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# Geographic variation in stable isotopic and fatty acid composition of anguilliform leptocephali and particulate organic matter in the South Pacific

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ABSTRACT: The feeding ecology of leptocephali has remained poorly understood because they apparently feed on particulate organic matter (POM), which varies in composition, and it is unclear which components of the POM they assimilate. The $\delta^{13}C$ and $\delta^{15}N$ stable isotope (SI) and fatty acid (FA) compositions of 3 families of leptocephali and POM were compared in 3 latitudinal current zones of the western South Pacific. The $\delta^{15}N$ signatures of leptocephali and POM overlapped, with both having their lowest values in the southern current zone. POM in general (across all zones) contained 38 FAs and was rich in saturated FAs (SFA) (16:0, 18:0, 14:0), while leptocephali contained 50 FAs, with high proportions of 16:0, and higher contributions of 22:6ω3, 20:5ω3, 18:1ω9, 16:1ω7 and other FAs than found in the POM. Serrivomeridae leptocephali in the north had higher $\delta^{15}N$ signatures and were also distinguished from Nemichthyidae and Muraenidae larvae by their FA compositions (higher SFAs, lower 22:6ω3 and 20:5ω3). Although SI signatures of the Serrivomeridae larvae did not clearly vary with size, 16:0 and 18:0 FA proportions decreased with increasing larval size, and 22:6ω3 and 16:1ω7 increased in larger larvae. Correspondences between the latitudinal variations in nitrogen SI signatures and FA compositions of POM with those of leptocephali and the presence of FA markers of both autotrophic and heterotrophic organisms were consistent with leptocephali feeding on POM. POM can contain various materials from primary producers and heterotrophic microorganisms, but differences in the SI signatures and FA compositions in leptocephali remain to be explained through further research.

KEY WORDS: Leptocephali · Fatty acids · Stable isotopes · Biomarkers · Trophic ecology · Oceanic currents

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## INTRODUCTION

Leptocephali are the larvae of approximately 15 families of anguilliform fishes and their close relatives, which are widely distributed in the oceans from low-latitude temperate zones to tropical latitudes (Böhlke 1989, Miller & Tsukamoto 2004, Miller 2009). They have highly laterally compressed, transparent bodies and a unique physiology compared to other fish larvae (Pfeiler 1999, Bishop et al. 2000). All eel families except the Anguillidae are almost exclusively marine and live in habitats ranging from the meso- and bathypelagic zones (e.g. Nemichthyidae, Serrivomeridae) to coastal areas (e.g. Muraenidae) (Miller & Tsukamoto 2004). All leptocephali, however, are found in the first 300 m of the water column in the open ocean (Castonguay & McCleave 1987, Miller 2009). Dependent on their taxa and adult habitats, the pelagic phase lasts for several months to >1 yr before the larvae undergo metamorphosis into juveniles (Marui et al. 2001).

Despite their wide distributions and the extensive literature on their morphology (e.g. Böhlke 1989), the feeding ecology of leptocephali is still poorly resolved. During their larval feeding and growth period, leptocephali accumulate energy storage compounds such as lipids and glycosaminoglycans (GAG) in a gelatinous body matrix, which are then used for building new tissues during metamorphosis (Padrón et al. 1996, Pfeiler 1999, Pfeiler et al. 2002). A few studies have examined the gut contents of leptocephali and have observed materials that resemble particulate organic matter (POM), such as marine snow, discarded appendicularian houses or faecal pellets (Otake et al. 1993, Mochioka & Iwamizu 1996, Miller et al. 2011). Carbon and nitrogen stable isotope studies of leptocephali indicate they feed at a low trophic level (Otake et al. 1993, Miyazaki et al. 2011, Feunteun et al. 2015), which was confirmed using amino acid nitrogen isotopes (Miller et al. 2013).

Other observations have suggested that leptocephali might also feed on microplanktonic organisms such as protozoans (Tanaka et al. 1995, Govoni 2010), and the DNA sequences of various organisms, including gelatinous zooplankton, have been detected in leptocephalus stomach contents (Riemann et al. 2010). Marine snow, however, can be composed of multiple materials and/or organisms, due to both aggregation and colonization processes (Alldredge & Silver 1988, Shanks & Walters 1997, Kiørboe 2000). Therefore, the hypothesis that leptocephali feed on POM and assimilate some of the components of marine snow, including colonising microorganisms, appears most likely (Deibel et al. 2012, Miller et al. 2013, Feunteun et al. 2015). Differences in the isotopic compositions of leptocephali taxa have been detected and clear correspondences between POM signatures and expected enrichment values in leptocephali have not always been observed (Miyazaki et al. 2011, Feunteun et al. 2015). Thus, it is unclear whether leptocephali feed on POM opportunistically or whether there are selective differences in the types of POM consumed and/or the types of compounds assimilated from ingested POM between different taxa.

An important component of the POM and organisms that potentially contribute to the diet of leptocephali are lipids, but only a few studies have examined the lipid content of leptocephali (Padrón et al. 1996, Deibel et al. 2012). Lipids are important for marine organisms because they are their major metabolic energy reserves (Falk-Petersen et al. 2000, Lee et al. 2006) and play key roles in cell structure and metabolism (Dalsgaard et al. 2003). Fatty acids (FAs) are a specific class of lipids that are energy rich molecules (Dalsgaard et al. 2003). Most consumers are unable to synthetize essential FAs (EFAs: 20:4ω6, 20:5ω3, 22:6ω3) — which are polyunsaturated FAs (PUFAs) with a terminal end omega-3 (ω3) or omega-6 (ω6) — due to their lack of specific enzymes and therefore they acquire these EFA molecules exclusively from the food they eat (Canuel et al. 1995, Styrishave & Andersen 2000, Meziane et al. 2002, Dalsgaard et al. 2003).

Biochemical tracers, such as FAs and stable isotope (SI) ratios, are commonly used in trophic ecology studies because they provide information about the food sources that are assimilated by the organism over time (Pitt at al. 2009). SI ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are used to evaluate the food sources of organisms (Fry & Sherr 1984, Canuel et al. 1995) and to characterize organic matter transfer through food webs (Fry & Sherr 1984, Canuel et al. 1995, Abrantes & Sheaves 2009). FAs can be used as biomarkers of specific organisms (Meziane et al. 2007), or to determine the food sources exploited by consumer organisms (Meziane et al. 1997, Meziane & Tsuchiya 2000, Mortillaro et al. 2015). They can also be used to follow organic matter transfer through food webs (Dalsgaard et al. 2003, Budge et al. 2006, Hall et al. 2006) and can provide information on larval ontogenetic changes (Plante et al. 2007).

This study used bulk SI and FA composition to provide information on the possible food sources of leptocephali and was specifically intended to investi-

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gate (1) how leptocephali, belonging to 3 families, may assimilate POM and the associated microorganisms colonizing POM (e.g. protozoa, microalgae, bacteria), and (2) whether the diet of leptocephali changes with size and/or with latitude and/or current systems in the western South Pacific Ocean.

## MATERIALS AND METHODS

### Study area and sample collection

Leptocephali were collected in the western South Pacific (WSP) during Leg 1 of the KH-13-2 research cruise of the RV 'Hakuho Maru' from 4–24 February 2013. Stations (n = 34) were sampled, with some exceptions, at every 2° of latitude/longitude between 5° S–30° S and 165° E–175° W, in the region that includes New Caledonia, Fiji and Samoa (Fig. 1A). Leptocephali were collected using an Isaacs-Kidd midwater trawl (IKMT) with an 8.7 $m^2$ mouth opening and 0.5 mm mesh. Sampling at each station was conducted following 2 strategies: (1) during the night, a step tow fished in the upper 120 m (10 min. steps at 120 m, 70 m and 50 m); and (2) during the day, an oblique tow fished from the surface to 200 m. Both types of tows took less than 1 h. Temperature, conductivity, depth (CTD) profiles were made at 21 of the stations to a depth of 500 m.

Leptocephali from each IKMT sample were sorted immediately, measured, and identified to the lowest possible taxonomic level in accordance with Miller & Tsukamoto (2004). A total of 538 leptocephali from 13 families were collected, but only the 3 most abundant families were analysed in this study. After identification and measurement, specimens to be used for SI and FA analyses were stored at –80°C. In this study, Family is the only taxonomic level considered, and 101 leptocephali from the 3 most abundant families — Muraenidae (n = 27; length 25.3–83.0 mm,

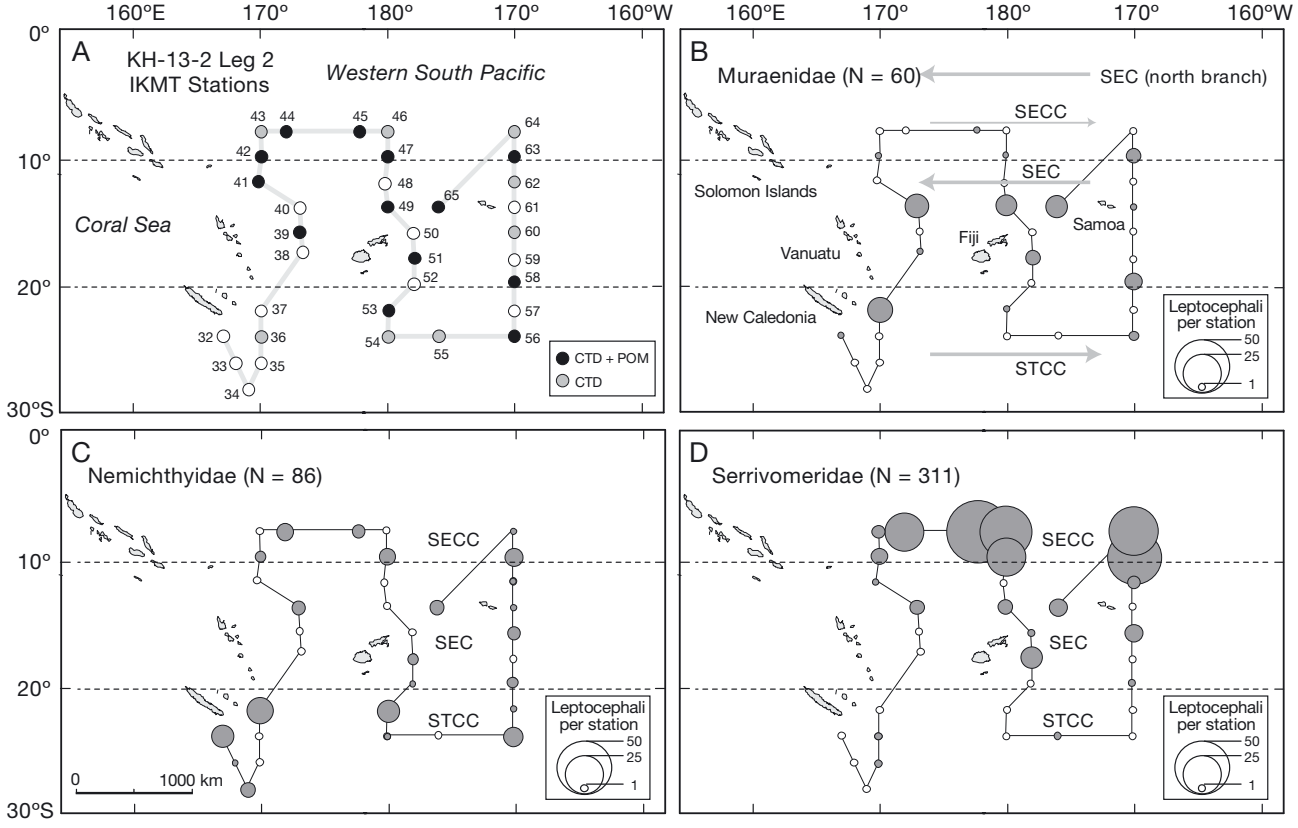


Fig. 1. KH-13-2 sampling survey of the RV 'Hakuho Maru' showing (A) the Isaacs-Kidd midwater trawl (IKMT) stations for collecting leptocephali (Stns 32–65) and stations (21 in total) with CTD casts with particulate organic matter (POM) sampling (black circles) or without POM sampling (grey circles). Number of leptocephali caught per station of (B) Muraenidae, (C) Nemichthyidae, and (D) Serrivomeridae are represented by the relative sizes of the shaded circles. General locations of the South Equatorial Current (SEC), South Equatorial Countercurrent (SECC) and South Tropical Countercurrent (STCC) are shown with arrows, and the dotted lines separate the 3 current zones used for analyzing latitudinal differences in SI and FA compositions of leptocephali and POM

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mean ± SD 50.1 ± 13.4 mm), Nemichthyidae (n = 28; length 30.0–259.0 mm, 122.0 ± 60.5) and Serrivomeridae (n = 46; length 9.2–62.0 mm, 28.4 ± 14.4 mm) — were analysed for SI and FA composition (Fig. 1). Five of the Serrivomeridae larva samples consisted of 2 of the smallest larvae (<10 mm) pooled together to obtain enough tissue for analysis. Each leptocephalus specimen or pooled sample was used for both SI and FA analyses. Serrivomeridae larvae were used to examine differences in SI and FA composition in relation to leptocephalus size because of the insufficient specimens of different sizes of Muraenidae and Nemichthyidae larvae, and the Serrivomeridae larvae were mostly from the same current zone.

For the POM analyses, seawater samples were collected in triplicate at 13 stations (Fig. 1A) in 12 l Niskin bottles attached to the CTD rosette multisampler at the depth of the chlorophyll maximum as measured by a fluorometer on the CTD. The depth of the chlorophyll maximum differed depending on the station, and was chosen because it is likely to have high concentrations of particulate material that may be suitable for feeding by leptocephali. No POM was sampled at the first CTD station in the southwest due to its proximity to the start of the cruise (and hence to the coast or land). POM was collected by filtering each 12 l of seawater sample, using separate precombusted glass fibre filters (GF/F, 47 mm diameter, 0.7 µm mesh). Large organisms such as copepods were removed manually and filters were immediately stored frozen at −80°C until analysed.

### Stable isotopes analysis

All samples were lyophilised before analysis. GF/F filters were fumigated with 10% HCl for 4 h to remove inorganic carbon (Lorrain et al. 2003). Leptocephali samples were weighed. Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) SI analysis were performed by the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California at Davis) using a PDZ Europa ANCA-GSL elemental analyser for leptocephali analysis (half body, excluding the head region except for the smallest larvae) and an Elementar Vario EL Cube or Micro Cube elemental analyser (Elementar Analysensysteme) for the GF/F filter POM samples (half filter). Each elemental analyser was interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Isotopic data are reported using standard delta notation ($\delta^{13}C$ or $\delta^{15}N$), defined as parts per thousand deviation (‰) from an international standard (Vienna Peedee belemnite for $\delta^{13}C$ and atmospheric $N_2$ for $\delta^{15}N$) as defined by the equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000 \quad (1)$$

Where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$ of the sample or standard (Peterson & Fry 1987). The analytical precision (standard deviation for repeated measurements of internal standards) was ±0.2‰ and ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

### Fatty acid analysis

Lipids were extracted following the procedure of Meziane et al. (2007). Briefly, lipid samples (half bodies or filters) were extracted twice with a mixture of distilled water, methanol (MeOH) and $CHCl_3$ (1:2:1, v:v:v). Saponification and methylation were performed successively under reflux following Meziane & Tsuchiya (2000). Saponification was achieved using a 2 M NaOH:MeOH solution (1:2, v:v) and methylation with 1 ml of 14% $BF_3$-MeOH. The fatty acid methyl esters (FAMEs) were separated and quantified by gas chromatography (Varian CP-3800) using a flame ionisation detector at the National Museum of Natural History (MNHN-Paris, Resaqua Laboratory). Separation was performed with an Agilent J&W GC VF-WAXms column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) with He as carrier gas. Most of the FA peaks were identified by comparing their retention times with those of known standards (Supelco™ 37 FAME mix; Sigma-Aldrich®) and confirmed with a GC-mass spectrometer (Varian 450-GC 220-Ion Trap MS; He as carrier gas). FAs are designated as X:YωZ, where X is the number of carbon atoms, Y is the number of double bonds and Z is the position of the ultimate double bond with respect to the terminal methyl group. The concentration of each FA was calculated using 23:0 as an internal standard in accordance with Schomburg (1987): $C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$, where $A_S$ is the peak area of the FA, $A_{IS}$ the peak area of the internal standard, $C_{IS}$ the concentration of the standard and $W_S$ the dry weight of the sample.

### Latitudinal regions of the study area

The study area was located within the northwestern part of the WSP subtropical gyre (Qiu & Chen 2004). The water temperature in this region decreases from the north to south (Miller et al. 2006), and during the survey it ranged from 29°C (surface)

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and 21–23°C (200 m depth) at northern stations (8° N) to 25°C and 18°C, respectively, in the south (24° S). This region includes 3 main surface currents at different latitudes, which are (1) the westward South Equatorial Current (SEC, Equator to 20° S), (2) the narrow eastward South Equatorial Countercurrent (SECC) at 5 to 10° S (between 2 branches of the SEC), and (3) the eastward South Tropical Countercurrent (STCC) at latitudes south of Fiji and north of New Zealand (Qiu & Chen 2004, Ganachaud et al. 2014). The SECC was present north of 10° S according to acoustic Doppler current profiler (ADCP) observations made during the cruise, with mixed westward flow at central latitudes and mixed eastward flow in the south (T. Otake et al. unpubl. cruise report). The assemblages of leptocephali vary latitudinally in this region, with Serrivomeridae larvae being most abundant in the north, species such as Muraenidae being abundant at central latitudes, and Nemichthyidae being more equally distributed across latitudes (Miller et al. 2006) as they were during the present survey (Fig. 1B–D). According to the general pattern of currents, the cruise stations were separated into 3 latitudinal zones for analyses of SI and FA compositions of leptocephali and POM, which generally correspond with the SECC, SEC, and STCC current systems (Fig. 1B).

### Data analysis

All SI and FA data are expressed as mean values ± standard deviation (SD). Prior to statistical comparison, data were first evaluated for homocedasticity (Bartlett test) and normality of distribution (Shapiro-Wilk test), which were not confirmed in most cases. Therefore, non-parametric Kruskal-Wallis (KW) tests were used to compare FA and SI group values, followed by pairwise comparisons with Mann–Whitney Wilcoxon (MWW) tests and Bonferroni correction to correct significance thresholds. When the sample size in a current zone was too low, only KW tests were used.

All FAs were used in the analyses and no transformations were performed on the dataset to prevent excessive weighting of low proportion FAs. Data matrices (% total fatty acid [TFA] per sample) were used to create triangular dissimilarity matrices with the Bray-Curtis dissimilarity coefficient, followed by non-metric multidimensional scaling (NMDS). Stress values < 0.2 were considered robust (Clarke 1993). Groups used as factors for the analysis were family (Muraenidae, Nemichthyidae, Serrivomeridae) and size groups (≤10.0, 10.1–20.0, 20.1–40.0, 40.1–60.0 mm, Serrivomeridae only), as well as current zones (SEC, SEC, STCC) for both leptocephali and POM. Differences in FA composition among groups was statistically tested using separate one-way ANOSIM computed after 5000 permutations. Average dissimilarity (AD) between groups, determined by SIMPER analysis (PRIMER®5 software module), was used to identify which fatty acids determine the observed differences within or between groups.

Multivariate analyses were performed with PRIMER®5 software (Clarke & Warwick 2001) and univariate tests using R software (R Development Core Team 2014, Vegan package, Oksanen et al. 2014). For all univariate tests, the probability α was set at 0.05.

## RESULTS

### Isotopic and fatty acid composition of POM and leptocephali

#### Particulate organic matter (POM)

SI values of POM (Fig. 2) ranged from −27.8 to −25.0‰ for $\delta^{13}C$ (mean ± SD = −26.6 ± 0.7‰) and from 2.5 to 12‰ for $\delta^{15}N$ (6.1 ± 2.3‰) (Table 1). A total of 38 FAs were identified in the POM (Table 1). The average saturated fatty acid (SFA) contribution to POM was 75.2 ± 4.6% of total FAs, with 16:0 showing the highest contribution (35.9 ± 2.3%). The main monounsaturated fatty acids (MUFAs) were 18:1ω9 (5.3 ± 1.1%) and 16:1ω7 (4.4 ± 0.9%). All relative contributions of polyunsaturated fatty acids (PUFAs) were low with maximum values of 1.7 ± 0.4% for 16:2ω4, 1.7 ± 0.8% for 22:6ω3 and 1.7 ± 0.5% for 18:4ω3. Similarly, essential fatty acids (EFAs) 20:5ω3 and 20:4ω6 had low contributions to TFAs (about 1% or less). The contribution of branched-chain fatty acids (BrFAs) was 2.4 ± 0.4% of TFAs.

#### Leptocephali

For the 3 families of leptocephali (Fig. 2), $\delta^{13}C$ stable isotope values ranged from −23.1‰ to −18.8‰ and $\delta^{15}N$ ranged from 0.7‰ to 14.5‰ (Table 1). There was a significant difference in $\delta^{13}C$ composition between Serrivomeridae (−21.0 ± 0.8‰) and Nemichthyidae (−19.9 ± 0.8‰) leptocephali (KW: $p < 0.001$; followed by MWW: $p < 0.001$). $\delta^{15}N$ signatures were significantly different among each of the 3 fam-

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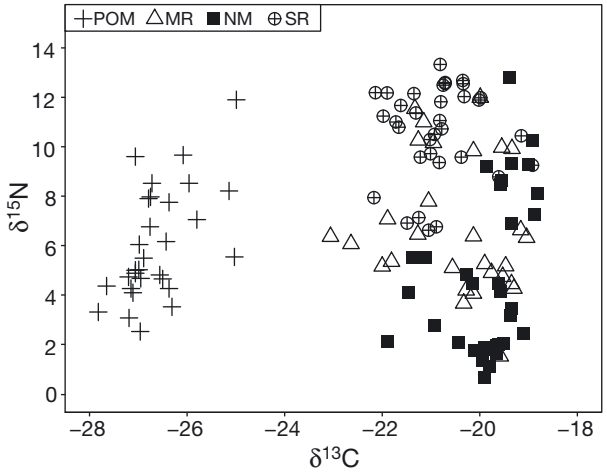


Fig. 2. Carbon and nitrogen stable isotope values of particulate organic matter (POM) and the leptocephali of the Muraenidae (MR), Serrivomeridae (SR) and Nemichthyidae (NM)

ilies (KW: $p < 0.001$; MWW: $p < 0.01$) with an average $\delta^{15}N$ of 4.7 ± 3.3‰ for Nemichthyidae, 6.7 ± 2.7‰ for Muraenidae and 10.9 ± 1.9‰ for Serrivomeridae.

A total of 50 FAs was identified in leptocephali (Table 1), with a large contribution of SFAs, MUFAs, and PUFAs to TFAs. The same 6 FAs (16:0, 16:1ω7, 18:0, 18:1ω9, 20:5ω3 and 22:6ω3) were most abundant in all 3 families, with all the other FAs each contributing less than about 4% to the TFAs. Muraenidae had higher levels of FAs by weight (11.7 ± 7.4 mg $g^{-1}$) than Nemichthyidae (5.2 ± 2.5 mg $g^{-1}$) and Serrivomeridae (5.4 ± 4.0 mg $g^{-1}$).

The 3 families also showed differences regarding their FA compositions (ANOSIM: $p < 0.001$), with Muraenidae showing no overlap with the Serrivomeridae and Nemichthyidae, but these both overlapped to some degree in the NMDS plot (Fig. 3). FA profiles of leptocephali exhibited average dissimilarity values (AD; SIMPER analysis) that were more similar between Muraenidae and Serrivomeridae (16.6%) than between Muraenidae and Nemichthyidae (14.3%) and between Nemichthyidae and Serrivomeridae (14.6%). The observed differences were mostly due to the relative contributions of 16:0, 16:1ω7, 18:0, 18:1ω9, 20:5ω3 and 22:6ω3 to TFAs (Table 1). SFAs contributed a higher proportion of TFAs in Serrivomeridae leptocephali (44.8 ± 7.5%) than PUFAs (33.8 ± 5.7%), whereas the PUFA contribution was slightly higher in Muraenidae and Nemichthyidae (42.3 ± 3.7 and 39.4 ± 4.2%, respectively) than the SFA contribution (38.5 ± 3.7 and 37.4 ± 4.2%, respectively) (Fig. 4A,B). The total ω3 FA contribution was significantly different (KW: $p < 0.001$; MWW: $p < 0.05$) among the 3 families, ranging from 23.7 ± 5.5% for Serrivomeridae to 32.5 ± 3.8% for Muraenidae (Fig. 4C). BrFAs contributed less than 1% of TFAs, but significantly differed in their contribution to TFAs (KW: $p < 0.001$; MWW: $p < 0.001$) between the Muraenidae (0.5 ± 0.1%) and the other families (Nemichthyidae: 0.8 ± 0.1% and Serrivomeridae: 0.9 ± 0.2%) (Fig. 4D).

Fatty acid trophic markers (FATMs) of the leptocephali included 2 predominant 16:0 and 22:6ω3 FAs, which contributed on average 40% of the TFAs (Table 1). The proportions of the PUFAs 20:5ω3 and 22:6ω3 were significantly lower for Serrivomeridae (5.1 ± 2.1% and 14.8 ± 3.2%, respectively) than for the 2 other families (KW: $p < 0.001$; MWW: $p < 0.001$). Muraenidae had the highest percentage of 22:6ω3 of the 3 families. The contribution of 18:1ω9 to TFAs was lower for Muraenidae (5.8 ± 0.9%; KW: $p < 0.001$; MWW: $p < 0.001$) than for Nemichthyidae and Serrivomeridae (8.3 ± 1.7% and 8.8 ± 1.3%, respectively).

### FA compositions and SI signatures of Serrivomeridae size groups

The 4 size groups of Serrivomeridae leptocephali were compared for their FAs and SI signatures. No significant differences were found between size groups for $\delta^{15}N$ (Table 2) and $\delta^{13}C$ values were only significantly different for ≤10 mm larvae (−19.9 ± 0.6‰) and 40–60 mm larvae (−21.4 ± 0.5‰) (KW: $p < 0.01$; MWW: $p < 0.05$).

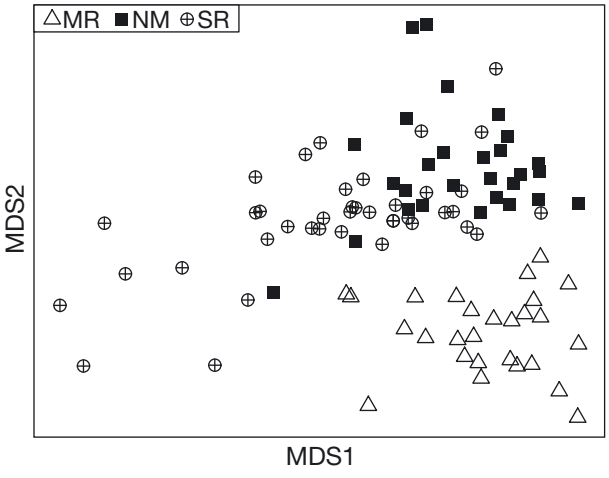


Fig. 3. Non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis similarity distance of total fatty acid composition for leptocephali of the Muraenidae (MR), Serrivomeridae (SR) and Nemichthyidae (NM). Stress = 0.12

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Table 1. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) mean isotopic signatures (‰) and fatty acid (FA) relative contribution (%) of particulate organic matter (POM) for 13 stations (3 replicates per station) and all leptocephali tissues for the 3 families Muraenidae (MR), Nemichthyidae (NM) and Serrivomeridae (SR) at 21 stations. All values are mean ± SD; n is the number of samples; –: FA not found

| | POM (n = 39) | MR (n = 27) | NM (n = 28) | SR (n = 41) |
|---|---|---|---|---|
| **Stable isotopes** | | | | |
| $\delta^{15}N$ | 6.1±2.3 | 6.7±2.7 | 4.7±3.3 | 10.9±1.9 |
| $\delta^{13}C$ | −26.6±0.7 | −20.5±1.1 | −19.9±0.8 | −21.0±0.8 |
| **Saturated FA** | | | | |
| 12:0 | 0.51±0.25 | 0.08±0.12 | 0.04±0.06 | 0.07±0.1 |
| 13:0 | 0.11±0.03 | 0.02±0.02 | 0.02±0.01 | 0.03±0.1 |
| 14:0 | 14.05±1.71 | 3.99±1.32 | 4.63±0.86 | 4.12±0.6 |
| 15:0 | 1.7±0.22 | 1.52±0.23 | 1.71±0.37 | 2.1±0.2 |
| 16:0 | 35.91±2.28 | 20.94±2.44 | 24.04±2.64 | 26.63±4.3 |
| 17:0 | 0.67±0.11 | 1.73±0.18 | 1.26±0.18 | 1.75±0.3 |
| 18:0 | 19.47±2.31 | 9.32±1.25 | 5.14±1.7 | 9.1±2.9 |
| 19:0 | 0.46±0.09 | 0.31±0.1 | 0.2±0.09 | 0.34±0.2 |
| 20:0 | 0.85±0.1 | 0.27±0.07 | 0.14±0.03 | 0.35±0.2 |
| 21:0 | 0.06±0.02 | 0.04±0.01 | 0.03±0.05 | 0.03±0 |
| 22:0 | 0.58±0.13 | 0.19±0.04 | 0.14±0.03 | 0.17±0.1 |
| 24:0 | 0.58±0.25 | 0.08±0.06 | 0.04±0.03 | 0.09±0.1 |
| **Σ SFA (%)** | **75.23±4.58** | **38.5±3.65** | **37.41±4.2** | **44.79±7.5** |
| **Monounsaturated FA** | | | | |
| 16:1ω5 | 0.07±0.03 | 0.14±0.03 | 0.15±0.03 | 0.13±0 |
| 16:1ω7 | 4.42±0.88 | 5.71±1.14 | 7.6±1.77 | 6.59±1.3 |
| 16:1ω9 | 2.07±0.5 | 0.54±0.09 | 0.66±0.13 | 0.63±0.1 |
| 17:1ω7 | 0.37±0.08 | 0.45±0.12 | 0.49±0.11 | 0.32±0.1 |
| 17:1ω9 | – | 0.45±0.1 | 0.73±0.15 | 0.75±0.1 |
| 18:1ω5 | – | 0.1±0.03 | 0.1±0.03 | 0.11±0 |
| 18:1ω7 | 0.71±0.16 | 4.18±0.77 | 3.93±0.63 | 2.82±0.7 |
| 18:1ω9 | 5.3±1.06 | 5.84±0.94 | 8.33±1.72 | 8.77±1.3 |
| 19:1ω9 | – | 0.03±0.02 | 0.02±0.02 | 0.03±0 |
| 20:1ω7 | – | 0.18±0.08 | 0.12±0.04 | 0.09±0.1 |
| 20:1ω9 | – | 0.22±0.21 | 0.13±0.07 | 0.1±0.1 |
| 22:1ω11 | 0.38±0.13 | 0.09±0.1 | 0.03±0.02 | 0.06±0 |
| 22:1ω9 | – | 0.05±0.02 | 0.01±0.01 | 0.02±0 |
| 24:1ω9 | – | 0.08±0.05 | 0.06±0.05 | 0.07±0.1 |
| **Σ MUFA (%)** | **13.33±2.66** | **18.69±1.15** | **22.38±2.9** | **20.49±2.5** |

| | POM (n = 39) | MR (n = 27) | NM (n = 28) | SR (n = 41) |
|---|---|---|---|---|
| **Polyunsaturated FA** | | | | |
| 16:2ω4 | 1.7±0.41 | 0.57±0.19 | 0.61±0.17 | 0.72±0.33 |
| 16:2ω6 | – | 0.05±0.02 | 0.09±0.02 | 0.04±0.02 |
| 16:4ω3 | 0.22±0.06 | 0.05±0.05 | 0.02±0.03 | 0.05±0.04 |
| 18:2ω6 | 1.21±0.26 | 2.76±0.47 | 2.97±0.44 | 2.55±0.6 |
| 18:3ω3 | 0.45±0.09 | 1.15±0.33 | 1.17±0.56 | 0.8±0.28 |
| 18:3ω6 | 0.42±0.08 | 0.28±0.06 | 0.23±0.06 | 0.39±0.1 |
| 18:4ω3 | 1.69±0.52 | 1.24±0.26 | 1.37±0.44 | 1.16±0.37 |
| 20:2ω6 | – | 0.24±0.1 | 0.11±0.05 | 0.08±0.05 |
| 20:3 | – | 0.15±0.04 | 0.15±0.04 | 0.09±0.03 |
| 20:3ω6 | – | 0.37±0.08 | 0.24±0.06 | 0.27±0.1 |
| 20:4ω3 | 0.09±0.02 | 1.4±0.77 | 0.86±0.3 | 0.96±0.69 |
| 20:4ω6 | 0.13±0.04 | 2.82±0.79 | 2.81±0.51 | 3.49±0.96 |
| 20:5ω3 | 1.02±0.22 | 7.85±0.97 | 8.52±1.22 | 5.05±2.07 |
| 22:2ω6 | 0.27±0.14 | – | – | – |
| 22:4ω6 | – | 0.36±0.15 | 0.27±0.21 | 0.12±0.13S |
| 22:5ω3 | – | 2.79±1.58 | 0.95±0.51 | 0.93±0.59 |
| 22:5ω6 | 0.19±0.11 | 2.18±0.65 | 2.24±0.37 | 2.34±0.63 |
| 22:6ω3 | 1.67±0.79 | 18.1±2.13 | 16.8±2.58 | 14.8±3.16 |
| **Σ PUFA (%)** | **9.1±2.1** | **42±3.7** | **39±4.2** | **34±5.7** |
| **Branched-chain FA** | | | | |
| 14:0iso | 0.35±0.1 | 0.01±0.01 | 0.22±0.01 | 0.03±0.01 |
| 15:0anteiso | 0.45±0.06 | 0.03±0.01 | 0.04±0.02 | 0.05±0.03 |
| 15:0iso | 0.37±0.05 | 0.12±0.03 | 0.14±0.03 | 0.17±0.05 |
| 16:0iso | 0.22±0.04 | 0.08±0.02 | 0.1±0.04 | 0.13±0.04 |
| 17:0anteiso | 0.68±0.17 | 0.08±0.02 | 0.1±0.03 | 0.11±0.04 |
| 17:0iso | 0.05±0.01 | 0.12±0.03 | 0.26±0.05 | 0.27±0.06 |
| 18:0iso | 0.26±0.09 | 0.07±0.05 | 0.16±0.06 | 0.13±0.05 |
| **Σ BrFA (%)** | **2.38±0.3** | **0.5±0.1** | **0.8±0.1** | **0.9±0.2** |
| Total FA (mg $g^{-1}$) | 3.6±0.97 | 11.7±7.44 | 5.22±2.51 | 5.41±3.99 |
| Σ ω3 (%) | 5.14±1.46 | 32.5±3.77 | 29.7±3.84 | 23.7±5.51 |
| Σ ω6 (%) | 1.95±0.12 | 9.06±1.44 | 8.96±0.83 | 9.28±1.62 |
| Σ EFA (%) | 2.82±0.35 | 28.7±1.3 | 28.1±1.44 | 23.3±2.06 |

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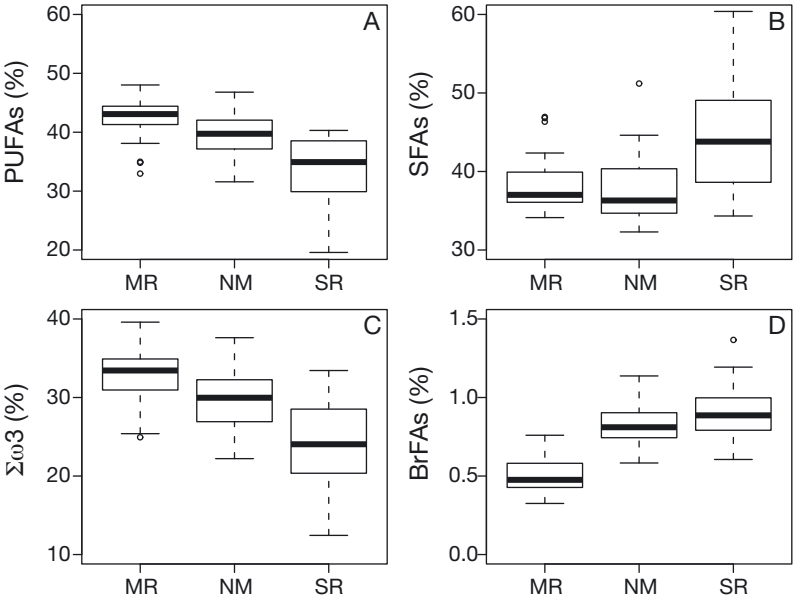


Fig. 4. Fatty acid proportions (midline: median; box limits: first and third quartiles; whiskers: 5th and 95th percentiles; points: outliers) of (A) polyunsatured fatty acids (PUFAs), (B) saturated fatty acids (SFAs), (C) sum of ω3 fatty acids (Σω3), and (D) branched-chain fatty acids (BrFAs), for the leptocephali of the Muraenidae (MR), Nemichthyidae (NM) and Serrivomeridae (SR)

The FA compositions of the Serrivomeridae leptocephali showed various percentage changes with increasing size, with SFAs and BrFAs tending to decrease, and MUFAs and PUFAs tending to increase with increasing leptocephali size (Table 2). The total weight of FA in the body of the leptocephali tended to decrease only slightly from the smallest to largest size classes (5.8 to 4.4 mg $g^{-1}$) but differences were not significant. The 40–60 mm and ≤10 mm sizes classes were significantly different in FA composition (ANOSIM: $p < 0.05$; NMDS) (Fig. 5) with an AD of 23.2%. The SFA contribution to ≤10 mm larvae was higher (56.7 ± 0.9%) and PUFA contribution was lower (25.2 ± 0.6%) than in 40–60 mm larvae (37.1 ± 0.3% for SFA and 39.0 ± 0.4% for PUFA) mainly due to the contribution of the SFAs 16:0 and 18:0, the MUFA 16:1ω7 and the PUFAs 22:6ω3 and 20:5ω3 (Table 2). The contribution of ω3 FAs increased with size, from 16.4 ± 1.0% for ≤10 mm to 30.0 ± 0.7% for 40–60 mm larvae.

### POM and leptocephali SI signatures in current zones

The POM exhibited variation in $\delta^{15}N$ values, which ranged from 7.7 ± 2.3‰ in the SECC in the north, to 4.1 ± 1.0‰ in the STCC in the southeast (Table 3), although the SEC values overlapped with those of the other zones (Fig. 6D). The $\delta^{13}C$ values showed less variation with mean values that ranged from −27.0 ± 0.2‰ to −26.2 ± 0.9‰, but some of the SECC values were lower (Fig. 6D). When FA compositions of POM were separated according to the 3 current zones, samples from the SECC (5–15°S) were significantly different (ANOSIM: $p < 0.05$) from those of both the SEC (15–20° S) and STCC (20–30° S) with an AD of 12.5% between the SECC and SEC, and 13.2% between SECC and STCC. These differences mostly resulted from variations in the proportions of the SFAs 18:0, 16:0 and 14:0 (AD between 1.4 and 2.6%), the MUFAs 18:1ω9 and 16:1ω7 (AD between 0.9 and 0.7%) and PUFAs 22:6ω3 and 18:4ω3 (AD between 0.8 and 0.5%). In the SECC, the SFA contribution was higher (78.8 ± 3.9%) and MUFA and PUFA contributions lower (11.7 ± 2.9% and 7.1 ± 1.4%) than in the other zones (Table 3).

Leptocephali also showed differences in isotopic signatures between the 3 current zones, with $\delta^{15}N$ values being different (KW and MWW: $p < 0.05$). Some separation of the $\delta^{15}N$ signatures in the 3 zones

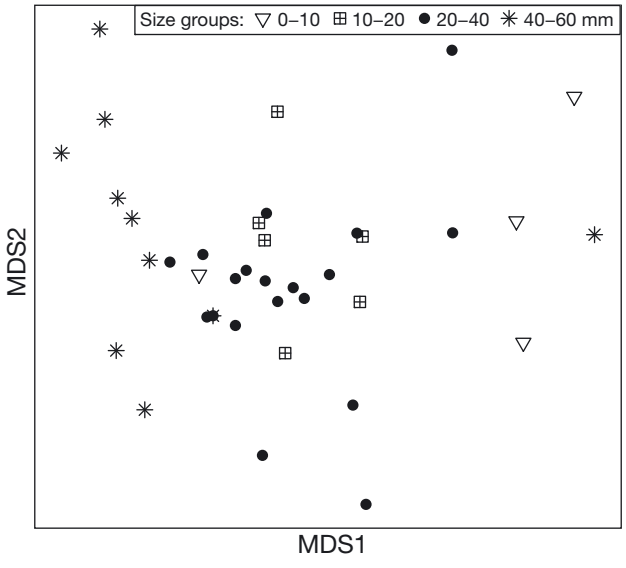


Fig. 5. Non-metric Multidimensional scaling (NMDS) based on Bray-Curtis similarity distance of total fatty acid concentration (mg $g^{-1}$) of Serrivomeridae leptocephali for 4 different size groups (see Table 2 for exact size ranges). Stress = 0.08

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Table 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) mean isotopic signature 8‰) and fatty acid (FA) relative contribution (%) of Serrivomeridae leptocephali from 4 different size groups (≤10, 10.1–20.0, 20.1–40.0 and 40.1–60.0 mm). The 5 samples of ≤10 mm larvae each include 2 larvae pooled together. All values are mean ± SD; n is the number of samples

| | ≤10 mm (n = 5) | 10.1–20.0 mm (n = 6) | 20.1–40.0 mm (n = 20) | 40.1–60.0 mm (n = 10) |
|---|---|---|---|---|
| **Stable isotopes** | | | | |
| $\delta^{15}N$ | 11.1±1.4 | 11.8±0.8 | 10.6±1.9 | 10.0±2.3 |
| $\delta^{13}C$ | −19.9±0.57 | −21.3±1.0 | −20.6±0.8 | −21.4±0.5 |
| **Saturated FA** | | | | |
| 12:0 | 0.13±0.11 | 0.06±0.06 | 0.07±0.05 | 0.04±0.02 |
| 13:0 | 0.05±0.02 | 0.04±0.03 | 0.03±0.01 | 0.03±0.01 |
| 14:0 | 4.5±0.54 | 3.84±0.88 | 3.99±0.55 | 4.35±0.61 |
| 15:0 | 2.25±0.29 | 2.07±0.27 | 2.16±0.16 | 1.95±0.12 |
| 16:0 | 33.23±5.29 | 27.01±1.33 | 27.22±2.67 | 21.95±1.67 |
| 17:0 | 1.79±0.14 | 1.76±0.24 | 1.83±0.27 | 1.56±0.21 |
| 18:0 | 13.18±3.9 | 9.9±2.25 | 9.17±2.13 | 6.42±0.62 |
| 19:0 | 0.69±0.24 | 0.32±0.14 | 0.3±0.09 | 0.24±0.08 |
| 20:0 | 0.46±0.15 | 0.48±0.39 | 0.31±0.07 | 0.32±0.09 |
| 21:0 | 0.04±0.02 | 0.03±0.01 | 0.04±0.01 | 0.03±0.01 |
| 22:0 | 0.24±0.07 | 0.16±0.03 | 0.17±0.03 | 0.14±0.03 |
| 24:0 | 0.18±0.08 | 0.13±0.11 | 0.07±0.06 | 0.08±0.07 |
| **Σ SFA (%)** | **56.73±0.91** | **45.79±0.48** | **45.36±0.51** | **37.09±0.3** |
| **Monounsaturated FA** | | | | |
| 16:1ω5 | 0.08±0.04 | 0.13±0.02 | 0.14±0.03 | 0.13±0.02 |
| 16:1ω7 | 4.95±0.81 | 6.54±1.14 | 6.31±0.68 | 8.01±1.24 |
| 16:1ω9 | 0.49±0.14 | 0.63±0.05 | 0.65±0.08 | 0.67±0.06 |
| 17:1ω7 | 0.3±0.03 | 0.27±0.07 | 0.34±0.1 | 0.33±0.08 |
| 17:1ω9 | 0.6±0.15 | 0.77±0.08 | 0.73±0.11 | 0.84±0.09 |
| 18:1ω5 | 0.05±0.03 | 0.11±0.01 | 0.11±0.03 | 0.13±0.02 |
| 18:1ω7 | 2.4±0.3 | 2.77±0.32 | 2.63±0.78 | 3.47±0.38 |
| 18:1ω9 | 7.81±2.11 | 9.33±0.64 | 8.76±1.23 | 8.92±0.94 |
| 19:1ω9 | 0.02±0.02 | 0.03±0.03 | 0.01±0.02 | 0.06±0.04 |
| 20:1ω7 | 0.06±0.02 | 0.06±0.03 | 0.07±0.02 | 0.15±0.07 |
| 20:1ω9 | 0.07±0.03 | 0.07±0.04 | 0.08±0.03 | 0.19±0.12 |
| 22:1ω11 | 0.08±0.05 | 0.06±0.03 | 0.06±0.03 | 0.05±0.02 |
| 22:1ω9 | 0.02±0.01 | 0.02±0.01 | 0.02±0.02 | 0.02±0.01 |
| 24:1ω9 | 0.1±0.03 | 0.05±0.04 | 0.07±0.05 | 0.07±0.07 |
| **Σ MUFA (%)** | **17.03±0.27** | **20.84±0.18** | **19.98±0.23** | **23.02±0.23** |

| | ≤10 mm (n = 5) | 10.1–20.0 mm (n = 6) | 20.1–40.0 mm (n = 20) | 40.1–60.0 mm (n = 10) |
|---|---|---|---|---|
| **Polyunsaturated FA** | | | | |
| 16:2ω4 | 0.36±0.21 | 0.83±0.39 | 0.68±0.3 | 0.94±0.24 |
| 16:2ω6 | 0.01±0.01 | 0.04±0.02 | 0.04±0.02 | 0.05±0.03 |
| 16:4ω3 | 0.05±0.05 | 0.07±0.04 | 0.05±0.04 | 0.03±0.02 |
| 18:2ω6 | 1.93±0.73 | 2.81±0.3 | 2.78±0.26 | 2.25±0.82 |
| 18:3ω3 | 0.48±0.3 | 0.76±0.2 | 0.79±0.26 | 1.1±0.2 |
| 18:3ω6 | 0.32±0.05 | 0.43±0.06 | 0.42±0.1 | 0.37±0.14 |
| 18:4ω3 | 0.6±0.41 | 1.17±0.29 | 1.23±0.32 | 1.24±0.34 |
| 20:2ω6 | 0.1±0.12 | 0.06±0.02 | 0.07±0.02 | 0.12±0.03 |
| 20:3 | 0.07±0.04 | 0.07±0.02 | 0.09±0.02 | 0.12±0.02 |
| 20:3ω6 | 0.16±0.05 | 0.25±0.05 | 0.25±0.06 | 0.4±0.08 |
| 20:4ω3 | 0.41±0.24 | 0.69±0.22 | 0.67±0.16 | 1.96±0.7 |
| 20:4ω6 | 3.73±1.25 | 3.69±0.77 | 3.89±0.66 | 2.46±0.74 |
| 20:5ω3 | 4.04±1.55 | 5.02±0.69 | 4.47±2.42 | 6.75±0.98 |
| 22:4ω6 | 0.03±0.06 | 0.11±0.11 | 0.09±0.12 | 0.22±0.13 |
| 22:5ω3 | 0.44±0.17 | 0.66±0.1 | 0.77±0.19 | 1.67±0.77 |
| 22:5ω6 | 2.12±0.68 | 2.36±0.4 | 2.51±0.75 | 2.08±0.3 |
| 22:6ω3 | 10.25±4.35 | 13.54±1.81 | 14.99±2.22 | 17.36±1.95 |
| **Σ PUFA (%)** | **25.17±0.6** | **32.55±0.32** | **33.78±0.47** | **39.01±0.44** |
| **Branched-chain FA** | | | | |
| 14:0iso | 0.04±0.01 | 0.02±0.01 | 0.03±0.01 | 0.02±0.01 |
| 15:0anteiso | 0.11±0.02 | 0.05±0.02 | 0.05±0.02 | 0.03±0.01 |
| 15:0iso | 0.21±0.07 | 0.15±0.02 | 0.17±0.04 | 0.18±0.04 |
| 16:0iso | 0.17±0.07 | 0.12±0.02 | 0.13±0.04 | 0.14±0.03 |
| 17:0anteiso | 0.15±0.09 | 0.11±0.03 | 0.1±0.02 | 0.08±0.02 |
| 17:0iso | 0.27±0.05 | 0.25±0.04 | 0.27±0.07 | 0.29±0.05 |
| 18:0iso | 0.13±0.05 | 0.12±0.09 | 0.13±0.05 | 0.14±0.03 |
| **Σ BrFA (%)** | **1.07±0.05** | **0.82±0.03** | **0.88±0.04** | **0.88±0.03** |
| Total FA (mg $g^{-1}$) | 5.82±1.08 | 5.19±1.92 | 4.62±1.41 | 4.36±1.54 |
| Σ ω3 (%) | 16.36±1.01 | 21.9±0.48 | 22.97±0.8 | 30.01±0.71 |
| Σ ω6 (%) | 8.39±0.37 | 9.74±0.22 | 10.05±0.25 | 7.95±0.28 |
| Σ EFA (%) | 18.02±2.38 | 22.25±1.09 | 23.35±1.77 | 26.57±1.20 |

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Table 3. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) mean isotopic signature (‰) and fatty acid (FA) relative contribution (%) of particulate organic matter (POM) from 3 different currents: South Equatorial Countercurrent (SECC), South Equatorial Current (SEC), and Sub-Tropical Countercurrent (STCC). All values are mean ± SD; n is the number of samples

| | POM in SECC (n = 15) | POM in SEC (n = 15) | POM in STCC (n = 9) |
|---|---|---|---|
| **Stable isotopes** | | | |
| $\delta^{15}N$ | 7.7±2.3 | 6.1±2.2 | 4.1±1.0 |
| $\delta^{13}C$ | −26.2±0.9 | −26.6±0.5 | −27.0±0.2 |
| **Saturated FA** | | | |
| 12:0 | 0.48±0.19 | 0.6±0.32 | 0.41±0.23 |
| 13:0 | 0.1±0.03 | 0.13±0.03 | 0.07±0.01 |
| 14:0 | 14.05±1.48 | 14.6±1.74 | 13.14±2.03 |
| 15:0 | 1.71±0.25 | 1.64±0.25 | 1.8±0.11 |
| 16:0 | 37.75±1.9 | 34.87±2.43 | 34.59±2.63 |
| 17:0 | 0.74±0.11 | 0.64±0.13 | 0.61±0.06 |
| 18:0 | 21.31±2.2 | 18.29±2.56 | 19.53±2.08 |
| 19:0 | 0.45±0.08 | 0.42±0.08 | 0.54±0.12 |
| 20:0 | 0.94±0.13 | 0.79±0.08 | 0.79±0.08 |
| 21:0 | 0.06±0.02 | 0.07±0.02 | 0.05±0.02 |
| 22:0 | 0.62±0.17 | 0.56±0.11 | 0.55±0.11 |
| 24:0 | 0.6±0.26 | 0.56±0.2 | 0.59±0.3 |
| **Σ SFA (%)** | **78.83±3.86** | **73.16±4** | **72.7±6.72** |
| **Monounsaturated FA** | | | |
| 16:1ω5 | 0.08±0.04 | 0.08±0.03 | 0.07±0.03 |
| 16:1ω7 | 3.62±0.73 | 5.62±1.1 | 3.76±0.75 |
| 16:1ω9 | 1.99±0.49 | 2.2±0.46 | 1.99±0.57 |
| 17:1ω7 | 0.39±0.1 | 0.34±0.04 | 0.37±0.12 |
| 18:1ω7 | 0.65±0.16 | 0.73±0.14 | 0.8±0.2 |
| 18:1ω9 | 4.63±0.91 | 5.32±0.71 | 6.36±1.9 |
| 22:1ω11 | 0.36±0.11 | 0.39±0.12 | 0.42±0.17 |
| **Σ MUFA (%)** | **11.71±2.87** | **14.68±2.1** | **13.8±3.32** |

| | POM in SECC (n = 15) | POM in SEC (n = 15) | POM in STCC (n = 9) |
|---|---|---|---|
| **Polyunsaturated FA** | | | |
| 16:2ω4 | 0.97±0.32 | 1.98±0.4 | 2.45±0.56 |
| 16:4ω3 | 0.26±0.08 | 0.22±0.04 | 0.18±0.07 |
| 18:2ω6 | 1.1±0.21 | 1.24±0.21 | 1.34±0.45 |
| 18:3ω3 | 0.44±0.07 | 0.44±0.08 | 0.49±0.14 |
| 18:3ω6 | 0.4±0.09 | 0.43±0.07 | 0.42±0.06 |
| 18:4ω3 | 1.16±0.24 | 1.93±0.55 | 2.16±0.92 |
| 20:4ω3 | 0.09±0.03 | 0.08±0.01 | 0.1±0.03 |
| 20:4ω6 | 0.11±0.03 | 0.14±0.05 | 0.16±0.04 |
| 20:5ω3 | 0.88±0.21 | 0.9±0.18 | 1.43±0.3 |
| 22:2ω6 | 0.31±0.2 | 0.32±0.13 | 0.13±0.05 |
| 22:5ω6 | 0.21±0.15 | 0.15±0.08 | 0.21±0.11 |
| 22:6ω3 | 1.17±0.51 | 1.71±0.76 | 2.43±1.31 |
| **Σ PUFA (%)** | **7.11±1.44** | **9.53±1.96** | **11.5±3.47** |
| **Branched-chain FA** | | | |
| 14:0iso | 0.34±0.12 | 0.35±0.08 | 0.37±0.09 |
| 15:0anteiso | 0.46±0.08 | 0.4±0.05 | 0.5±0.06 |
| 15:0iso | 0.39±0.06 | 0.34±0.04 | 0.37±0.04 |
| 16:0iso | 0.21±0.06 | 0.21±0.04 | 0.23±0.02 |
| 17:0anteiso | 0.64±0.18 | 0.99±0.26 | 0.23±0.02 |
| 17:0iso | 0.06±0.01 | 0.05±0.02 | 0.04±0.01 |
| 18:0iso | 0.23±0.09 | 0.28±0.06 | 0.29±0.13 |
| **Σ BrFA (%)** | **2.34±0.46** | **2.62±0.36** | **2.03±0.08** |
| Total FA (mg $g^{-1}$) | 2.86±0.83 | 4.49±0.77 | 3.33±1.53 |
| Σ ω3 (%) | 4.00±0.87 | 5.27±1.49 | 6.81±2.4 |
| Σ ω6 (%) | 2.14±0.14 | 2.28±0.11 | 2.26±0.14 |
| Σ EFA (%) | 2.16±0.25 | 2.75±0.33 | 4.02±0.55 |

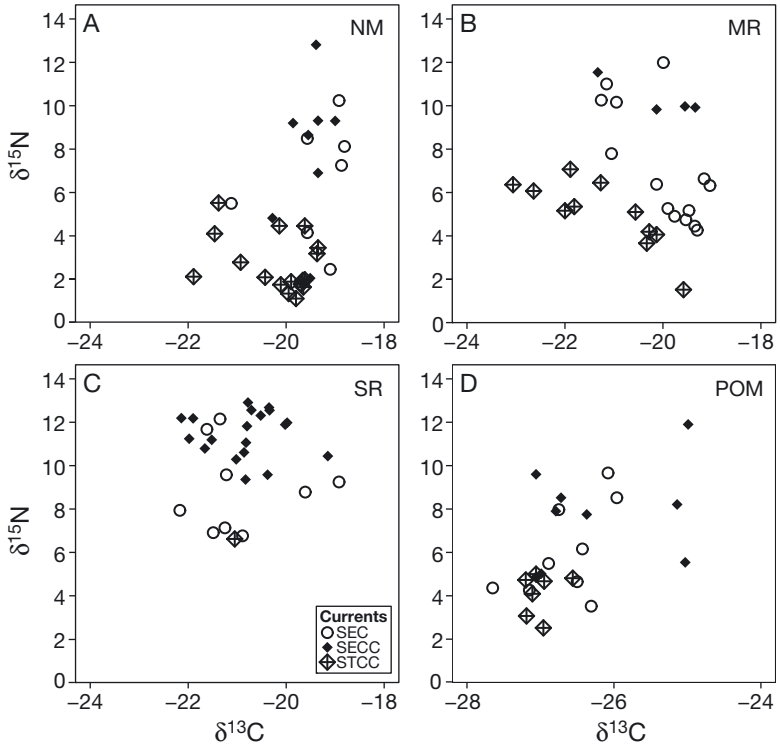


Fig. 6. Carbon and nitrogen stable isotope values of (A) Nemichthyidae (NM), (B) Muraenidae (MR), (C) Serrivomeridae (SR) leptocephali and (D) POM from the 3 current zones South Equatorial Countercurrent (SECC), South Equatorial Current (SEC), and South Tropical Countercurrent (STCC)

was also seen within families, except that there was only 1 Serrivomeridae larva analysed from the STCC (Fig. 6A–C). For Serrivomeridae leptocephali there was no significant difference in $\delta^{13}C$ values between larvae caught in the different current zones, whereas $\delta^{15}N$ signatures were different between larvae from the SECC and SEC (*t*-test: $p < 0.05$) (Fig. 6C). Muraenidae from the SEC and SECC had differences in both their $\delta^{13}C$ and $\delta^{15}N$ values (KW and MWW: $p < 0.05$) (Fig. 6B). There were also differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures of Nemichthyidae found in the STCC and those in the SECC

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and SEC, respectively (KW and MWW: $p < 0.05$) (Fig. 6A). FA profiles for the leptocephali caught in the 3 different currents were not significantly different (KW and MWW: $p > 0.1$).

## DISCUSSION

### FA and SI signatures of leptocephali and POM

This study examined the variation in the FA profiles and SI signatures of 3 families of leptocephali and their likely food source of POM. Samples were collected across a wide range of latitudes and longitudes of the WSP. Previous studies in the western North Pacific (Miyazaki et al. 2011) and western Indian Ocean (Feunteun et al. 2015) analysed leptocephali and POM from much more limited areas of the ocean.

The chemical composition of the different taxa of leptocephali is generally comparable (Pfeiler 1999, Bishop et al. 2000) as confirmed by the FA profiles of the 3 families of leptocephali studied in the WSP that show many similarities. There were however, also some differences. The Nemichthyidae, Muraenidae, and Serrivomeridae larvae had the same number of major FAs and categories of FA markers showed similar proportions. Deibel et al. (2012) also found similar FA compositions for 6 other taxa of leptocephali (members of the 4 families: Congridae, Muraenesocidae, Ophichthidae and Nettastomatidae) from coastal, shelf or deep slope habitats off northwest Australia. However, despite these overall similarities, the present study found small but significant differences between the FA profiles of Muraenidae larvae and those of Nemichthyidae and Serrivomeridae. The similarities in FA compositions of the 3 families were largely due to the FAs that were present in large proportions: 16:0, 18:0, 22:6ω3, 20:5ω3, 16:1ω7, and 18:1ω9. The same 6 FAs also had the highest proportions in leptocephali from northwestern Australia (Deibel et al. 2012).

SFAs 16:0 and 18:0 (mean ~24 and 8% of TFAs in all 3 families of leptocephali) are the most abundant FAs in nature, as they are products of lipogenesis in all organisms (Dewick 1997). Similarly, ω3 FAs are abundant in marine zooplankton (Lee et al. 2006) and fish larvae (Grote et al. 2011), as they were in the leptocephali, where they represented 24 to 33% of total FAs, depending on family. Amongst the ω3 FAs, the EFAs 20:5ω3 and 22:6ω3 are major components of cell membrane phospholipids (Sargent et al. 1993) and are also important for larval growth and development (Sargent et al. 1993, Furuita et al. 2006, Grote et al. 2011). Marine consumers, however, cannot synthetize essential ω3 and ω6 PUFAs (Dalsgaard et al. 2003, Lee et al. 2006, Kattner et al. 2007), so they need to obtain them through feeding (Canuel et al. 1995, Styrishave & Andersen 2000, Meziane et al. 2002). Thus, high proportions of ω3 and ω6 in leptocephali suggest that leptocephali feed on material containing or originating from autotrophic organisms or primary consumers in which ω3 and ω6 are abundantly present (Scott et al. 2002, Dalsgaard et al. 2003, Lee et al. 2006). There is also the possibility that leptocephali, as do some heterotrophic organisms (Dalsgaard et al. 2003, Canuel et al. 1995), synthesize these FAs from a precursor such as 18:1ω9. This MUFA is abundant in leptocephali (mean of 7.6 ± 1.6% for the 3 Families combined in this study, and 6.4 ± 1.3% in Deibel et al. 2012) and can be obtained from feeding on zooplankton or other heterotrophic sources in pelagic food webs (Dalsgaard et al. 2003, Lee et al. 2006).

In this study, POM was characterized by a strong contribution of SFAs (~75% of TFAs) and a low contribution of ω3 (~5%), MUFAs (~13%) and PUFAs (~9%), which typically indicates a low nutritional quality for the bulk organic matter, as suggested by other studies in diverse aquatic systems (e.g. North Sea: Boon & Duineveld 1996; Amazon River: Mortillaro et al. 2011). Interestingly, the important FATMs 18:1ω9 and 16:1ω7 (markers of heterotrophs and autotrophic microplankton, respectively) were the main unsaturated FAs that contributed to the TFAs of the POM (~5 and 4%, respectively). Also, BrFA markers made a low contribution to TFAs (~2%) in the POM. Most of these FATMs are typically biosynthesized in large amounts by bacteria (Kaneda 1991, Ederington et al. 1995, Meziane & Tsuchiya 2000) and because decaying bulk POM is rapidly colonised by bacteria (Skerratt et al. 1995, Najdek et al. 2002), it would be expected to be rich in these BrFAs. Thus, the FA composition may indicate that the POM sampled within the chlorophyll maximum layer during this study is likely to be composed of a large non-living POM fraction (e.g. detritus, faecal pellets) with a low bacterial influence and to a lesser extent living organic matter content (autotrophic and heterotrophic microplankton) than that which leptocephali would preferentially assimilate.

The diet of an organism can usually be examined by assuming a mean isotopic trophic enrichment of 1‰ in $\delta^{13}C$ (DeNiro & Epstein 1978, Rau et al. 1983) and of 3.4‰ in $\delta^{15}N$ (DeNiro & Epstein 1981, Minagawa & Wada 1984) from food source to consumer.

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The $\delta^{15}N$ values of POM (2.5 to 12‰) and leptocephali (0.5 to 13‰) in the present study heavily overlapped, so no clear trophic enrichment between $\delta^{15}N$ signatures of POM and leptocephali was evident, except perhaps for Serrivomeridae. One explanation for this is that there could be a mismatch between the POM sampled on the filters and the types of POM that leptocephali consume and what they assimilate from this, as mentioned above and described further below. A similar result was also described by Otake et al. (1993), who found that the $\delta^{15}N$ signature of Congridae leptocephali (*Conger myriaster*) overlap with that of POM (~11–13‰). A wide range of POM $\delta^{15}N$ values overlapping with those of leptocephali was also seen in studies in the western North Pacific (Miyazaki et al. 2011) and western Indian Ocean (Feunteun et al. 2015). Other studies examining geographic variations in SI signatures of POM and organisms found similarly high values of $\delta^{15}N$ for POM (Waite et al. 2007, Lorrain et al. 2015). This is presumably a result of regional variations that occur globally in the $\delta^{15}N$ values of primary producers that contribute to POM, with some areas such as parts of the South Pacific likely having higher values than other regions of the world (Somes et al. 2010). The $\delta^{13}C$ gap of about 7‰ between mean values (2‰ gap for closest values) of POM and leptocephali is also clearly larger than the expected 1‰ gap if leptocephali were consuming POM within the chlorophyll maximum and assimilating all of it. The values of $\delta^{13}C$ of the POM we collected within the chlorophyll maximum (about −25 to −27‰) were lower than those usually reported for oceanic environments (e.g. Waite et al. 2007, Hwang et al. 2009, Miyazaki et al. 2011). However, values of $\delta^{13}C$ of bulk POM as low as −25 to −29‰ have been reported at similar depths in several offshore areas (Jeffrey et al. 1983, Druffel et al. 2003, Feunteun et al. 2015, Soares et al. 2015). These low $\delta^{13}C$ bulk POM values are measured within or just below the depth of the chlorophyll maximum and this may be a common pattern in the open ocean (Jeffrey et al. 1983, Druffel et al. 1998, Hwang et al. 2009, Close et al. 2014). Moreover, POM is considered to be organic material that usually forms bigger particles commonly called 'marine snow' that contains various living organisms (Alldredge & Silver 1988, Shanks & Walters, 1997, Kiørboe 2000). Therefore, bulk POM composition in the open ocean likely depends on plankton communities, but also on the non-living degraded part of the organic matter as well as bacterial communities. Isotopic fractionation and thus, the signature of the bulk POM, can differ due to variations in physiological processes between phytoplanktonic groups (metabolic $^{13}C$ enrichment, C-fixation) that are related to cell size, physiology and growth rate (Goericke & Fry 1994, Popp et al. 1998). POM with widely varying $\delta^{13}C$ and $\delta^{15}N$ signatures can be found at different depths (Jeffrey et al. 1983, Waite et al. 2007, Miyazaki et al. 2011, Feunteun et al. 2015), so our POM isotopic values may be related to the specific signatures within the chlorophyll maximum, which is not targeted in studies that only sample at particular depths. Or the POM isotopic values could be partly related to aspects of the biological communities in our study area which have not yet been studied. These factors might explain the large range of values in carbon and nitrogen signatures of POM in the WSP.

It is also possible that leptocephali do not feed exclusively within the chlorophyll maximum or do not consume every type of POM. It is more likely that leptocephali select specific types of POM particles to eat, such as some types of marine snow or the appendicularian houses that have been directly observed in the intestines of leptocephali (Mochioka & Iwamizu 1996, Miller et al. 2011). In addition, some materials such as zooplankton faecal pellets observed in gut contents (Otake et al. 1993, Miller et al. 2011) are unlikely to be digested. Further differences between the SI signatures of the POM sample collected on a filter and those that leptocephali consume and assimilate might be related to the transparent exopolymer particles (i.e. TEP; Passow 2002) produced by microorganisms (phytoplankton, bacteria) that aggregate within POM. TEPs are mainly composed of carbohydrate molecules that aggregate into marine snow (Holloway & Cowen 1997, Skoog et al. 2008, Engel et al. 2012) and are at the interface between dissolved and particulate organic carbon (Passow 2002). Therefore, a significant fraction of TEP is likely not retained on the GF/F filters used to collect POM from the water samples, leading to these compounds being only partially included in POM carbon measurements; whereas they would have been present in the POM materials assimilated by leptocephali. In addition, carbohydrates are molecules that have no nitrogen, thus only the $\delta^{13}C$ signature of these compounds would be reflected in leptocephali if they were assimilated (Feunteun et al. 2015). Also, leptocephali have a long larval duration, so as previous studies (Miyazaki et al. 2011, Feunteun et al. 2015) have noted, larger larvae could be transported into collection areas after originating from other areas where they have fed on POM with different isotopic signatures.

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### Feeding ecology of leptocephali

Considering the various possible factors discussed above, the SI and FA analyses of this study may still be generally consistent with the hypothesis that leptocephali feed on POM, which has been indicated by direct observations of their gut contents (Otake et al. 1993, Miller et al. 2011) and an analysis of their trophic position (Miller et al. 2013). The observational studies of the gut contents of leptocephali that have found POM components such as amorphous materials, appendicularian and other zooplankton fecal pellets, and discarded appendicularian houses (Otake et al. 1993, Mochioka & Iwamizu 1996, Miller et al. 2011), provide direct evidence of POM consumption by a variety of eel larvae taxa. Also, Feunteun et al. (2015) characterised SI signatures of many taxa of zooplankton along with 12 taxa of leptocephali and POM and did not find evidence of leptocephali feeding on zooplankton. However, as in previous SI studies (Miyazaki et al. 2011, Feunteun et al. 2015) it remains unclear what types and components of POM are used by various taxa of leptocephali, and methodological issues related to the filtration and analysis of POM may make it difficult to see clear linkages using SI analyses.

The FATM compositions determined in the present study and those of Deibel et al. (2012) indicate that leptocephali obtain their nutrition from various sources that include both phytoplankton (16:1ω7, 20:5ω3), dinoflagellates (22:6ω3) and heterotrophic microorganisms such as bacteria and protozoans (18:1ω9) (Dalsgaard et al. 2003), all of which are likely to contribute to POM composition (Alldredge & Silver 1988, Shanks & Walters, 1997, Kiørboe, 2000). As explained above and also suggested in other studies (Dalsgaard et al. 2003, Pitt et al. 2009), the composition of the food contents in the intestines of leptocephali could be different from what they assimilate from ingested food. Our FA results suggest that it is the materials from living organisms associated with POM that are mainly assimilated by leptocephali (e.g. FATM of autotrophic and heterotrophic organisms). Indeed, Muraenidae larvae were rich in PUFAs and ω3, meaning even if they feed on FA-poor food sources, they are able to preferentially assimilate and store the FA-rich components of the POM. Conversely, Serrivomeridae larvae had the highest SFA and lowest PUFA and ω3 proportions, which suggests that they may feed on POM opportunistically, with no selective assimilation or FA storage.

### FA and SI composition of Serrivomeridae size classes

Although no major differences were found in the isotopic compositions of the 4 size classes of Serrivomeridae leptocephali, clear changes in their FA composition were detected. The only significant SI differences were that $\delta^{13}C$ values differed between the largest (–21.4‰) and smallest (–19.9‰) size classes, but no significant differences were found in $\delta^{15}N$ values. In contrast, all the major categories of FAs showed at least minor changes with size, either increasing MUFA and PUFA or decreasing SFA contributions to TFAs.

The dynamic nature of lipid metabolism and the influence of dietary lipids when larvae first feed can cause FA composition shifts during ontogeny (Wiegand 1996, Rainuzzo et al. 1997, Plante et al. 2006, Grote et al. 2011), and shifts in the growth strategies of leptocephali when they reach large sizes may result in physiological changes (Pfeiler 1999, Bishop et al. 2000). The decrease in percentage of SFAs in larger Serrivomeridae larvae might be caused by 16:0 and 18:0 fatty acids being used as major substrates for energy production (Sargent 1995). MUFAs and PUFAs are energy sources for development and growth, especially during the larval stage when fish need energy for organogenesis, fast growth and basal metabolism (Abi-ayad et al. 2004, Plante et al. 2007), and these tended to increase in proportion in the leptocephali. Moreover, the PUFA 20:5ω3 is an important energy substrate, along with 22:6ω3, which is also one of the principal components of cell membranes (Dalsgaard et al. 2003). In the family Serrivomeridae, the increase of MUFA and PUFA percentages with size suggest that these energetically rich FAs are not preferentially used to provide energy during larval growth. Thus, larvae may be preferentially storing energy dense FAs, such as long chain FAs and PUFAs that they cannot synthesize and which are in low concentrations in POM, to support the later high-energy requirements of metamorphosis or to use during the early juvenile stage, when food availability may be uncertain. In bonefish leptocephali, SFAs, MUFAs, and PUFAs were used during metamorphosis (for example, a 30% use of 16:0, 12% of 16:1ω7, 7% of 18:5ω3), but most of the fatty acid 22:6ω3 was conserved (Padrón et al. 1996). More species of leptocephali, including those that reach larger sizes than the Serrivomeridae, should be examined in future research to further evaluate how FA composition changes during growth and metamorphosis.

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### Regional differences in leptocephali and POM

The isotopic signatures of leptocephali in the 3 current zones showed variations that appeared to be related to the POM signatures in each zone. Both $\delta^{15}N$ and $\delta^{13}C$ values of Nemichthyidae and Muraenidae larvae decreased to some extent from north (SECC) to south (STCC) and the same pattern was seen in POM signatures, especially for $\delta^{15}N$. The compositions of the FAs of POM and leptocephali also varied significantly among the current zones. This suggests there is regional variability in the nutritional quality (SFA and 22:6ω3 contributions) and availability (18:1ω9, 16:1ω7 and 18:4ω3) of POM. For example, the SECC POM had the highest percentage of SFAs and the lowest percentage of MUFAs and PUFAs, which indicates a lower nutritional quality. This may be reflected in the FA compositions of Serrivomeridae larvae that were mostly collected in the SECC, since they also had high SFA content and low PUFA content compared to the other, more widely distributed families. In contrast, the SEC and STCC tended to have POM with higher nutritional quality with larger PUFA and ω3 contributions.

These observed differences in POM may have affected the composition of the leptocephali because many studies indicate that diversity and bioavailability of POM play a key role in ecosystem functioning by supporting the trophic network (Grémare et al. 1997, Carlier et al. 2007), with regional variability in trophic resources being reflected in the consumers (Cartes et al. 2014, Chouvelon et al. 2014). Our sampling was spread across 20 degrees of latitude and crossed 3 current systems, so it is not surprising that geographic variations were observed in both the SI signatures and FA compositions of POM and leptocephali. This variation was likely due to latitudinal differences in planktonic community structure in the different current systems and temperature regimes because differences in the $\delta^{15}N$ of primary producers can cause variations in the $\delta^{15}N$ values of POM and organisms between regions (Somes et al. 2010, Cartes et al. 2014, Lorrain et al. 2015, Soares et al. 2015). Longitudinal differences in the $\delta^{15}N$ of mesozooplankton have also been observed around New Caledonia (Hunt et al. 2015).

It is clear, though, that questions remain about the feeding ecology of leptocephali because this study and the study in the western Indian Ocean (Feunteun et al. 2015) have found differences in the compositions of other taxa of leptocephali collected from the same area. Feunteun et al. (2015) found that Nemichthyidae leptocephali had lower $\delta^{15}N$ values than Muraenidae and Serrivomeridae leptocephali (and other families), and similar differences were seen between Nemichthyidae and Muraenidae larvae in the STCC zone in the present study. Similar $\delta^{15}N$ differences were also seen between *Anguilla japonica* and Congridae larvae of the genus *Ariosoma* (Miyazaki et al. 2011) in the western North Pacific. Possible reasons proposed for these differences are similar to those already discussed above, such as different types of POM being selected, the taxa feeding at different depths where the POM is slightly different, differences in assimilation of materials from the POM, or physiological differences among leptocephali (Miyazaki et al. 2011, Feunteun et al. 2015). To begin to evaluate these possibilities, more research is needed using SI and FA analyses of leptocephali and other food web components in a wider range of areas to gain a better understanding of the feeding ecology of these fish larvae.

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