

Distribution and stable isotope ratio characteristics of Japanese eel leptocephali in relation to hydrographic structure of their Pacific Ocean spawning area

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3 **Distribution and stable isotope ratio characteristics of Japanese eel leptocephali**
4 **in relation to hydrographic structure of their Pacific Ocean spawning area**
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7 Shingo KIMURA^{1,*}, Sachie MIYAZAKI¹, Hiroaki ONDA¹, Takashi KITAGAWA¹,
8 Yoichi MIYAKE^{1, 3}, Michael J. MILLER², Katsumi TSUKAMOTO²
9

10
11 ¹Graduate School of Frontier Sciences / Atmosphere and Ocean Research Institute,
12 The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa, Chiba 277-8564, Japan
13

14 ²Atmosphere and Ocean Research Institute,
15 The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa, Chiba 277-8564, Japan
16

17 ³ Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 2-17-5
18 Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan
19

20
21 *Corresponding author e-mail address: s-kimura@aori.u-tokyo.ac.jp
22
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24 **Abstract**

25 To understand the larval distribution, size variation and stable isotope ratios of Japanese eel
26 leptocephali in relation to the salinity front and their feeding ecology, larvae from 7 research
27 cruises (2002–2013) in the North Equatorial Current (NEC) spawning area were examined.

28 The smallest early-stage larvae were distributed south of or near the salinity front,

29 confirming that the salinity front is an important oceanic feature to understand spawning
30 locations of the Japanese eel. Larger size larvae tended to distribute into higher latitudes.

31 Transport to northern latitudes with their growth would facilitate transport into the Kuroshio
32 region, but retention in the Subtropical Countercurrent (STCC) might be detrimental.

33 Preleptocephalus isotope ratios reflected maternal ratios, but feeding-stage leptocephali (8–
34 56 mm) tended to have higher $\delta^{15}\text{N}$ values at lower latitudes typically in areas south of a
35 salinity front. Feeding larvae quickly assimilate isotope ratios from the NEC after spawning
36 and early growth. Large differences of $\delta^{13}\text{C}$ values of larvae between the NEC and STCC
37 might vary with spatial baselines in the two currents. However, diel vertical migrations
38 should be considered, because the isotope ratios in particulate organic matter distinctly
39 depend on the depth. Comparisons among Japanese eel larvae and other taxa of leptocephali
40 in the NEC illustrate the need for further studies on the trophic ecology of leptocephali.
41 215 words

42 **Key words:** Japanese eel; leptocephali; stable isotope ratios; North Equatorial Current;
43 salinity front; preleptocephali

44 **Running title:** Distribution of Japanese eel in Pacific

45

46 INTRODUCTION

47 The Japanese eel (*Anguilla japonica*) spawns to the west of the Mariana Islands in the
48 North Equatorial Current (NEC) about 3000 km away from their juvenile nursery areas in
49 East Asia (Japan, China, Taiwan and Korea) (Tsukamoto 1992). Adult Japanese eels reach
50 the spawning area in the open ocean after migrating long distances from where they grew in
51 East Asia. A salinity front located around 15°N in the NEC is thought to represent a
52 landmark for termination of spawning migration of the Japanese eel (Kimura et al. 1994,
53 2001; Kimura and Tsukamoto, 2006). In the far western tropical Pacific, the salinity front
54 forms at a salinity of 34.5 where it is generated at the boundary of southern low-salinity (<
55 34.2) water diluted by precipitation and northern high-salinity (> 34.8) water caused by
56 excessive evaporation (Delcroix and Henin 1991; Ando and McPhaden 1997; Delcroix
57 1998; Delcroix and Picaut 1998; Henin et al. 1998; Kao and Lagerloef, 2015).

58 The main flow of the NEC westward current occurs between 10°N and 18°N, and the
59 position of the salinity front can vary within the latitudes and among years. Even if no
60 distinct front exists, spawning still occurs within the lower salinity water (Aoyama et al.

61 2014; Takeuchi et al. 2021). Therefore, although larvae can occur at various latitudes, the
62 location of the salinity front seems to be an important determinant of spawning locations.

63 Based on collections of eggs, newly hatched larvae (preleptocephali), larvae
64 (leptocephali) and spawning-condition adult eels, spawning is considered to occur along the
65 western side of the West Mariana Ridge seamount chain (Chow et al. 2009, 2010; Kurogi et
66 al. 2011; Tsukamoto et al. 2011; Aoyama et al. 2014; Takeuchi et al. 2021) around the new
67 moon between late spring and autumn (Kawakami et al. 1998; Ishikawa et al. 2001;
68 Tsukamoto et al. 2003, 2011). After spawning, larvae drift westward within the NEC
69 towards where they will recruit as juveniles and grow. Part of the NEC flow turns to the
70 north along the eastern side of the Philippines to then enter the Kuroshio Current (Fig. 1). If
71 the Japanese eel larvae reach this NEC bifurcation zone and are transported into the
72 southward flowing Mindanao Current, they will not reach appropriate habitat to settle in
73 East Asia (Kimura et al. 1994, 1999). Therefore, hydrographic structure, particularly,
74 latitude of the salinity front, which moves southward during El Niño events (Kimura et al.
75 2001) might play an important role in the success of larval transport towards East Asia.
76 Transport modelling studies have evaluated the effects of El Niño including changes in
77 latitude of the bifurcation, and other oceanic changes might also be related to Japanese eel
78 recruitment (Kim et al. 2007; Zenimoto et al. 2009; Hsu et al. 2017; Chang et al. 2018;
79 Hsiung et al. 2018; Chang and Miller 2022), but no single factor has been definitively linked
80 to fluctuations in the recruitment.

81 In addition to the effect of larval transport, larval survival and eventual recruitment
82 might also be affected by larval feeding success and growth. The ecology of leptocephali
83 has been difficult to fully understand, because their diets and feeding behavior are poorly
84 known, but are consistent with feeding on marine snow based on the objects seen in their
85 intestines (e.g., Miller et al. 2011; Tsukamoto and Miller 2021) and the DNA sequences
86 found in their gut contents (Chow et al. 2019; Watanabe et al. 2021). Marine snow is a type
87 of particulate organic matter (POM) that consists of amorphous material, microorganisms,
88 and visible objects such as appendicularian houses and fecal pellets (Otake et al. 1993;

89 Mochioka and Iwamizu 1996; Miller et al. 2011, 2019; Tomoda et al. 2018), but a wide
90 range of tissues seem to aggregate into the marine snow consumed by leptocephali based on
91 DNA studies (Chow et al. 2019; Watanabe et al. 2021). However, visual gut content
92 observations or DNA sequence studies do not provide information about what parts of
93 consumed materials are digested and assimilated by leptocephali.

94 Carbon and nitrogen stable isotope ratio ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) analyses have also been used
95 to study the diets of the Japanese eel and other species of leptocephali. These types of
96 studies provide information on the trophic level and source of food being consumed by a
97 predator (Deniro and Epstein 1978; Minagawa and Wada 1984; Post 2002). $\delta^{15}\text{N}$ values
98 reflect trophic positions of prey and $\delta^{13}\text{C}$ values reflect the characteristics of primary
99 producers at the base of the food web for an area (Layman et al. 2012).

100 Isotope ratios of the Japanese eel larvae and POM in 2002 revealed differences in
101 isotope ratios on either side of the salinity front (Kimura and Tsukamoto 2006). The
102 Japanese eel larvae may also feed more on marine snow at depths between 5 and 50 m, but
103 sometimes deeper in the NEC based on comparisons to POM isotopic ratios (Miyazaki et al.
104 2011). Anguillid leptocephali (including *A. japonica*) were more abundant near the top of
105 the thermocline at 70–100 m at night (Onda et al. 2017), but there is not enough vertical
106 distribution data to know at what depths leptocephali are feeding. POM isotope ratios
107 differed by depth in two Indo-Pacific studies (Feunteun et al. 2015; Ghinter et al. 2020),
108 possibly affecting the isotope ratios of leptocephali that fed at different depths, similar to
109 what Miyazaki et al. (2011) reported for the western North Pacific.

110 Aims of this study are to examine the possible effects of the salinity front on
111 distribution of the Japanese eel larvae and food web components of the larvae using
112 hydrographic data and stable isotope analyses data in the NEC region. Larvae were obtained
113 from collections made during 7 research surveys over more than 10 years, from within and
114 downstream of their NEC spawning area. We also perform stable isotope analyses on newly-
115 hatched non-feeding preleptocephali, whose isotope ratios will more likely be affected by
116 maternal influences, to help understand isotope ratios of leptocephali after they have

117 commenced feeding. Because previous studies (Kimura and Tsukamoto 2006; Miyazaki et
118 al. 2011) have not focused on spatial (latitude and longitude) differences, we described the
119 salinity structure of each of the 7 years in relation to larval catch data in this study.

120

121 **MATERIALS AND METHODS**

122 Seven research surveys from 2002–2013 by the R/V *Hakuho Maru* (operated by Japan
123 Agency for Marine-Earth Science and Technology) of the Ocean Research Institute
124 (currently, Atmosphere and Ocean Research Institute) of the University of Tokyo, to study
125 the spawning area of the Japanese eel and their larval migration in the NEC were included in
126 the present study. Survey areas, sampling station transect lines, and locations where larvae
127 were collected are shown in Fig. 1. Each survey included sampling for leptocephali (larval
128 stage) and newly hatched preleptocephali (pre-feeding larval stage).

129 Depending on the cruise, sampling was conducted with an Isaacs-Kidd Midwater
130 Trawl (IKMT) with a mouth opening of 8.7 m² or a newly designed 3 m ORI net with a
131 mouth opening of 7.1 m² (large ring net developed by the Ocean Research Institute,
132 University of Tokyo), both with a 0.5 mm mesh, in deployments to depths of about 300 m.
133 Except for samplings in STCC in 2013, samplings were conducted just west of about 143°E
134 along the western side of the West Marina Ridge. Conductivity, temperature and depth
135 measurements (CTD) were also made during surveys across the NEC, but not always in long
136 transects with equally spaced stations. Transects along 137°E had been treated as an
137 intensive and representative section through the surveys since the 1991 research cruise when
138 many Japanese eel leptocephali were collected at that longitude (Tsukamoto 1992). The
139 salinity front has been defined by salinity 34.5 as an absolute value. However, since there
140 was not always a distinct salinity front, we defined locations of the largest horizontal
141 gradient of surface salinity as the salinity front in this study.

142 Determination process of the salinity front locations are as follows. 1) average vertical
143 salinity upper 50 m in each observational station, 2) calculate horizontal salinity gradients
144 dividing the vertically averaged salinity differences among neighboring two observational

145 stations (salinity in northern station minus salinity in southern station) by distance between
146 the two stations, 3) seek locations of the largest horizontal gradient, 4) average latitude of
147 the observational stations where the largest horizontal gradient is located.

148 In addition to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data from larvae collected in two previous studies, a
149 research survey in 2002 (Kimura and Tsukamoto 2006) and research surveys from 2004–
150 2009 (Miyazaki et al. 2011), newly reported larval data from a research survey in 2013
151 (Onda 2017) were also used to determine spatial and temporal variation in the Japanese eel
152 larvae. In Miyazaki et al. (2011), half of stable isotope ratio data in 2005 were analyzed
153 because of no enough machine time at that time. Thus, in this study, another half data
154 analyzed after the study were added.

155 Upon collection, leptocephali were identified and their total length measured, and were
156 then frozen at -80°C . Subsequently, at the Atmosphere and Ocean Research Institute
157 research laboratory at the University of Tokyo, leptocephali were ground to a fine powder
158 using a spatula after drying in an oven at 60°C for 24 h. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were then determined
159 using 0.5–1.0 mg of each sample in an elemental analyzer interfaced with a mass
160 spectrometer (without de-lipidization). We express isotope ratios as per mill (‰) deviation
161 according to international standards of Vienna Pee Dee Belemnite (VPDB) for carbon and
162 atmospheric N_2 for nitrogen, for which $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = (\text{R}_{\text{sample}} / \text{R}_{\text{standard}} - 1) \times 1000$ where R
163 $= {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{15}\text{N} / {}^{14}\text{N}$. Measurement error was within $\pm 0.25\%$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
164 analyses. Linear regressions were performed on isotope ratio data and were tested for
165 significance using null hypothesis significance tests.

166 Of 130 pre-leptocephali collected around 14°N and 142°E in the 2005 survey
167 (Tsukamoto 2006) we randomly selected 30 for analysis. Because preleptocephali were too
168 small to be individually analyzed for stable isotope ratios, samples were pooled. In 2009
169 (Tsukamoto et al. 2011) more than 100 preleptocephali were collected from the same areas
170 (around 13°N and 141°E , south of the salinity front), from which we randomly selected 50
171 individuals for analysis into two samples of 25 individuals. Table 1 details the numbers,

172 total lengths (TL), and collection details of leptocephali used in stable isotope analyses, at
173 sites shown in Fig. 1.

174

175 **RESULTS**

176 *Hydrographic structure and larval distribution*

177 The larvae were distributed widely between 11°N and 17°N in the main stream of the
178 NEC (Fig. 1, 2). Sampling locations varied among years, but larvae were usually caught at
179 various latitudes and longitudes. Although sampling in 2013 occurred in November when it
180 is late spawning season, the distribution in the NEC did not differ from the distribution in
181 other years when surveys were done in main spawning season. In 2013, an additional survey
182 was conducted to the northwest in the Subtropical Countercurrent (STCC) area (Fig. 1a) and
183 several large larvae were collected (Table 1).

184 Figure 2 shows vertical sections of salinity along 137°E or nearby longitudes. Since
185 the section in 2005 was not very long, the CTD profile data along 137°E was merged with
186 the profiles along 139°E. Hydrographic data along 140°E were used in 2009, because there
187 were no CTD observations along 137°E during that cruise. Higher salinity water occurred in
188 the upper 300 m in the north, and a more saline subsurface core of water termed North
189 Pacific Tropical Water (NPTW) occurred at around 150 m. The surface layer above the
190 NPTW was low in salinity and formed the salinity fronts in the surface NEC as shown in
191 Fig. 2 by white arrows with the salinity values labeled. Latitude of the salinity front (largest
192 gradient) varied between 12.5°N and 15.5°N. The salinity of these locations changed
193 between 34.2 and 34.7, with 34.5 as the average of the 7 years.

194 The locations of the smallest larvae are indicated by red arrows with their TL labeled,
195 and they ranged from 8.5 mm in 2002 to 13.5 mm in 2008, with the smallest larva in 2013
196 being 26.3 mm. All of the smallest and average larvae were located south of the salinity
197 front or very close to it.

198

199 *Latitudinal and longitudinal larval distribution associated with their growth*

200 Figure 3 shows the relationship between total length and latitudinal/longitudinal locations of
201 the Japanese eel larvae. The figure indicates that the TL of larvae significantly increased
202 with latitude ($P < 0.01$, $R^2 = 0.50$) and with western longitude ($P < 0.01$, $R^2 = 0.43$). Smaller
203 larvae were concentrated around 12–13°N and 137 and 140°E, and large larval dispersion
204 occurred from north of 15°N and west of 137°E. Therefore, a location around 13°N and
205 140°E is estimated to be an average main spawning area. After the spawning, the larvae
206 were transported by the NEC and eddies northward and westward. However, the figure and
207 Fig. 1 also indicates that a part of smaller larvae are transported southward and would
208 probably be entrained into the Mindanao Current, such as two 25 mm larvae collected at 11–
209 12°N along 130°E in 2008.

210

211 *Spatial variation in larval isotope ratios*

212 Combining all of the larvae from all years, there were weak significant tendencies for
213 both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values to be higher in the south ($P < 0.01$, $R^2 = 0.09$ and $P < 0.01$, $R^2 = 0.15$,
214 respectively) (Fig. 4). However, there were no strong relationships between longitude and
215 both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values ($R^2 = 0.04$ and $R^2 = 0.0005$, respectively).

216 Four larvae collected in STCC show almost the same values of $\delta^{15}\text{N}$ as an average
217 value of other smaller larvae in the NEC (average = 5.5). However, the value of $\delta^{13}\text{C}$ in the
218 northwest STCC area were distinctly different from other smaller larvae collected in the
219 NEC (average = -20.8). According to comparison of Fig. 3 with Fig. 4, it seems that there is a
220 tendency that larval $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ decrease with growth. However, there were no
221 significant relationships between both larval $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and total length pooled for
222 2002–2013 ($P > 0.01$; not shown).

223 Isotope ratios of ~ 5 mm TL preleptocephali (~ 2–3 days old) likely resemble maternal
224 isotope ratios because feeding had not commenced, and they differed from leptocephali.
225 Preleptocephalus $\delta^{15}\text{N}$ values of 11.3‰ to 14.3‰ (2005, $n = 30$, 11.3‰; 2009 [2 samples,
226 each $n = 25$], 12.3‰, 14.3‰) were much higher than those of leptocephali (almost all <
227 8‰). $\delta^{13}\text{C}$ values of preleptocephali, -21.4‰ to -22.5‰ (2005, $n = 30$, -21.5‰; 2009 [2

228 samples, each $n = 25$], -21.4‰ , -22.5‰), were mostly out of the range of larvae collected
229 in the NEC. Considering effect of the salinity front on larval distribution, larvae were
230 separated by latitudinal groups, south or north of the salinity front based on latitude of the
231 salinity front (largest gradient) shown in Fig. 2. Figure 5 shows the C-N map of the larvae
232 separated by north and south groups. $\delta^{13}\text{C}$ values heavily overlap and there are no
233 significant difference (north = $-20.7 \pm 0.1\text{‰}$, south = $-20.8 \pm 0.2\text{‰}$, $P > 0.5$, t-test). $\delta^{15}\text{N}$
234 values also overlap (north = $4.9 \pm 0.6\text{‰}$, south = $5.7 \pm 0.7\text{‰}$), but there are lower values
235 among larvae collected in the north. As a result, north and south larvae $\delta^{15}\text{N}$ differ
236 significantly ($P < 0.001$, t-test).

237

238 **DISCUSSION**

239 *Geographic variation of larval distribution and isotope ratios*

240 Seven years of research cruise results of latitudinal location of the salinity front and
241 salinity structure in relation to the Japanese eel larvae indicate that the latitude of the salinity
242 front varies between 12.5°N and 15.5°N . All of the smallest and the positions of the average
243 latitude of larvae smaller than 20 mm in each cruise were located south of the salinity front
244 or very close to it. According to Kuroki et al. (2014), 20 mm TL larvae correspond to ages
245 of 35–40 days. If current velocity around the spawning area is typically about $15\text{--}20 \text{ cm s}^{-1}$
246 (Kimura et al., 1994), the larval transport distance from the spawning area around the West
247 Mariana Ridge is calculated to be 500–700 km during 35–40 days. That distance would
248 correspond to be around $137\text{--}139^\circ\text{E}$, which corresponds to the center of the main
249 observational lines through the research cruises in this study. Later research cruises that only
250 targeted the eggs and preleptocephali along the range of spawning latitudes along the west
251 side of the seamount ridge found spawning only occurred south of the front in single months
252 in 2011 and 2014, but spawning occurred over a wider range of latitudes when no front was
253 present in 2 surveys in 2012 (Aoyama et al. 2014; Takeuchi et al. 2021). All of these results
254 strongly confirm the salinity front is an important oceanic feature to understand the
255 spawning migration of the Japanese eel.

256 Our data and a general analysis of all Japanese eel larvae collected in the western
257 North Pacific up to 2007 (Shinoda et al. 2011) show that small larvae were only collected
258 along the western side of the West Mariana Ridge seamount chain at latitudes within the
259 NEC. The two studies also show that larger larvae had a tendency to be distributed in higher
260 latitudes and farther to the west. This indicates that early-stage larvae that hatched in
261 southern latitudes were transported to northern latitudes with their growth, probably due to
262 the actions of eddies that may generally move westward with the NEC. According to several
263 studies on larval transport modelling, after being spawned along the seamount ridge, the
264 larvae are transported westward by the NEC (Kimura et al. 1999; Kim et al. 2007; Zenimoto
265 et al. 2009; Hsu et al. 2017; Chang et al. 2018; Hsiung et al. 2018), although some larvae
266 were retained near the spawning area even as they grow, as seen in our observations. The
267 hydrographic sections suggest that the basic oceanographic structure of this region is
268 relatively stable, but variations do occur, which are uniquely shown in our presentation of 7
269 salinity sections. The stable westward flow of the NEC typically extends up to about 16–
270 17°N (Kaneko et al. 1998; Oka et al. 2018), but the eastward countercurrent, the STCC,
271 flows within 18–25°N (Qiu and Chen 2010). Therefore, large size larvae in water masses
272 north of the salinity front are likely influenced by the STCC and possibly transported too far
273 to metamorphose into glass eels in coastal nursery areas.

274 These distributions showing mixing of larvae into different water masses and currents
275 also appear to be reflected in the isotopic values of the leptocephali. $\delta^{15}\text{N}$ values show
276 significant relationships with latitude and higher values are recognized in the south where
277 larvae tend to be smaller. However, larger larvae collected in the STCC in 2013 did not have
278 $\delta^{15}\text{N}$ values on the regression line because of their much higher latitude. There is a
279 significant linear tendency between latitude and $\delta^{13}\text{C}$ values in the NEC, but larger larvae
280 collected in the STCC had distinctly lower $\delta^{13}\text{C}$ values than those in the NEC. $\delta^{15}\text{N}$ values
281 reflect trophic level of prey and $\delta^{13}\text{C}$ values are related to carbon sources within food webs
282 under different environmental conditions (Layman et al. 2012). Therefore, even if the larvae
283 grew up to large size just before metamorphosis to glass eels and were transported into the

284 Kuroshio and STCC, the late-stage larvae probably ingest the similar trophic level of diets
285 as the initial stage of larvae. However, since $\delta^{15}\text{N}$ values range was considerably wide
286 between 3-9‰ in the NEC, other factors should be considered even if the diet condition is
287 the same. In addition, differences of $\delta^{13}\text{C}$ between the NEC and STCC suggests different
288 marine snow consumed by larvae as they grow during their westward transport in the NEC.

289 The higher $\delta^{15}\text{N}$ values of larvae in more southern latitudes may be related to several
290 factors. For example, the salinity front that determines the latitude at which spawning of the
291 Japanese eel occurs might separate surface layer water masses with different baseline
292 isotope ratios. Considering this variability of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, early-stage larvae would be
293 classified into two groups north or south of the salinity front in each year. Yang et al. (2017)
294 suggests that isotope ratio of zooplankton and POM in the western North Pacific (120–
295 135°E) were different and depended on latitude and current systems. According to that
296 study, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the NEC were consistently higher than isotope ratios in the
297 STCC, and a greater abundance of nitrogen-fixing cyanobacteria (*Trichodesmium* spp.) in
298 the STCC in the western North Pacific may be primarily responsible for lower $\delta^{15}\text{N}$ values
299 at northern latitudes. $\delta^{15}\text{N}$ values of zooplankton also appear to be affected by
300 *Trichodesmium* abundance across the North Atlantic (Momepean et al. 2013). Spatial
301 differences in *Trichodesmium* abundance were also suggested to influence isotope ratios in
302 leptocephali and other species in the western South Pacific (Ghinter et al. 2020). Kimura and
303 Tsukamoto (2006) reported POM $\delta^{13}\text{C}$ values in south of the salinity front is higher than that
304 in north in 2002, which were possibly related to cyanobacteria. This suggests that different
305 baseline isotope ratios exist in northern and southern NEC areas that might roughly
306 correspond with the latitude of the salinity front, but other factors such as diet composition
307 should also be considered.

308

309 *Isotope ratios of preleptocephali compared with leptocephali*

310 In contrast to leptocephali, the distinctly different isotope ratios of preleptocephali
311 from the spawning area would not likely be related to baseline isotope ratios of the oceanic

312 environment in which they were collected. Newly hatched 2005 and 2009 pre-feeding stage
313 preleptocephali had much higher $\delta^{15}\text{N}$ and mostly lower $\delta^{13}\text{C}$ values than leptocephali that
314 had been feeding in the NEC. Because migrating silver eels do not feed (Chow et al. 2010)
315 and isotope ratios are transmitted to offspring (Starrs et al. 2014), the preleptocephalus
316 isotope ratios resemble maternal ratios and the continental habitats in which they lived.
317 Although it was often said that the preleptocephalus does not ingest diet, we could confirm it
318 based on the stable isotope analysis in this study.

319 Leptocephali of 9–10 mm TL already had lower $\delta^{15}\text{N}$ (> 50% lower) and mostly
320 higher $\delta^{13}\text{C}$ ratios than preleptocephali (~ 5 mm TL). Based on new moon spawning dates
321 and estimated larval growth rates of ~ 0.5 mm/day (Ishikawa et al. 2001; Kuroki et al.
322 2014), the leptocephali were 1–2 weeks in age, which indicates that preleptocephali quickly
323 assimilate isotope ratios in the NEC. This sized early-stage larvae were also collected 137°E
324 line where is far from estimated spawning area around the West Mariana Ridge. Since it is
325 difficult to reach 137°E line from the spawning area within 1–2 weeks even if the maximum
326 current speed in the NEC is supposed, another spawning area located much downstream area
327 in the NEC would be considered.

328

329 *Possible factors affecting isotope ratios of leptocephali*

330 Differences in marine snow among depth ranges could affect larval isotope ratios,
331 because the ratios of POM have been found to vary with depth. The depths at which
332 leptocephali feed are not known, but some species perform diel vertical migrations (DVM)
333 from deeper depths during the day to shallower depths at night (Castonguay and McCleave
334 1987; Otake et al. 1998). Therefore, because POM can have different isotope ratios at
335 different depths within the upper few hundred meters of the ocean (*e.g.*, Miyazaki et al.
336 2011; Feunteun et al. 2015; Ghinter et al. 2020), the depths at which leptocephali feed could
337 influence isotope ratios. Miyazaki et al. (2011) compared various patterns in POM isotope
338 ratios and indicated clearly different isotope ratios occurred between depths at 50 m and 150
339 m. POM at 150 m tended to have higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values than those at 50 m.

340 Further east in the North Pacific subtropical gyre, POM $\delta^{15}\text{N}$ was lowest near the surface (<
341 2‰ above 50 m), and values were higher (> 8‰) below about 120 m (Hannides et al. 2013).
342 Similarly, in the southeastern North Pacific high $\delta^{15}\text{N}$ values occurred below about 100 m
343 (Williams et al. 2014). POM in the western South Pacific also had higher $\delta^{15}\text{N}$ at depths
344 ranging 200–260 m compared with the chlorophyll maximum layer or at the surface,
345 although surface POM had distinctly higher $\delta^{13}\text{C}$ values (Ghinter et al. 2020). Therefore,
346 lower POM $\delta^{13}\text{C}$ values for larger larvae in 2013 might be related to increased DVM
347 behavior of larger anguillid leptocephali suggested by Castonguay and McCleave (1987).
348 However, since $\delta^{15}\text{N}$ values in 2013 were not considerably high, further POM stable isotope
349 analyses in downstream region of the NEC and the STCC are necessary to clarify the effect
350 of DVM certainly.

351

352 *Differences in isotope ratios of leptocephali among taxa*

353 Variation in POM isotope ratios at different depths might explain differences in
354 isotope ratios among taxa or sizes of leptocephali within an area if they feed at different
355 depths. Possibly related to this is a recurring pattern in leptocephalus taxa isotope ratios,
356 which have been designated as Group 1 with high $\delta^{15}\text{N}$ and low $\delta^{13}\text{C}$ (including the families
357 Anguillidae, Congridae, Muraenidae, and Serrivomeridae) and Group 2 with low $\delta^{15}\text{N}$ and
358 high $\delta^{13}\text{C}$ (including species with large larvae in the Nemichthyidae (*Avocettina*,
359 *Nemichthys*) and *Ariosoma*-type leptocephali in the congrid subfamily Bathymyrinae)
360 (Feunteun et al. 2015). Isotope ratios of the Japanese eel larvae and *Ariosoma* were found to
361 be consistent with these two groupings (Miyazaki et al. 2011). According to a wider range of
362 taxa collected in the 2013 cruise, *Anguilla*, *Conger*, Muraenidae, *Kaupichthys*, *Derichthys*
363 and Serrivomeridae of Group 1, and *Ariosoma* and *Nemichthys* of Group 2 could be
364 classified. Figure 6 shows a summarized C-N map based on results of the 2002–2013
365 research cruises for Japanese eel leptocephali and mostly in 2013 for other species. Average
366 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for all POM analyzed in the NEC region from 2006–2009 suggests
367 that Group 2 leptocephali may feed at shallower depths than those of Group 1 including the

368 Japanese eel larvae. These two groups were also reported from the western South Pacific
369 (Liénart et al. 2016; Ghinter et al. 2020) and Gulf of Mexico in the western North Atlantic
370 (Quattarini et al. 2019).

371

372 **CONCLUSION**

373 Using hydrographic data and stable isotope ratios of nitrogen and carbon of the
374 Japanese eel larvae in 2002–2013 research cruises, spatial differences of the larval
375 distribution and isotope ratios were described in relation to the salinity front in this study.
376 Larger larvae collected in the STCC had different $\delta^{13}\text{C}$ values, possibly related to
377 geographic differences, including more intense ontogenetic DVM behavior. Isotopic values
378 of larvae overlap between north and south of the salinity front, but $\delta^{15}\text{N}$ values tended to be
379 higher in the south. Probably, increased DVM behavior and mixing of larvae from the south
380 to the north with their growth make clear patterns difficult to detect especially when the
381 latitude of the fronts and the spawning locations varies among years.

382 Further studies on levels and types of primary productivity as discussed recently
383 (Chang and Miller 2022), community structure, and types of marine snow present in each
384 area or year are needed to determine the factors that contribute to differences in stable
385 isotope ratios. Differences in isotope ratios of leptocephali taxa are interesting and need
386 further investigation to determine if they are due to depth of feeding or might also be related
387 to other factors.

388 Gut content analysis of a complete size range of the Japanese eel larvae is necessary to
389 determine ontogenetic and/or geographic differences in their diet, preferably using a variety
390 of techniques, such as microscopic, next generation sequencing, and new chemical analyses
391 to determine the content of ingested materials. This information can then be compared with
392 the biological and oceanographic characteristics of the spawning area and areas downstream
393 in the NEC and STCC where larvae are transported and grow. These studies will help
394 identify the diets and feeding behavior of Japanese eel larvae, and through a better
395 understanding of its life history, contribute to its conservation.

396

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405

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407 cruises that collected the leptocephali, SK, TK, and YM managed research efforts on the
408 stable isotopic compositions of leptocephali that were conducted by SM and HO, SK drafted
409 the manuscript with the assistance of MJM, and the authors participated in the cruises and
410 critically revised the review.

411

412 **Compliance with ethical standards**

413 **Conflict of interests** The authors declare they have no conflict of interests. Funding was
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415 **Ethical approval** All applicable national and/or institutional guidelines for sampling for
416 the study have been followed.

417 **Data availability statement** The stable isotope ratio data in this manuscript are available
418 from the corresponding author upon reasonable request.

419

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

Year	Cruise	Sampling area	Sampling date	<i>n</i>	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	26.3–48.0
2013	KH-13-6	STCC	17 Oct. –28 Nov.	4	55.0–56.9

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600

601 **Figure captions**

602

603 **Figure 1** Survey area and collection sites of the Japanese eel leptocephali in this study :
604 North Equatorial Current (NEC); southward flowing Mindanao Current (MC); and northern
605 branch of the NEC that becomes the Kuroshio Current (a), where the Japanese eel
606 leptocephali from each year were collected (b–h). A rectangle in (a) depicts the area
607 included in (b–h). Red circles (main map) and red squares (individual years) in all panels
608 indicate locations where leptocephali were collected. Preleptocephali were collected around
609 regions indicated by blue squares (individual years). Thin lines indicate observational cruise
610 lines.

611

612 **Figure 2** Hydrographic section plots of salinity along 137°E obtained from the 7 research
613 cruises showing the TL of the individual larvae that were collected at the stations plotted in
614 Fig.1. Sections in 2005 and 2009 also used hydrographic data from 139°E and 140°E,
615 because not enough CTD observations conducted along 137°E. White arrows with salinity
616 values indicate the salinity front defined by the largest horizontal gradient in the upper 50 m
617 of surface salinity. Green arrows indicate average latitude of larvae smaller than 20 mm TL,
618 and red arrows and TL values indicate areas where the smallest larvae in each section were
619 observed. The sections were made using Ocean Data View (odv.awi.de).

620

621 **Figure 3** Relationship between the total length and latitudinal (top panel) /longitudinal
622 (bottom panel) collection location of the Japanese eel larvae. Red lines indicate linear
623 regression lines.

624

625 **Figure 4** Relationships between nitrogen stable isotope ratios ($\delta^{15}\text{N}$) of the Japanese eel
626 larvae and latitude (top panel), and between carbon stable isotope ratios ($\delta^{13}\text{C}$) and latitude
627 (bottom panel). Red lines indicate linear regression lines.

628

629 **Figure 5** Plots of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotope ratios of the Japanese eel
630 leptocephali classified by north and south of the salinity front.

631

632 **Figure 6** Plots of average POM nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotope ratios from
633 50 m and 150 m in 2006–2009 (from Miyazaki et al. (2011)), of *Ariosoma* leptocephali
634 collected 2007–2009 (from Miyazaki et al. (2011)), *Anguilla marmorata*, Muraenidae,
635 *Conger*, *Ariosoma*, *Kaupichthys*, *Derichthys*, Serrivomeridae and *Nemichthys* collected in
636 2013 (from Onda (2017)), and all *A japonica* collected 2002–2013 (from Kimura and
637 Tsukamoto (2006); Miyazaki et al. (2011); Onda (2017)). Leptocephalus isotope ratios were
638 separated into two isotopic groups (red: Group 1, blue: Group 2) of taxa according to
639 Feunteun et al. (2015). Bars show standard deviations.