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Contribution to the Themed Section: '*Mesopelagic resources—potential and risk*'

Original Article

Feeding habits estimated from weight-related isotope variations of mesopelagic fish larvae in the Kuroshio waters of the northeastern East China Sea

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Bulk carbon and nitrogen stable isotope (SI) ratios (δ^{13}C and δ^{15}N) were analysed to investigate the feeding habits of six taxa of mesopelagic fish larvae inhabiting the Kuroshio waters of the northeastern East China Sea. Large variation in tissue SI during early larval periods suggested maternal effects from parent fishes, and non-selective feeding on a variety of plankton species due to poor swimming ability. The similarity between SI ratios measured in larval tissues and those estimated for eggs of an "income breeder" in the spawning area support an "income breeder" strategy in *Diaphus* slender type and *Vinciguerria nimbaria*, while *Lipolagus ochotensis* seemed to show "capital breeder"-like characteristics. SI ratios of the fish larvae studied became relatively constant at species-specific body dry-weights (0.5–1.0 mg), probably due to the commencement of selective feeding, meaning SI ratios during late larval periods could be used for trophic position analysis. There was great overlap (44.6–76.5%) in trophic niche among the larval fishes within the same taxonomic family of Myctophidae. Even if principal diet components cannot be identified with gut contents analyses, diet information from other fish species occupying a similar isotopic niche can thus improve our understanding of the diets of larval fishes.

Keywords: breeder type, feeding habit, Kuroshio water, maternal effect, mesopelagic fish larvae, weight-related isotope variation

Introduction

Mesopelagic fishes, with a biomass of at least 10 billion tons, dominate the world's total fish biomass (Irigoien *et al.*, 2014). These fishes are an important food source for commercially important fish species such as yellowfin tuna (Potier *et al.*, 2007), and, since most species show active diurnal vertical migration (Watanabe *et al.*, 1999; Luo *et al.*, 2000; Yatsu *et al.*, 2005), also have important implications for biogeochemical cycling in the ocean. For instance, mesopelagic fishes provide trophic connectivity and transport organic carbon between the surface and the mesopelagic layers (Kaartvedt *et al.*, 2012; Irigoien *et al.*, 2014).

The Kuroshio region in the northeastern East China Sea (ECS) is regarded as an important spawning and nursery ground, especially in winter and early spring, for commercial fishes such as Japanese sardine (*Sardinops melanostictus*) and chub mackerel (*Scomber japonicus*) (Sugisaki *et al.*, 2010; Chen *et al.*, 2014), whereas various larvae of mesopelagic fishes such as Japanese lanternfish (*Notoscopelus japonicus*) and Eared blacksmelt (*Lipolagus ochotensis*) also frequently occur and may compete for food resources and marine habitat (Sassa and Hirota, 2013). When larval fishes replace their diets with various exogenous sources after yolk absorption they still have a weak swimming ability due to

undeveloped body structures such as fins and muscles, and thus are at enhanced risk of predation. Hence, growth and nutrient condition are commonly considered to be the main factors determining larval fish survival rates (Anderson, 1988; Bailey and Houde, 1989). Therefore, clarifying the feeding habits of these mesopelagic fish species in their larval or juvenile stage is important for understanding growth and survival rates, which contribute greatly to the abundance of fishery resources.

Several studies have shown that six taxa of mesopelagic fishes, *Diaphus* slender type, *Myctophum asperum*, and *N. japonicus* (Myctophidae), *L. ochotensis* (Microstomatidae), *Sigmops gracilis* (Gonostomatidae), and *Vinciguerria nimbaria* (Phosichthyidae) are dominant species in the northeastern ECS during winter, with the species composition, spatial and vertical distributions, and reproductive seasonality of these mesopelagic fishes larvae having been described in detail (Sassa *et al.*, 2004; Watanabe *et al.*, 2010; Sassa and Hirota, 2013; Sassa and Konishi, 2015). Specifically, the depth preferences of these fish larvae show large variation: *Diaphus* slender type, *N. japonicus*, *M. asperum*, and *V. nimbaria* occurred mainly in the 25- to 80-m depth layers, while *L. ochotensis* and *S. gracilis* were centered in relatively deeper layers at 30- to 100- and 55- to 100-m depth, respectively (Boehlert *et al.*, 1992; Watanabe *et al.*, 2010). Feeding habits of these mesopelagic fishes have been investigated mainly based on stomach contents. For example, *V. nimbaria* in its adult stage mainly preys upon Oncaeidae and Corycaeidae in the equatorial zone (Champalbert *et al.*, 2008). In the larval stage, *M. asperum* feeds mainly on ostracods and polychaetes, while *Diaphus spp.* are reported to feed on appendicularian houses, copepod nauplii, calanoid copepodites and *Oithona* spp. depending on species and body size (Sassa and Kawaguchi, 2004, 2005). However, feeding habits of most of these mesopelagic fishes in the larval stage remain unclear.

Stable isotopes (SIs) are effectively used for elucidating the time-integrated structures and dynamics of food webs, based on their enrichments between each trophic level (e.g. Minagawa and Wada, 1984; Post, 2002; McCutchan *et al.*, 2003). For example, stable carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N) has been used to demonstrate spatial variations in the diet of Lanternfish (Myctophidae, 42- to 122-mm standard body length (SL)) (Flynn and Kloser, 2012), as well as differences in trophic positions within the overlapping distribution of 18 dominant mesopelagic fish species (13- to 193-mm SL) in the western Mediterranean (Valls *et al.*, 2014).

SIs in fish tissues have also been studied with other biotic factors (e.g. body length, body weight, and body-size ratio) to clarify the shift in trophic level, diet, habitat, and migration between juvenile and adult stages in epipelagic fishes (Deudero *et al.*, 2004; Wells and Rooker, 2009; Laiz-Carrión *et al.*, 2015) and mesopelagic fishes (Cherel *et al.*, 2010; Choy *et al.*, 2012). Shifts in tissue SI with body size in the larval stage have also been studied for some epipelagic fishes, such as *Sardina pilchardus* (Laiz-Carrión *et al.*, 2011), *Engraulis encrasicolus* (Quintanilla *et al.*, 2015) and *Thunnus thynnus* (García *et al.*, 2017), but the presence of such shifts are still largely unknown for mesopelagic fish larvae.

When SI variations are examined for fishes before the flexion stage, there is the potential for a maternal effect on larval tissue SI, i.e. the SI in fish larvae at this time reflect two factors: the isotopes of their diets, and the isotopes of parent fishes (Uriarte *et al.*, 2016). McBride *et al.* (2015) reported that δ^{13}C and δ^{15}N differences among the fish species during the early period of larval stage may be linked to energy acquisition and allocation to egg production. Some fish species (e.g. Atlantic salmon and Winter flounder) spawn and feed in separate areas, during different seasons by storing energy and utilizing it later for reproduction (termed as "capital breeder"), whereas some other fish species (e.g. Zebrafish and Bay anchovy) spawn using energy acquired locally, allocating energy directly to reproduction (termed an "income breeder") (McBride *et al.*, 2015). Based on diet-switching feeding experiments, Tanaka *et al.* (2016) successfully demonstrated that Japanese anchovy (*Engraulis japonicus*) were "income breeders," because the δ^{13}C and δ^{15}N ratios in eggs closely follow the isotope ratios of the food of adult fish (hereafter, termed as "$\delta_{adultfood}$") which the fishes had incorporated just before spawning (i.e. $\Delta\delta^{13}C = \delta^{13}C_{egg}—\delta^{13}C_{adultfood} = 0.1–1.6‰$, $\Delta\delta^{15}N = \delta^{15}N_{egg}—\delta^{15}N_{adultfood} = 0.9–2.0‰$). Even in the field, therefore, the difference or similarity between the SI ratios measured in early larval fishes and the SI ratios estimated for "income breeder" eggs from that spawning ground represents a potentially useful tool for identifying the breeding type of targeted fishes. Recently, a quantitative analytical approaches in SIBER (Stable Isotope Bayesian Ellipses in R) was introduced by Jackson *et al.* (2011), in which SI data sets are used for comparing isotopic niches among and within communities. In this study, we examined δ^{13}C and δ^{15}N of six taxa of larvae of mesopelagic fishes (i.e. *M. asperum*, *N. japonicus* and *Diaphus* slender type, *L. ochotensis*, *S. gracilis*, and *V. nimbaria*) which are dominant in the micronektonic fish communities of the Kuroshio region of the East China Sea during the late winter, and aimed to assess: (i) biotic and environmental factors affecting the weight-specific isotopic shifts in their larval stages; and (ii) the species-specific feeding habits based on the isotopic niche overlaps indicated by the SIBER approach.

Material and methods

Study area and sample collection

Larval fish samples were collected at 112 stations in the shelf-break region of the northeastern ECS (Figure 1) during cruises of the *TV Wakatori-Maru* (Tottori Prefecture, Japan) from 01 to 24 February 2009 and 28 January to 21 February 2010. A paired bongo net (Posgay and Marak, 1980) with 70-cm mouth diameter, 335-μm mesh, flowmeters, and a depth metre were used for quantitative sampling. A double-oblique tow was conducted at each station from the surface down to 100-m depth or 10 m above the bottom at shallow stations. Because the targeted species are generally distributed in the epipelagic layer without diel vertical migration to mesopelagic layer in their larval stages (Moser and Smith, 1993; Sassa *et al.*, 2002, 2004), sampling was performed regardless of day or night conditions. In order to consider the maternal effect on isotope ratios in the larval fishes, adult fish samples of *Diaphus* slender type, *L. ochotensis* and *M. asperum* were collected using midwater otter trawls (9-mm mesh) during a cruise of the *RV* Kaiyo-Maru No.7 (Nippon Kaiyo Co. Ltd., Japan) between 18 February and 12 March 2008. Adult fish samples of *S. gracilis* and *V. nimbaria* were collected using Matsuda-Oozeki-Hu trawl tows (MOHT, 1.59-mm mesh net) during a cruise of the *RV* Yoko-Maru (Japan Fisheries Research and Education Agency) from 18 to 28 February 2015. Adult *N. japonicus* were not caught during the surveys. The position of the Kuroshio axis during each cruise was determined based on the location of 16.5°C isotherm at 200-m depth (Kawai, 1972). The sampling area in the study was thus divided into two parts: the area east of the Kuroshio axis and the area west of the Kuroshio

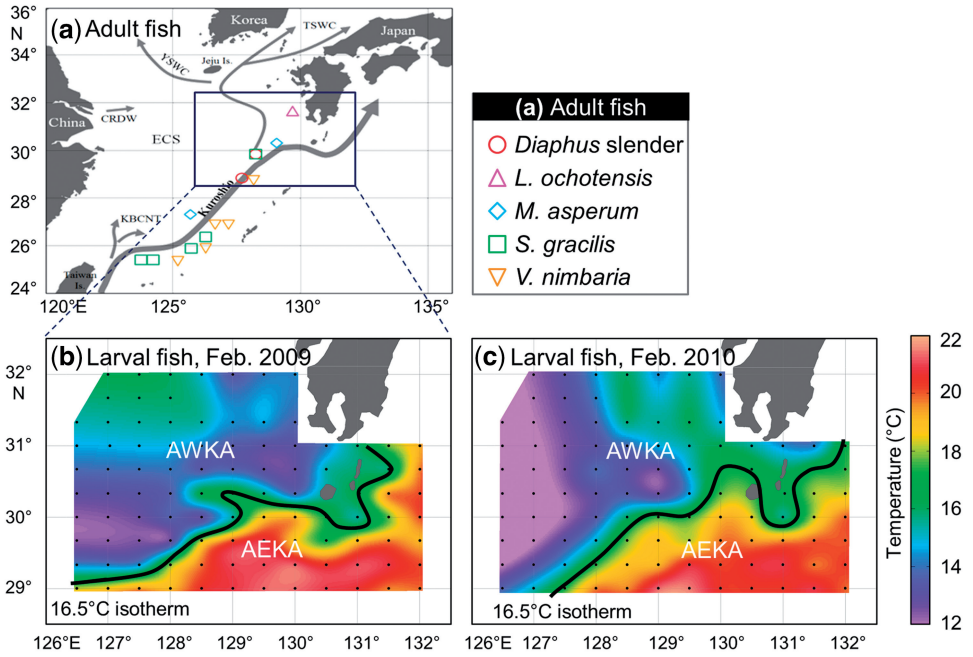

Figure 1. Adult fish sampling stations (a) for *Diaphus* slender type, *L. ochotensis*, and *M. asperum* in February and March 2008 and *S. gracilis* and *V. nimbaria* in February 2015, and larval fish sampling stations (black dots) in (b) February 2009 and (c) February 2010 in the Kuroshio waters of the northeastern East China Sea, on a map of sea water temperature at 200-m depth layer. ECS, East China Sea; KBCNT, Kuroshio Branch Current North of Taiwan; TSWC, Tsushima Warm Current; YSWC, Yellow Sea Warm Current; CRDW, Changjiang River Diluted Water. The black solid lines in (b) and (c) represent the position of the Kuroshio axis as defined by the 16.5°C isotherm at 200-m depth (Kawai, 1972). The sampling areas were defined as AEKA, the area east of the Kuroshio axis including the axis, and AWKA, the area west of the Kuroshio axis (Sassa *et al.*, 2004).

axis (hereafter "AEKA" and "AWKA," respectively; Sassa *et al.*, 2004) to describe the spatial differences in SIs.

All larval fish specimens were first fixed in 5% buffered formalin seawater for 6 h and then transferred to 70% isopropyl alcohol after rinsing formalin with freshwater on board. After all samples were identified in laboratory, they were transferred to 90% ethanol for preservation. All adult fish specimens were first fixed in 10% buffered formalin seawater on board, and transferred to 10% formalin for preservation after identification in laboratory. The six mesopelagic fishes belonging to four families [i.e. *Diaphus* slender type, *M. asperum*, and *N. japonicus* (Myctophidae), *L. ochotensis* (Microstomatidae), *S. gracilis* (Gonostomatidae), and *V. nimbaria* (Phosichthyidae)] were sorted and identified according to Okiyama (2014). Standard body lengths of the larval and adult fish samples were measured to the nearest 0.01 mm with a digital calliper. Samples preserved with ethanol were rinsed with distilled water and vacuum-freeze dried (DRT140FB, ADVANTEC) overnight after removing gut contents. The body weights (hereafter dry-weight) were measured to the nearest 0.1 μg with ultra-microbalances (Mettler Toledo: XP2U, Switzerland).

A total of 19 428 larval fish specimens (i.e. $n = 10\,048$ in 2009 and $n = 9380$ in 2010, respectively) were sampled for the six targeted taxa. Of these, 1– 2 larval fish individuals for each taxon at each station (in total, $n = 354$ in 2009 and $n = 279$ in 2010, respectively) were subjected to stable isotopes analysis (SIA). In addition, 8–10 adult fish individuals for each taxon were used for SIA. The SL of the larval fish samples used for SIA during 2009 and 2010 ranged from 3.1 mm in *M. asperum* to 17.8 mm in *V. nimbaria*. The dry-weight ranged from 0.04 mg in *V. nimbaria* to 2.99 mg in *S. gracilis*. The SL measured for all fishes showed strong nonlinear correlations with dry-weight (Supplementary Table S1).

Sample preparation and analysis

Lipids were removed using a chloroform-ethanol solution (2:1, V/V) for 12 h to remove the potential effect of lipids on $\delta^{13}C$ (Rau *et al.*, 1992). Samples were then dried on a hotplate (60°C, 4 h) to remove any remaining solvent. Whole fish samples (for small larvae fish with 0.02–0.8 mg dry weight) or 0.6–0.8 mg subsamples of the dorsal muscle (for large fish including adult fish) were weighted into 5 × 8 mm silver capsules, and dried at 60°C after acidification with a few drops of 1.0 N HCl to remove inorganic carbon (e.g. $CaCO_3$). SIA was conducted using a continuous-flow elemental analyzer/isotope-ratio mass spectrometer (Flash EA, Delta V Advantage, Thermo Fisher Scientific) with narrow-diameter customized combustion and reduction columns δ or ultra-sensitive analysis of small samples (Ogawa *et al.*, 2010).

SI ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed in δ notation defined as follows: δ

$$\delta^{13}C, \delta^{15}N\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where the term R denotes the ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and Vienna Pee Dee Belemnite and atmospheric nitrogen were used as standards for carbon and nitrogen isotopes, respectively.

Quality assurance of SI ratios was tested by running one known standard (L-Alanine SS09, SI Science Co., Ltd., Japan) for each five unknown (fish) samples. Based on replicate measurement of this

standard, analytical precision was generally better than ±0.2‰ *SD* for both δ^{13}C in sample sizes > 18 μg-C and δ^{15}N in sample sizes > 7 μg-N. The δ^{13}C and δ^{15}N of fish samples are presented without any correction for the effect of organic solvent preservation, except where fish SI ratios are compared with other organic matter [e.g. particulate organic matter (POM)] in which case corrected values are used (see "Discussion" and Supplementary Material sections).

Statistical analysis

Normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) of data were verified before statistical analysis. Spearman's rank correlation was conducted to test the relationship between isotopes in fish tissues and other variables (i.e. sea surface temperature and salinity in their sampling locations, body length, body weight and CN ratio of the targeted fishes). Isotopic distributions were compared between the sampling locations (i.e. AWKA and AEKA) using a Mann-Whitney U test. Isotopes shifts between the two years were compared for the early larval fishes and late larval fishes using Mann-Whitney U test (see "Results" section for the definitions of "early larval" and "late larval" fishes). Median SI ratios of the late larval fishes were compared among the six taxa using the Kruskal test followed by Dunn *post-hoc* test.

SIBER analyses in the R package SIAR were conducted to compare isotopic niche overlap among the six taxa of late larval fishes, including: (i) the standard ellipse area (SEA) for core isotopic niche width (a proxy for trophic niche width), SEAc for SEA after small sample size correction; and (ii) isotopic niche overlap area and overlap percentage, based on comparison of isotopic niche width using a Bayesian modelling approach (Jackson *et al.*, 2011; Layman *et al.*, 2012). Statistical analyses were conducted using R 3.3.0 (www.R-project.org).

Results

Isotopic compositions and its shift with body weight

The δ^{13}C of the larval fishes sampled in 2009 varied from −21.6‰ in *L. ochotensis* to −18.8‰ in *V. nimbaria*, while the δ^{15}N varied from 4.2‰ in *M. asperum* to 10.1‰ in *L. ochotensis*. The ranges in δ^{13}C and δ^{15}N were similar in 2010 (−21.5 to −18.6 and 4.2–9.6‰, respectively). The median SL, dry-weight, δ^{13}C and δ^{15}N of larval fishes were significantly different among the six taxa in 2009 and 2010 (Table 1).

The δ^{13}C and δ^{15}N of larval fishes showed large variation (Table 1), but SI ratios became relatively constant as body weight increased, especially in terms of δ^{15}N (Figure 2). When these constant values were defined as being SI ratios with *SD*s < 0.4‰, the dry-weight above which SI ratios become constant (hereafter W1), was estimated as follows for each species: ca. 0.8 mg (SL: 8.2 mm) for *Diaphus* slender type; ca. 0.7 mg (6.8 mm) for *M. asperum*; ca. 0.5 mg for *L. ochotensis* (10.2 mm), *N. japonicus* (6.6 mm) and *S. gracilis* (9.6 mm); ca. 1.0 mg (12.7 mm) for *V. nimbaria*. In this study, the fish larvae of "post-larval stage" were categorized into two growth periods based on W1 (Supplementary Figure S1): "early larval period" with dry-weight less than W1 and "late larval period" with dry-weight larger than W1.

During the early larval period, δ^{13}C showed a dispersed distribution and varied between −21.6 and −18.6‰ (Figure 2a–f). Likewise, δ^{15}N varied between 4.2 and 8.0‰ for most of the species, with only *L. ochotensis* having higher values up to 10.1‰ (Figure 2g–l). During the late larval period, three taxa (*M. asperum*, *Diaphus* slender type and *N. japonicus*) had similarly lower median δ^{13}C (ca. −20.5‰) and δ^{15}N (ca. 5.7‰), while two species (*S. gracilis* and *V. nimbaria*) had similarly higher median δ^{13}C (ca. −19.6‰) and δ^{15}N (ca. 6.2‰) (Supplementary Table S2). Although the median δ^{13}C of *L. ochotensis* was varied from −21.2 to −20.8‰, the median δ^{15}N (ca. 6.9‰) was relatively higher than the other taxa (Supplementary Table S2).

For the adult fishes, δ^{13}C was similar across fish species [*D. fulgens* (*Diaphus* slender type) = −21.1 ± 0.5‰ (mean ± *SD*, $n = 8$); *L. ochotensis* = −20.2 ± 0.3‰ ($n = 8$); *M. asperum* = −21.0 ± 0.5‰ ($n = 8$); *S. gracilis* = −20.1 ± 0.6‰ ($n = 10$) and *V. nimbaria* = −20.5 ± 0.3‰ ($n = 8$)] (Figure 2a–f). The δ^{15}N ratios in adult fishes were highest for *S. gracilis* [12.1 ± 0.8‰ (mean ± *SD*, $n = 10$)], followed by *D. fulgens*, *M. asperum*, and *L. ochotensis* [δ^{15}N = 9.6 ± 0.7, 9.4 ± 0.6, and 9.3 ± 1.0‰ in 2008 ($n = 8$ for each fish species)], while they were lowest for *V. nimbaria* [7.9 ± 0.3‰ ($n = 10$)] (Figure 2g–l).

Spatial distributions of δ^{13}C and δ^{15}N

Although the species-specific diet should be considered using late larval period samples, during which time isotope ratios become relatively constant, the number of such late stage fish samples (all fishes, $n \leq 5$ in AWKA, except *S. gracilis* in 2010, $n = 13$ in AWKA) was too small for statistical analysis comparison of δ^{13}C and δ^{15}N between AEKA and AWKA. Therefore, the spatial distributions of δ^{13}C and δ^{15}N between AWKA and AEKA were compared based on datasets including both early and late larval periods (Supplementary Table S3). During 2009, δ^{13}C in *L. ochotensis*, *N. japonicus*, *S. gracilis*, and *V. nimbaria* was significantly higher in AWKA (*t* test, all $p < 0.006$), while only δ^{15}N in *N. japonicus* was significantly higher in AEKA (*t* test, $t_{62} = -2.212$, $p = 0.0307$). During 2010, δ^{13}C in *M. asperum*, *N. japonicus*, and *V. nimbaria* was significantly higher in AWKA (*t* test, all $p < 0.012$), while δ^{15}N in *M. asperum*, *S. gracilis*, and *V. nimbaria* was also significantly higher in AWKA (t test, all $p < 0.017$).

Isotopic comparisons of the larval fishes between 2009 and 2010

The δ^{13}C of early larval period fishes showed no significant difference between 2009 and 2010 (Mann-Whitney U test, all $p > 0.05$), while the δ^{15}N of early larval period fishes showed significant difference for some fish species (Mann-Whitney U test, $p < 0.05$ for *N. japonicus*, *S. gracilis*, and *V. nimbaria*) (data not shown). For late larval period fishes, on the other hand, δ^{15}N showed no significant difference (Mann-Whitney U test, all $p > 0.21$), whereas δ^{13}C only showed significant differences for two fish species (Mann-Whitney U test, $p < 0.02$ for *M. asperum* and *V. nimbaria*) (Supplementary Table S2).

Isotopic niches overlap among six species fishes in the late larval period

Since there were no significant differences between February 2009 and 2010 for δ^{15}N of all species and δ^{13}C of most species during the late larval period, the isotopes ratios representing only during the later larval period from the 2 different years were compiled together and used for isotopic niche analysis (Figure 3). The δ^{13}C of *S. gracilis* and *V. nimbaria* were significantly higher than the other four species (Kruskal test and Dunn *post-hoc* test,

Table 1. Isotopic compositions (δ^{13}C and δ^{15}N) of six taxa of mesopelagic fish larvae in the ECS.

			SL (mm)		Dry-W (mg)		δ^{13}C (‰)		δ^{15}N (‰)	
Years	**Fish species**	***n***	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
2009	*M. asperum*	61	4.5^{c}	3.1–8.8	0.103^{c}	0.048–1.504	-20.3^{bc}	−21.4 to −19.3	6.0^{d}	4.2–7.0
	Diaphus slender	61	5.4^{b}	3.7–10.0	0.164^{ab}	0.054–1.896	-20.4^{c}	−21–2 to −19.6	6.1^{cd}	4.7–7.6
	N. japonicus	64	4.5^{c}	3.1–6.7	0.144^{bc}	0.054–0.602	-20.2^{b}	−21.0 to −19.1	6.2^{c}	4.8–7.5
	L. ochotensis	30	6.8^{a}	6.0–14.0	0.244^{a}	0.089–1.348	-20.9^{d}	−21.6 to −20.2	7.7^{a}	5.7–10.1
	V. nimbaria	64	7.0^{a}	4.3–17.8	0.156^{a}	0.036–2.720	-19.8^{a}	−20.5 to −18.8	6.1^{c}	4.8–7.8
	S. gracilis	74	6.6^{a}	3.8–17.6	0.210^{a}	0.066–2.990	-19.8^{a}	−20.3 to −19.1	6.6^{b}	5.7–7.5
2010	*M. asperum*	51	5.1^{c}	3.1–8.6	0.223^{c}	0.070–1.410	-20.2^{c}	−21.0 to −18.7	5.6^{de}	4.2–7.5
	Diaphus slender	38	5.5^{c}	4.2–8.5	0.151^{d}	0.083–1.136	-20.2^{c}	−21.2 to −19.4	6.0^{cd}	5.2–6.8
	N. japonicus	54	5.1^{c}	4.1–8.5	0.231^{c}	0.068–0.968	-20.4^{c}	−21.3 to −18.6	5.5^{e}	4.3–8.0
	L. ochotensis	30	8.3^{b}	6.2–13.0	0.218^{c}	0.105–0.894	-20.8^{d}	−21.5 to −19.5	7.3^{a}	4.9–9.6
	V. nimbaria	41	10.3^{a}	7.0–17.4	0.500^{a}	0.181–2.730	-19.5^{a}	−20.5 to −18.7	6.0^{bc}	5.0–7.3
	S. gracilis	65	8.6^{b}	6.2–14.2	0.343^{b}	0.151–1.500	-19.8^{b}	−20.8 to −19.1	6.1^{b}	4.8–7.7

Superscripts "a, b, c, d, e" indicate significant differences at $\alpha = 0.05$ level. SL, standard body length; dry-W, dry weight after removing gut contents and lipids; *n*, number of samples.

all $p < 0.05$). The standard ellipses area (SEAc, $‰^2$) as a proxy of trophic niches were 0.40, 1.10, 0.44, 0.60, 0.51, and 0.63 for *Diaphus* slender type, *L. ochotensis*, *M. asperum*, *N. japonicus*, *S. gracilis*, and *V. nimbaria*, respectively.

Furthermore, we quantified the isotopic niche overlap among the six taxa of fishes during the late larval period using SIBER (Table 2). The fishes' isotopic niches of the taxonomic family Myctophidae (i.e. *M. asperum*, *N. japonicus*, and *Diaphus* slender type) overlapped from 44.6% to 76.5%. Microstomatidae fish species, *L. ochotensis*, overlapped only 0–4.7% with the other five taxa of fishes. Fishes from different taxonomic families, *S. gracilis* (Gonostomatidae) and *V. nimbaria* (Phosichthyidae), which belong to the same taxonomic order of Stomiiformes, also showed high overlap (33.6–41.2%) with each other. Moreover, all six taxa showed little trophic niche overlap (0–13.1%) among the different taxonomic families or orders.

Discussion

Correlations between isotopes and other variables

Fish tissue δ^{13}C and δ^{15}N is often correlated with body size, reflecting a shift in the trophic level of food sources as they grow (Deudero *et al.*, 2004). Furthermore, these isotopes potentially change among habitats because the isotopes in phytoplankton, which is the basis of many open ocean food webs, also shift depending on several environmental and physiological conditions [e.g. the availability of terrestrial dissolved inorganic nitrogen (DIN) and dissolved inorganic carbon (DIC), and growth rate]. Therefore, environmental (salinity and temperature) and biotic (dry-weight and SL) parameters were compared with both isotopes (δ^{13}C and δ^{15}N) for all fish species in the larval stage (Table 3) to understand factors causing the variation observed in tissue isotopes.

Decreases in δ^{13}C in POM are observed in western Kyushu when primary productivity decreases due to nutrient depletion associated with salinity increases (i.e. negative relationship; Ozaki, 2016), partly because (i) the δ^{13}C of phytoplankton-derived organic matter often decreases during bacterial decomposition (Lehmann *et al.*, 2002) and (ii) relative isotopic discrimination increases at lower growth rates (Rau *et al.*, 1996). This isotopic change in POM can generally be reflected in the SI ratios of large zooplankton and other consumers at higher trophic levels. Therefore, the negative correlation between δ^{13}C in larval tissues and salinity may indicate that larval fishes inhabiting the AWKA and on the continental shelf where relatively lower salinities occur have higher δ^{13}C (Supplementary Table S3) due to feeding on plankton supported by phytoplankton with higher δ^{13}C.

On the other hand, SI ratios of most larval fishes showed a negative correlation with both SL and body weight in our study. A similar result was observed for bluefin tuna larvae in the Eastern and Western Gulf of Mexico, although the reason was not clearly identified (Laiz-Carrión *et al.*, 2015). Because both SL and body weight did not show any correlation with salinity in our dataset, the negative correlation between SL and δ^{13}C in the larval tissues is not likely to indicate larval migration to AEKA (i.e. high salinity zone) during larval growth. Potential reasons are further considered from the viewpoint of maternal effects in the following section.

Fish species- and weight-specific δ^{15}N ratios in the larval stage

The isotope ratios of fishes during the early larval period showed large within species variation depending on the fish species, but trends reached a relative constant in all the species when weights were larger than W1, especially for δ^{15}N. Here we considered the potential factors causing these species-, and weight-specific variations in δ^{13}C and δ^{15}N in the larval stage from the viewpoint of (i) the effect of nutrition from parent fish, and (ii) diet shifts during morphological development.

Maternal effect

Uriarte *et al.* (2016) reported that the yolk sac of *T. thynnus* were fully consumed at ca. 3.0-mm NL (notochord length), while its effect on SI ratios continued to ca. 4.0-mm NL. In other words, there was little or no isotopic discrimination between fish egg and yolk-sac tissue before ca. 4.0-mm NL. In the case of *M. asperum*, its yolk sac was reported to be fully consumed before ca. 3.0-mm NL (Sassa and Kawaguchi, 2004). Because the SL range of *M. asperum* in this study was 3.1–8.8 mm (Supplementary Table S1), SI ratios of fish larvae may continue to reflect the SI ratios of egg for several days (e.g. before ca. 4.0-mm NL). Therefore, the SI ratios of the smallest larvae of several fish species were used as a

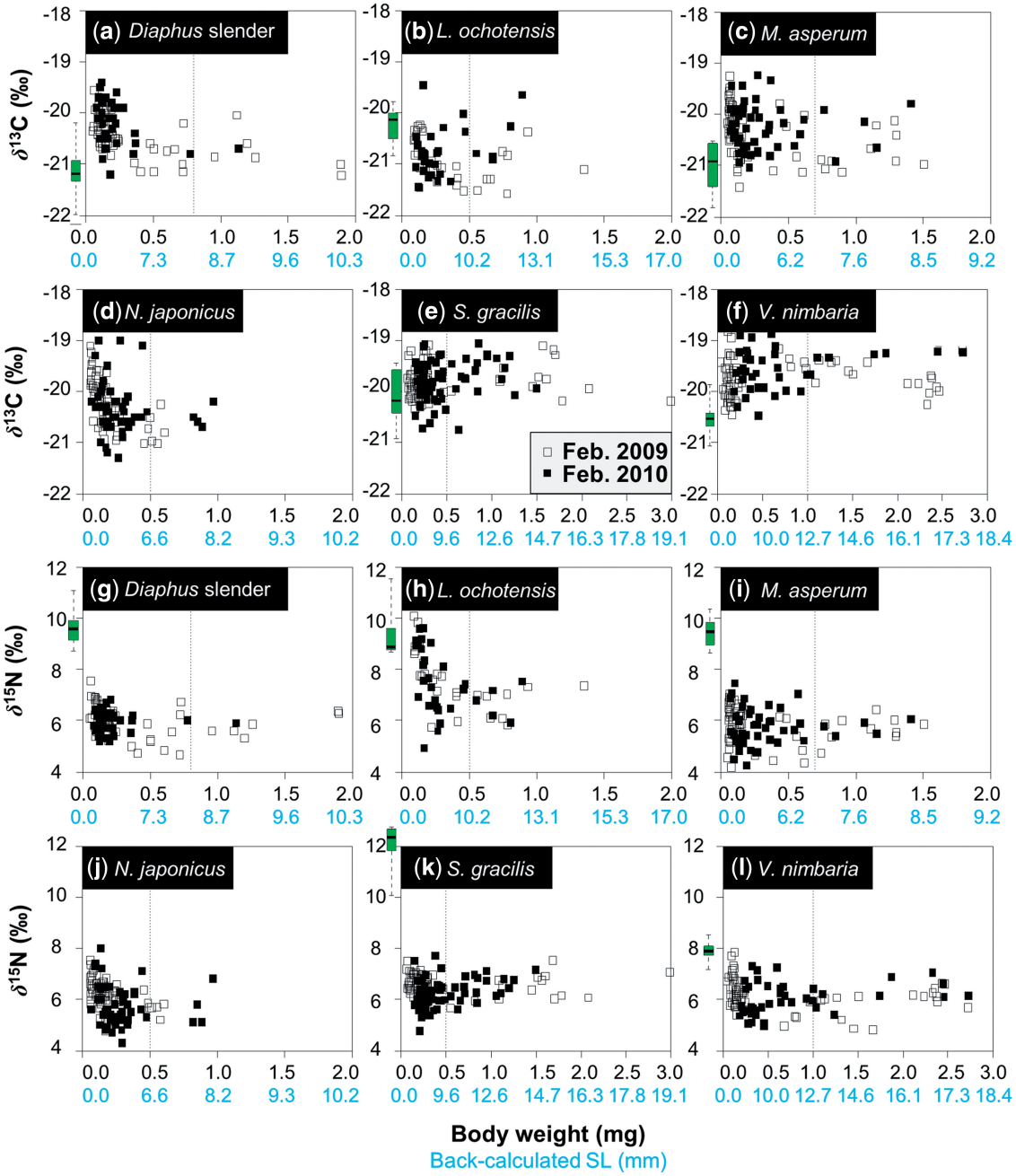

Figure 2. Relationships between body weight (dry-weight) and tissue isotopes (δ^{13}C and δ^{15}N) in six taxa of larval fishes in February 2009 and 2010. The scatter plots refer to larval fishes, while the boxplots on the left side of the y-axis denote the ranges (minimum, first quartile, median, third quartile and maximum) of isotopes ratios of adult fishes (no data for adult *N. japonicus*). Dotted lines indicate the body weight in which tissue SI become nearly constant (see text). The numeric values in blue colour under x-axis indicate the back-calculated SL (mm). And the back-calculated SL (mm) was calculated based on the average relationship between SL and dry-weight (mg) for each species, because the SL is variable depending on the morphology and fertility of each individual while the body weight simply increases with growth.

proxy of SI ratios of fish eggs, to assess the type of breeder strategy (see "Introduction" section and Supplementary Figure S2).

In the spawning area of this study (mainly AEKA), the estimated δ^{15}N of eggs from "income breeder" fishes (δ_{egg}) were ca. 5.0–6.1‰, based on the estimated SI ratios of potential food for parent fishes (i.e. large zooplankton in AEKA) and laboratory-determined isotopic enrichments between adult "income breeder" food and their eggs (see Figure 4 and Supplementary Material). However, δ^{15}N of the smallest larvae (δ_{larvae}) of *L. ochotensis* (8.6 ± 0.7‰) was significantly higher than the estimated SI ratios of eggs from "income breeder" fishes in AEKA (Mann-Whitney U test, $p < 0.001$). This clear discrepancy suggested that at least *L. ochotensis* in our sampling years did not show "income breeder"-like characteristics (Figure 4). *L. ochotensis* is known to migrate to the high productivity subarctic Oyashio region before returning to the subtropical Kuroshio

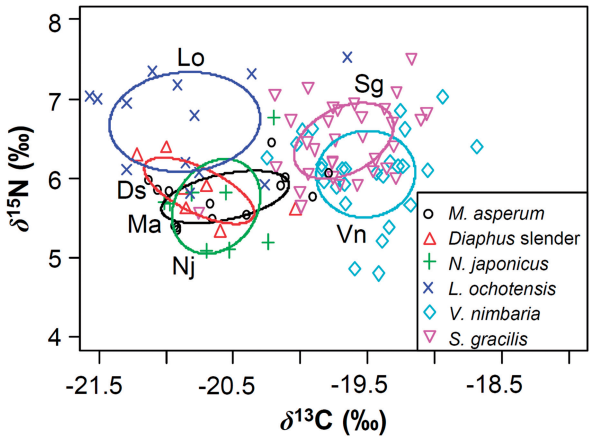

Figure 3. Plots for the core isotopic niche spaces of six taxa of fishes during the late larval period.

Table 2. Overlap percentage of SEAc among six taxa of mesopelagic fishes during late larval period (dry-weight > W1).

	Ds[a]	Ma[a]	Nj[a]	Lo[b]	Sg[c]	Vn[c]
Ds[a]		57.3%	67.1%	13.1%	0.0%	0.0%
Ma[a]	52.4%		76.5%	0.0%	0.0%	0.0%
Nj[a]	44.6%	55.6%		2.1%	0.0%	0.0%
Lo[b]	4.7%	0.0%	1.2%		0.0%	0.0%
Sg[c]	0.0%	0.0%	0.0%	0.0%		41.2%
Vn[c]	0.0%	0.0%	0.0%	0.0%	33.6%	

The table should be read horizontally, as each number in the cell refers to the percentage of overlap of the area of the group indicated in each row (e.g. 52.4% is the percentage of the SEAc of Ma that is overlapped with the Ds, while 57.3% is the percentage of the SEAc of the Ds overlapped with the Ma). SEAc, core isotopic niche width (SEA, standard ellipse area) after small sample size correction. Superscripts "a, b, c" refer to the same family Myctophidae fishes (Ds, *Diaphus* slender type; Ma, *M. asperum*; Nj, *N. japonicus*), family Microstomatidae fish (Lo, *L. ochotensis*) and the same order Stomiiformes fishes (Sg, *S. gracilis*; Vn, *V. nimbaria*).

region before the spawning season (Sassa *et al.*, 2004). If "capital breeder"-like characteristics contributed significantly to the spawning strategy of *L. ochotensis*, the energy stored in parents could be used for reproduction. This hypothesis is likely to explain the rapid shift in SI ratios of the *L. ochotensis* larvae from values close to those of adult fishes to values reflecting the food sources for their larvae in AEKA.

On the other hand, Sassa *et al.* (2016) found that all three species of *Diaphus* stubby type fishes (*Diaphus garmani*, *D. chrysorhynchus*, and *D. watasei*) were primarily "income breeders" based on multiple physiological characteristics (i.e. asynchronous oocyte development, multiple batch spawning, and active feeding during the spawning season). Therefore, it is expected that *Diaphus* slender type should mainly behave as an "income breeder." In the case of *Diaphus* slender type in this study, the SI differences between the early larval period fishes and the potential food of parent fish in AEKA (i.e. $\Delta\delta^{13}C_{Ds} = \delta^{13}C_{larvae} - \delta^{13}C_{adultfood} = 0.0–0.5‰$, $\Delta\delta^{15}N_{Ds} = \delta^{15}N_{larvae} - \delta^{15}N_{adultfood} = 0.0–1.5‰$) were equivalent to the differences in SI between eggs and foods of parent fishes just before the spawning period for the "income breeder" (i.e. $\Delta\delta = \delta_{egg} - \delta_{adultfood}$) reported in Tanaka *et al.* (2016). Therefore, the isotopes signatures of *Diaphus* slender type during the early larval period were consistent with the biological observations of Sassa *et al.* (2016) suggesting that *Diaphus* spp. are "income breeders." Similarly, *V. nimbaria* primarily belongs to the "income breeders" based on asynchronous oocyte development and multiple batch spawning (Stequert *et al.*, 2003). This characteristic also seems to be supported by the overlap between the $\delta^{13}C$ and $\delta^{15}N$ ranges of early larval *V. nimbaria* and the estimated isotopic ranges in eggs of "income breeders" that feed and spawn in AEKA.

Here, we tested only three taxa of fishes as a first approximation, focussing on those whose larvae had distinctly different SI ratios from the estimated SI ratios in eggs of an income breeder, or which had other evidence of being income breeders based on physiological characteristics. In other species, many uncertainties in the SI signatures used for the above estimations (i.e. lack of actual zooplankton and parent SI data, potential SI variation in space and time, and species-specific SI enrichment factors, etc.) might obscure the breeder type-depended isotopic characteristics. However, the good accordance between evidence from SI ratios and physiological characteristics suggests that SI ratios are a potentially effective tool for understanding reproductive strategies in the field, especially under conditions where energy acquisition and allocation to egg production is variable and depends on food availability during the spawning period (McBride *et al.*, 2015). Further studies, including accumulating SI and physiological evidence through the intensive field surveys and rearing experiments to check species-specific isotopic enrichment between food and eggs, could improve our understanding of the spawning strategy of mesopelagic fishes.

Effect of morphological development

The $\delta^{13}C$ and $\delta^{15}N$ in POM showed large variation (Supplementary Table S4), because POM consists of a variety of components (e.g. diazotrophs, phytoplankton, small zooplankton) which have different nitrogen sources [e.g. terrestrial and upwelling-derived DIN and dissolved organic nitrogen (DON)], have differing isotopic fractionation associated with DIN and DIC uptake (e.g. Rau *et al.*, 1996), and are at different stages of decomposition. Reflecting these variations, the SI ratios in zooplankton at each subsequent trophic step also differ between zooplankton groups, species and development stage (Koppelmann *et al.*, 2009). Therefore, large variation in SI ratios during early larval periods may be explained by non-selective feeding on a variety of organisms with different SI ratios due to poor swimming ability, in addition to maternal effect from adult fishes with a broad range of diets and thus tissue SI. When some morphological characteristics (e.g. caudal and anal fins) are completely developed, it is plausible that tissue SI become relatively constant because the fishes can then start to selectively prey on certain diet species with specific and relatively constant $\delta^{13}C$ and $\delta^{15}N$. Larvae of *Diaphus theta* and *M. asperum* in the transition region of the western North Pacific have been reported to be daytime visual feeders (Sassa and Kawaguchi, 2004, 2005), thus visual targeting of specific small zooplankton is possible in the larval stage. Actually, the caudal fin of *N. japonicus* is completely developed when its' size become > 7 mm (Okiyama, 2014), which is consistent with the SL at the timing of W1 of *N. japonicus* larvae.

Moreover, gut contents analyses (GCAs) in the transition region of the western North Pacific Ocean indicated that larval *D. theta*, which have morphological characteristics of the slender type in this study in the larval stage, started to change their diet from copepod *nauplii* to calanoid copepodites at body lengths

Table 3. Relationships (Spearman *r* values) between isotopes of fishes in the larval stage and other variables.

			Fish species					
Years	**Isotopes**	**Factors**	***M. asperum***	***Diaphus* slender**	***N. japonicus***	***L. ochotensis***	***V. nimbaria***	***S. gracilis***
2009	δ^{13}C	SST	−0.05	−0.03	−0.25*	−0.18	−0.03	−0.12
		SL	−0.42***	−0.48***	−0.54***	−0.43***	0.06	0.24*
		dry-W	−0.57***	−0.58***	−0.74***	−0.57***	0.16	0.19
		Salinity	−0.23	−0.21	−0.45***	−0.23	−0.52***	−0.31**
	δ^{15}N	SST	0.19	0.19	0.28*	0.23	0.03	0.2
		SL	−0.17	−0.37***	−0.29*	−0.72***	−0.23	−0.12
		dry-W	−0.23	−0.45***	−0.52***	−0.89***	−0.4***	−0.21
		Salinity	0.38***	0.11	0.12	0.01	0.34**	0.08
2010	δ^{13}C	SST	−0.24	0.11	−0.43***	0.19	−0.23	0
		SL	−0.09	−0.38*	−0.04	0.23	0.48***	0.23
		dry-W	−0.03	−0.32*	−0.18	0.2	0.42***	0.27*
		Salinity	−0.51***	−0.15	−0.42***	−0.09	−0.51***	−0.1
	δ^{15}N	SST	−0.36**	−0.2	−0.17	0.08	−0.38**	−0.22
		SL	0.05	−0.15	−0.12	−0.64***	0.55***	0.34**
		dry-W	0	0.07	−0.15	−0.64***	0.5***	0.42***
		Salinity	−0.28*	−0.35*	−0.17	0.19	−0.21	−0.23

SST, sea surface temperature; SL, standard body length; dry-W, dry-weight after removing gut contents and lipids. Asterisks indicate significant correlations between two variables. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

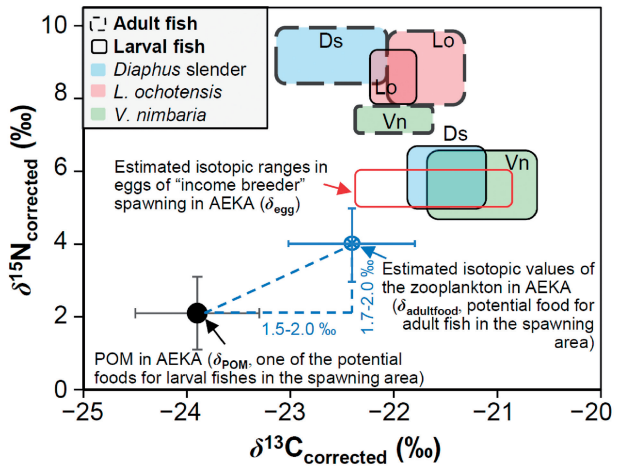

Figure 4. SIs measured in the targeted mesopelagic fish larvae, and those estimated for eggs of an "income breeder," for estimation of spawning strategy. For the targeted three species of mesopelagic fishes, SI ratios of only the smallest fish individuals with body weights <0.2 mg were used as the beginning of the early larval fishes (i.e. a proxy of fish egg), and compared with the estimated SI for eggs of an "income breeder" in the spawning AEKA area. Mesopelagic fish data were corrected based on the effect of organic solvent preservation. The borders of all boxes indicate the variations (mean ± 1 *SD*) of $\delta^{13}C_{corrected}$ and $\delta^{15}N_{corrected}$. POM SI ratios in the AEKA area were cited from Cheung *et al.* (2017) and H. Saito unpublished data (Supplementary Table S4). SI ratios for eggs of an "income breeder" were estimated based on POM SI ratios and the reported enrichment factors between phytoplankton and zooplankton, and between food for parent fish and their eggs. See more detailed explanations in Supplementary Material and Supplementary Figure S2.

> 8.0 mm (Sassa and Kawaguchi, 2005), which is consistent with the SL in the timing of W1 of *Diaphus* slender type larvae. This suggests that the timing of W1 based on SI values may be effective for determining the timing of feeding changes of fishes in the larval stage.

Food sources and isotopic niches overlap among six species fish larvae

As discussed earlier, tissue isotopes of fishes during the early larval period were related not only to food source isotopes but also maternal effects. Therefore, only isotope data obtained during the late larval period, during which selective feeding is expected to start, was considered suitable for trophic niche analysis (SIBER) of the larvae of targeted fishes.

The isotopic niche for late larval fishes within the same taxonomic family (family Myctophidae: *Diaphus* slender type, *M. asperum* and *N. japonicus*) was highly overlapped. This suggests that there is competition for diets and/or habitats for late larval fishes belonging to the same taxonomic family. However, even though *S. gracilis* (family Gonostomatidae) and *V. nimbaria* (family Phosichthyidae) belong to different taxonomic families, their larvae showed relatively high isotopic niche overlap with each other. Although the main habitat depths of these larvae show small differences between *S. gracilis* (55–100 m; Watanabe *et al.*, 2010) and *V. nimbaria* (25–75 m; Boehlert *et al.*, 1992), the vertical mixing of the Kuroshio waters (Watanabe *et al.*, 2010), sinking POM, and active vertical migration of zooplankton (Steinberg *et al.*, 2002; Bianchi *et al.*, 2013) may cause similar isotopic niches (food source) between these fishes.

GCAs of these larval fishes in the northwestern ECS were also conducted by C. Sassa (unpublished data). Most gut contents (80–95%) in *Diaphus* slender type, *N. japonicus* and *L. ochotensis* were digested materials and not identified. However, the high isotopic niche overlap among the species in the family Myctophidae and the GCA of *M. asperum* indicated that Copepoda and other Crustaceans may be the common diets of larval Myctophidae fishes in the northwestern ECS. On the other hand, the gut of larval *V. nimbaria* is straight, and it was almost impossible to find any remaining food. The diet of larval *S. gracilis* was almost completely identified as Calanoid copepodite and other Crustaceans. Therefore, some of the diets of the late larval *V. nimbaria* were also expected to be Calanoid copepodite and other Crustacean species similar to *S. gracilis*, because their isotopic niches

overlapped. Isotopic niche of larval *L. ochotensis* (Microstomatidae) overlapped only 0–4.7% with the other five taxa of larval fishes. The potential contributions from other minor diet components, such as appendicularian houses, may be identified with molecular-based analysis using the digested materials in the future (e.g. Hirai *et al.*, 2017).

Conclusion

(i) Tissue isotopes of mesopelagic fishes during the early larval period showed large variation, which probably reflected maternal transmission from parent fishes, and non-selective feeding on a variety of plankton species due to poor swimming ability.

(ii) The isotopes of late larval fish from six mesopelagic fish taxa could be appropriately used for trophic position analysis because the maternal effect was shown to disappear. Tissue isotopes became almost constant at the following body size: ca. 0.8 mg (back-calculated SL: 8.2 mm) for *Diaphus* slender type; ca. 0.7 mg (6.8 mm) for *M. asperum*; ca. 0.5 mg for *L. ochotensis* (10.2 mm), *N. japonicus* (6.6 mm) and *S. gracilis* (9.6 mm); ca. 1.0 mg (12.7 mm) for *V. nimbaria*.

(iii) Combined with physiological observations, the similarity or difference between measured tissue SI of the early larval fishes and the estimated SI ratios of eggs for "income breeder" in the spawning area supported the idea that *Diaphus* slender type and *V. nimbaria* are mainly "income breeders," while *L. ochotensis* didn't show "income breeder"-like characteristics in this study.

(iv) The isotopic niche overlap ranged from 44.6–76.5% among species in the same taxonomic family of Myctophidae larval fishes, followed by 33.6–41.2% overlap between *S. gracilis* and *V. nimbaria*. Consequently, there may be intense competition for diets or habitats in larval fishes within the same taxonomic family. Even if principal diets are not identified with GCAs due to digestion or evacuation, diet information from other fish species having similar isotopic niches can improve our understanding of the diets of larval fishes.

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Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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