (*Trichodesmium* spp.) in the STCC in the western North Pacific may be primarily responsible for lower $\delta^{15}N$ values at northern latitudes. $\delta^{15}N$ values of zooplankton also appear to be affected by *Trichodesmium* abundance across the North Atlantic (Mompean et al. 2013). Spatial differences in *Trichodesmium* abundance were also suggested to influence isotope ratios in leptocephali and other species in the western South Pacific (Ghinter et al. 2020). Kimura and Tsukamoto (2006) reported changed POM $\delta^{13}C$ values across the
salinity front in 2002. It possibly relates to cyanobacteria. This suggests that different baseline isotope ratios exist in NEC northern and southern areas that might roughly correspond with the latitude of the salinity front, but other factors should also be considered.

Isotope ratios of preleptocephali compared with leptocephali

In contrast to leptocephali, the distinctly different isotope ratios of preleptocephali from the spawning area would not likely be related to baseline isotope ratios of the oceanic environment in which they were collected. Newly hatched 2005 and 2009 pre-feeding stage preleptocephali had much higher $\delta^{15}N$ and mostly lower $\delta^{13}C$ values than did leptocephali that had been feeding in the NEC. Because migrating silver eels do not feed (Chow et al. 2010) and isotope ratios are transmitted to offspring (Starrs et al. 2014), the preleptocephalus isotope ratios resemble maternal ratios and the continental habitats in which they lived. Accordingly, isotope ratios of the preleptocephali were within ranges of juvenile eels living in Japan (Chow et al. 2010). Based on otolith Sr stable isotope analyses, adult Japanese eels captured in the spawning area also appear to have originated from Japan (Otake et al. 2019). According to surveys across wide areas of Japan, many Japanese eels inhabiting rivers and lakes are eels that have been released by fisheries cooperatives for stock conservation purposes (Yoneta et al. 2019). Therefore, if the origin of these eels affects larval recruitment and abundance, studies on the contribution of stocked eels to natural reproduction not only in Japan but also in East Asia would be a very important research topic.

Regardless of the origin of spawning adults, larvae quickly assimilate isotope ratios of the food web wherein they feed in the NEC. Leptocephali of 9–10 mm TL already have lower $\delta^{15}N$ (> 50% lower) and mostly higher $\delta^{13}C$ ratios than preleptocephali (~ 5 mm TL). Our leptocephali were usually at least 1 month in age, with the largest having been spawned several months earlier based on new moon spawning dates and estimated larval growth rates of ~ 0.5 mm/day (Ishikawa et al. 2001; Kuroki et al. 2014). According to several larval transport modelling studies, after being spawned along the seamount ridge the larvae are transported westward by the NEC (Kimura et al. 1999; Kim et al. 2007; Zenimoto et al. 2009; Hsu et al. 2017; Chang et al. 2018; Hsiung et al. 2018), although some larvae are retained near the spawning area, especially in northern waters (as for some larvae in 2013). Therefore, those larvae that we analyze were likely spawned at various latitudes and had different larval transport histories.

Possible factors affecting isotope ratios of leptocephali

In addition to the geographic factors discussed above, other factors such as the depth at which leptocephali feed, and differences in POM among depth ranges could affect leptocephalus isotope ratios. The depths at which leptocephali feed are not known, but some species perform diel vertical migrations (DVM) from deeper depths during the day to shallower depths at night (Castonguay and McCleave 1987; Otake et al. 1998). Therefore, because POM can have different isotope ratios at different depths within the upper few hundred meters of the ocean (e.g., Miyazaki et al. 2011; Feunteun et al. 2015; Ghinter et al. 2020), the depths at which leptocephali feed could influence isotope ratios. Miyazaki et al. (2011) compared those Japanese eel larvae from 2004–2009 that we include in this study to POM collected at
various depths. Various patterns in POM isotope ratios occurred at several depths each year, including some cases where clearly different isotope ratios occurred between depths such as at 50 m and 150 m as shown in Fig. 8. Isotope ratios of leptocephali collected at these locations in 2008 and 2009 were similar to POM at 50 m depth in having mostly higher $\delta^{15}$N and $\delta^{13}$C values. POM at 150 m tended to have higher $\delta^{15}$N and lower $\delta^{13}$C values, as observed along 140°E in 2008 (Fig. 8c), and POM at 5 m was similar to that at 50 m (Miyazaki et al. 2011). Lower POM $\delta^{13}$C values at 150 m and lower $\delta^{13}$C values for larger leptocephali in 2013 might be related to increased DVM behavior of larger anguillid leptocephali (Castonguay and McCleave 1987).

Further east in the North Pacific subtropical gyre, POM $\delta^{15}$N was lowest near the surface (< 2‰ above 50 m), and values were higher (> 8‰) below about 120 m (Hannides et al. 2013). Similarly, in the southeastern North Pacific below about 100 m, high $\delta^{15}$N values occurred (Williams et al. 2014). POM in the western South Pacific also had higher $\delta^{15}$N at depths ranging 200–260 m compared with the chlorophyll maximum layer or at the surface, although surface POM had distinctly higher $\delta^{13}$C values (Ghinter et al. 2020).

Variation in POM isotope ratios at different depths might explain differences in isotope ratios among taxa or sizes of leptocephali within an area if they feed at different depths (Miyazaki et al. 2011; Feunteun et al. 2015; Ghinter et al. 2020). Possibly related to this is a recurring pattern in leptocephalus taxa isotope ratios, designated Group 1 with high $\delta^{15}$N and low $\delta^{13}$C (including the families Anguillidae, Congridae, Muraenidae, and Serrivomeridae) and Group 2 with low $\delta^{15}$N and high $\delta^{13}$C (including species with large larvae in the Nemichthyidae (Avocettina, Nemichthys) and Ariosoma-type leptocephali in the congrid subfamily Bathymyrinae) (Feunteun et al. 2015). Isotope ratios of Japanese eel larvae and Ariosoma (of Miyazaki et al. 2011) are consistent with these two groupings (Fig. 9). These two groups were also reported by Onda (2017) for a wider range of taxa collected in 2013, including the Muraenidae and Congridae (excluding Ariosoma) of Group 1 and Nemichthys and Ariosoma of Group 2 (as shown in Fig. 9). Average $\delta^{15}$N and $\delta^{13}$C values for all POM analyzed in the NEC region from 2004–2009 by Miyazaki et al. (2011) suggests that Group 2 leptocephali may feed at shallower depth than those of Group 1, including Japanese eel larvae. These same two groups were also reported from the western South Pacific (Liénart et al. 2015; Ghinter et al. 2020) and Gulf of Mexico in the western North Atlantic (Quattarini et al. 2019).

Consideration of lower trophic levels in oceanic food webs

Leptocephali might be directly linked to lower trophic levels of oceanic food webs because they consume marine snow. In Japanese near-shore waters, leptocephalus gut contents included discarded appendicularian houses, zooplankton fecal pellets, and amorphous material (Otake et al. 1993; Mochioka and Iwamizu 1996). Similar gut contents have been reported from leptocephali elsewhere, including in the NEC (Miller et al. 2011, 2019; Tomoda et al. 2018). These discarded objects represent marine snow, but they likely aggregate with other materials. Amino acid isotope analysis of small Japanese eel
leptocephali from the NEC revealed their trophic position to be low, consistent with feeding on marine snow (Miller et al. 2013). Stable isotope analyses comparing various leptocephali taxa and zooplankton in the western Indian Ocean also indicate the trophic level of leptocephali to be below zooplankton (Feunteun et al. 2015).

Marine snow can originate from a variety of sources, and be colonized by various organisms (Alldredge and Silver 1988; Shanks and Walters 1997; Kiørboe 2000), leading to differences in isotopic values. Next Generation DNA sequencing (NGS) of leptocephalus gut contents from the Sargasso Sea (Ayala et al. 2018) and our western study area (Chow et al. 2019) revealed a wide range of eukaryotic and prokaryotic taxa to be ingested. An NGS study on gut contents of European eel leptocephali and an earlier study on this species’ larvae (Riemann et al. 2010) reported high proportions of hydrozoan DNA sequences, but mostly calycophoran siphonophores, which are most abundant in the Sargasso Sea (Ayala et al. 2018; Lüskow et al. 2019). Although Chow et al. (2019) reported possible contamination of these larval gut samples from the Sargasso Sea, they also reported hydrozoan DNA sequences from leptocephalus gut contents in the western North Pacific. These siphonophores occur widely in the upper few hundred meters of the water column in subtropical gyres, including in the NEC (Lo et al. 2012), and they have a temporary reproductive stage that dies after releasing eggs (Carré and Carré 1991) which could contribute to marine snow and possibly be ingested by leptocephali (Miller et al. 2020).

Siphonophore sequences occurred in large marine snow particles in the Sargasso Sea (Ayala et al. 2018, Lundgreen et al. 2019), but the occurrence of their DNA in leptocephalus gut contents does not mean that their nutritional value is greater than any other component of marine snow. For example, marine snow can include transparent exopolymer particles (TEP) that contain carbohydrates (Passow 2002; Skoog et al. 2008) that act as a glue to facilitate particle aggregation, which are highly likely to be nutritious. Marine snow can also contain heterotrophic and high fatty acid content thraustochytrid protists (Li et al. 2013; Bochdansky et al. 2017), which may have occurred in Sargasso Sea leptocephalus gut contents (Miller et al. 2019). Therefore, marine snow composition depends on the materials being produced at a given time and location, and the isotope ratios of leptocephali would vary accordingly.

Conclusion

Using stable isotope ratios of nitrogen and carbon we report the food source for Japanese eel larvae, marine snow, to potentially differ spatially (geographically and bathymetrically) and temporally either side of the salinity front, on the eastern and western sides of the NEC. Larger larvae collected in the STCC have different $\delta^{15}$C values, possibly related to geographic differences, including more intense ontogenetic DVM behavior (Castonguay and McCleave 1987). However, our data reveal no definitive evidence for geographic differences in feeding behavior of larger larvae. Further studies on levels and types of primary productivity, community structure, and types of marine snow present in each area or year are needed to determine those factors that contribute to differences in stable isotope ratios. $\delta^{15}$N baseline values may differ either side of the salinity front as also reported by Yang et al. (2017).
Nutrient levels might influence the balance between prokaryotic cyanobacteria and eukaryotic phytoplankton abundance (see Miller et al. 2016), which could affect the spatial and temporal abundance and composition of marine snow. Seasonal cycles or regional variations in primary or TEP production, or the abundance of zooplankton taxa contributing to marine snow, could also affect the isotope ratios of Japanese eel leptocephali.

Little is known of marine snow TEP composition or the feeding preferences of any leptocephali, such as their dietary preferences or depths at which they feed. While artificially reared Japanese eel leptocephali can ingest phytoplankton exudate TEP (Tomoda et al. 2015) and ocean-collected POM (Chow et al. 2017), and first-feeding larvae can eat rotifers (e.g., Tanaka et al. 1995), their natural feeding preferences are unknown. Gut content analysis of a complete size range of Japanese eel leptocephali is necessary to determine ontogenetic and/or geographic differences in their diet, preferably using a variety of techniques, such as microscopic, NGS, and new chemical analyses to determine ingested materials. This information can then be compared with the biological and oceanographic characteristics of the spawning area and areas downstream in the NEC and STCC where larvae are transported and grow. These studies will help identify the diets and feeding behavior of Japanese eel leptocephali, and by through a better understanding of its life history, contribute to its conservation.

Declarations

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Author contributions SK developed the concept of the study, KT and SK led the research cruises that collected the leptocephali, SK, TK, and YM managed research efforts on the stable isotopic compositions of leptocephali that were conducted by SM and HO, SK drafted the manuscript with the assistance of MJM, and the authors participated in the cruises and critically revised the review.

Compliance with ethical standards

Conflict of interests The authors declare they have no conflict of interests. Funding was provided by the Japan Society for the Promotion of Science (JSPS).

Ethical approval All applicable national and/or institutional guidelines for sampling for the study have been followed.
Data availability statement  The stable isotope ratio data in this manuscript are available from the corresponding author upon reasonable request.

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**Table**

**Table 1.** Survey and leptocephalus sampling data: \( n = \) number of specimens, \( TL = \) leptocephalus total length, \( NEC = \) North Equatorial Current, \( STCC = \) Subtropical Countercurrent.

| Year | Cruise   | Sampling area | Sampling date       | \( n \) | TL (mm) |
|------|----------|---------------|---------------------|-------|---------|
| 2002 | KH-02-2  | NEC           | 5 Jul.–15 Aug.      | 18    | 8.5–32.9|
| 2004 | KH-04-2  | NEC           | 13 May–6 Jul.       | 34    | 9.3–27.0|
| 2005 | KH-05-1  | NEC           | 27 May–16 Jul.      | 61    | 11.7–18.4|
| 2006 | KH-06-2  | NEC           | 26 Jun.–5 Sep.      | 16    | 16.7–38.3|
| 2008 | KH-08-1  | NEC           | 21 May–14 Jul.      | 13    | 13.5–28.0|
| 2009 | KH-09-1, 2 | NEC       | 14 Apr.–3 Jun.      | 12    | 9.1–18.2|
| 2013 | KH-13-6  | NEC           | 17 Oct.–28 Nov.     | 5     | 26.3–46.4|
| 2013 | KH-13-6  | STCC         | 17 Oct.–28 Nov.     | 4     | 55.0–56.9|

**Figures**
Figure 1

Survey area and collection sites of Japanese eel leptocephali: North Equatorial Current (NEC); southward flowing Mindanao Current (MC); and northern branch of the NEC that becomes the Kuroshio Current (a), where Japanese eel leptocephali from each year were collected (b–h). The rectangle and dashed line in (a) depict the area included in (b–h) and the 137°E repeat CTD observational line of the Japan Meteorological Agency (JMA) corresponding to sections in Fig. 2. Red circles (main map) and squares (individual years) in all panels indicate locations where leptocephali were collected. Thin lines indicate observational cruise lines. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

Hydrographic section plots of salinity along the JMA 137°E repeat CTD observational line in summer for survey years, and the 50 year mean. Red lines show the surface layer 34.5 isohaline associated with the salinity front, or low salinity water that appears to affect where Japanese eels spawn; red arrowheads mark the northern locations where the 34.5 isohaline reaches the surface; black rectangles show latitudes where Japanese eel larvae were collected. Sections modified from imagery plotted by JMA. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 3

Plots of capture latitudes and larval sizes used for stable isotopic analysis. Fewer larvae were included from 2013 and some were caught at higher latitudes in the northwest (Fig. 1) for which capture locations are described in the text.
Figure 4

Relationships between nitrogen (δ¹⁵N) and carbon (δ¹³C) stable isotope ratios of Japanese eel leptocephali collected in all years in relation to latitude of capture (a, b) and longitude (c, d). The regression lines exclude larvae collected in 2013, which include small numbers of larvae collected outside the main sampling area in a different season.
Figure 5

Relationships between nitrogen stable isotope ratios ($\delta^{15}N$) of Japanese eel leptocephali and latitude (top panels), and between total length and latitude (bottom panels) for 2002, 2004 and 2005, during which larvae were caught over a wide latitudinal range.
Figure 7

Plots of nitrogen (δ15N) and carbon (δ13C) stable isotope ratios for the Japanese eel leptocephali by year (a), larval size group (b), latitude group (c), and longitude group (d)
Figure 9

Plots of average POM nitrogen ($\delta^{15}$N) and carbon ($\delta^{13}$C) stable isotope ratios from 50 m and 150 m from 2004–2009 of Miyazaki et al. (2011) in relation to isotope ratios of Japanese eel leptocephali reported herein (2002 and 2005 separated into north and south latitudes; 2013 separated in northwestern station and all others), of Ariosa leptocephali collected 2007–2009 (from Miyazaki et al. (2011)), and Muraenidae, Congridae, Ariosa, and Nemichthys collected in 2013 (from Onda (2017)). Leptocephalus isotope ratios are separate into two isotopic groups of taxa defined by Feunteun et al. (2015). Bars show standard deviations