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The Trophic Relationships of Sympatric Fish Species of the Genera *Opisthocentrus* and *Pholidapus* (Perciformes: Opisthocentridae) in the Sea of Japan

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Abstract—Trophic relationships of four sympatric fish species of the family Opisthocentridae (Perciformes), that is, *Opisthocentrus ocellatus*, *O. tenuis*, *O. zonope*, and *Pholidapus dybowskii*, were studied by analyzing their stomach contents and signatures of stable nitrogen and carbon isotopes (δ^{15}N and δ^{13}C) in soft tissues. The mean carbon isotope signature values ranged from -20.04 ± 0.95 to -15.44 ± 0.98, which indicated a wide range of primary carbon sources. The highest nitrogen isotope signature values (10.71 ± 0.55) and the maximum isotope niche width were recorded for *Ph. dybowskii*. Amphipods are the major food item of all the fish species under study, which was confirmed by a significant overlap of their isotope niches. The partitioning of trophic niches is explained by the selective consumption of prey crustaceans that are specific to each of the fish species and also by the use of aquatic invertebrates from different taxonomic groups as an additional food supply. The Schoener's similarity index, calculated on the basis of relative abundances of food items identified to species, showed no competitive relationships between all of the fish species under study. The values of the index between the most phylogenetically close species *O. ocellatus* and *O. tenuis* were at a minimum.

INTRODUCTION

Fish of the genera *Opisthocentrus* and *Pholidapus* (Perciformes: Opisthocentridae) are typical members of coastal benthic ichthyocenes in the Far Eastern seas [15], which inhabit nearshore beds of macrophytes and seagrasses [1, 7−11, 37]. The seaweed gunnel *O. zonope* Jordan et Snyder, 1902 is a warmth-loving species common in southern regions of Primorsky Krai, where it is found among boulders and on rocky slopes [8]. The most phenotypically similar ocellated blenny *O. ocellatus* (Tilesius, 1811) and white-nosed gunnel *O. tenuis* Bean et Bean, 1897 (Shiogaki, 1984) are often found together in catches. The legless gunnel *Pholidapus dybowskii* (Steindachner, 1880) commonly occurs in more northerly waters of the Sea of Japan [16]. The young-of-the-year (YOY) of the above-mentioned fishes constitutes a significant portion in the coastal ichthyocene. Nevertheless, their biology and, in particular, trophic relationships have been extremely poorly studied so far [37].

Using examples of other cohabiting fish species, it has been demonstrated that differentiation of trophic niches between them can occur due to the temporal separation of their feeding activities [30, 32, 43], selectivity in food item size [23], differentiated habitat use [6, 36, 44], or specifics of biochemical processes of food consumption [28]. It has also been shown that the closer taxa are located to each other in the evolutionary tree, the greater the extent of disjunction becomes between their food preferences [13, 26]. Even high indices of diet range similarity do not always mean a high level of competitive relationships, since different strategies of food resource utilization may be involved [44], or a food supply is sufficient to support two or more species living together [34].

Data of stomach content analyses (SCA) [20] and results of stable isotope analyses (SIA) showing natural ratios of stable carbon (^{13}C/^{12}C) and nitrogen isotopes (^{15}N/^{14}N), defined as δ^{13}C and δ^{15}N signatures, respectively, are used to assess trophic relationships

[4, 40]. Both methods have certain advantages and disadvantages, but complement each other when applied together [38]. One of the advantages of SCA is that it provides information about the taxonomic affiliation of food items, which is unavailable in the case of SIA [35]. Nevertheless, SIA provides integrated data on the primary carbon sources and the position in the food chain on longer time scales than SCA does [39]. As an organism grows, its food preferences change and the trophic niche becomes subject to ontogenic shift [27], while the change in the diet affects the isotope signature of the organism [21, 29]. The use of both methods allows a more comprehensive assessment of the trophoecological relationships between organisms [24, 38, 48] and visualization of the ontogenic shift of their ecological niche [42].

Sympatric fish species of the family Opisthocentridae are a convenient model object for understanding how trophic niches of cohabiting and closely related species can be isolated. This work was aimed at studying the trophic relationships of four Opisthocentridae species from coastal waters of the Sea of Japan: the seaweed gunnel, ocellated blenny, white-nosed gunnel, and legless gunnel.

MATERIALS AND METHODS

The study was based on the material collected at the marine experimental stations in three areas of the distribution range (in the bays of Kievka, Vityaz, and Vostok Bay, Sea of Japan) in the period from May to October 2005 and 2006, and also in 2018. Fish were caught with a Sigsbee trawl (with a frame size of 2.5×0.7 m) at a depth of $0.5-5.0$ m and with a seine net (height, 1.3 m; opening, 7.25 m; mesh size, 0.5 cm; bolting cloth in the codend) at a depth of $0.5-1.3$ m, on silty/sandy sediments in communities of perennial plants *Zostera marina* + *Sargassum miyabei*, *Chorda filum*, and *Z. asiatica*. The area swept with nets was approximately $1000-1400$ m^2 depending on locality. A total of 438 fish specimens were analyzed. Of these, 82 specimens of ocellated blenny (15 juveniles/67 adults), 153 specimens of white-nosed gunnel (63/90), 30 specimens of seaweed gunnel (10/20), and 138 specimens of legless gunnel (36/102) were selected for SCA analysis. These fish species reach sexual maturity in the second year of life [37] and, therefore, YOY and adults (second year of life and older) were analyzed separately. Age was estimated using whole otoliths (sagittae) in transmitted light under a Zeiss Stemi DV4 microscope. For isotope analysis, weighed samples of muscle tissue were used: from ocellated blenny and white-nosed and seaweed gunnels (12 specimens of each) caught in the summer (July); from seaweed gunnel (1 specimen) and also from ocellated blenny and white-nosed and legless gunnels (5 specimens of each) caught in the autumn season (October).

Samples for SCA were processed according to the methods described in [12]. Stomach fullness was eval-

uated on a six-point scale [5]. All food items, except ostracods and harpacticoids, were weighed on a Pioneer PA 214 analytical balance (with a readability to 0.0001 g). The weights of ostracods and harpacticoids were estimated using nomograms [17].

To characterize the diet range, the following indices were used: frequency of occurrence (F, %), number of food items per gastrointestinal tract of one individual (N, %), proportion of each diet component in the total weight of food bolus (P, %) [12], and the modified [14] index of relative significance (IR):

$$IR = \left(\frac{FiPi}{\sum FiPi}\right) \times 100\%,$$

where Fi is the frequency of occurrence of each food item; Pi is the weight fraction, with i value varying from 1 to n (n is the number of species of prey organisms in food bolus). The index is normalized and, therefore, its fluctuations have a range from 0 to 100% regardless of the number of species of prey organisms.

The SIMPER analysis was used to identify discriminating food groups [33]. The one-way analysis of similarities (ANOSIM) [22] was carried out on the basis of data embedded into the Bray−Curtis similarity matrix [19]. The degree of similarity of diet ranges (IS) was calculated using the Schoener's index [45] by the following formula:

$$IS = 100(1 - 0.5\sum |p_{xi} - p_{yi}|),$$

where p_{xi} and p_{yi} are the relative amounts of the diet component i in stomachs of the species x and y. Food items were identified to species, which significantly increased the value of the analysis.

The isotope analysis was carried out at the Laboratory of Stable Isotopes, Far East Geological Institute, Far Eastern Branch, Russian Academy of Sciences. Relative contents of heavy ^{13}C and ^{15}N isotopes in the specimens were determined in a generally accepted way: as δ^{13}C and δ^{15}N signatures calculated with respect to the corresponding standards of isotope composition and expressed in ppm:

$$\delta X (‰) = \frac{(R \text{ sample} - R \text{ standard})}{R \text{ standard}} \times 1000,$$

where X is the stable carbon and nitrogen isotopes (^{13}C, ^{15}N) and R is the ratio of the contents of stable isotopes (^{13}C/^{12}C, ^{15}N/^{14}N). All the δ^{13}C and δ^{15}N values below were calculated with respect to generally accepted international standards of isotope composition: Pee Dee Belemnite (PDB) carbonate for carbon and atmospheric N$_2$ (AIR) for nitrogen. The accuracy of determination of the δ^{13}C and δ^{15}N signatures was $\pm 0.1‰$.

The lipid profile of tissues, calculated as the C/N ratio [35], showed a small variance for all specimens (3.25 ± 0.03), which allowed neglecting these values. Differences between the samples were estimated using the Kruskal−Wallis nonparametric analysis of vari-

Table 1. The taxa of food items and the quantitative characteristics of diet of four fish species from the family Opisthocentridae in coastal waters of the western Sea of Japan

Food item	*Opisthocentrus zonope,* n = 10/20*			*Opisthocentrus ocellatus,* n = 15/67			*Opisthocentrus tenuis,* n = 63/90			*Pholidapus dybowskii,* n = 36/102		
	F, %	P, %	IR, %	F, %	P, %	IR, %	F, %	P, %	IR, %	F, %	P, %	IR, %
Amphipoda	100/93	92.2/73.1	92.2/95.8	100/80	53.3/30.4	58.1/71.43	84/39	33.3/65.6	58.2/83.4	96/64	30.13/48.7	60.7/73.1
Ostracoda	100/93	92.2/73.1	92.2/95.8	100/80	53.3/30.4	58.1/71.43	84/39	33.3/65.6	58.2/83.4	96/64	30.13/48.7	60.7/73.1
Harpacticoida	100/36	0.15/01	0.15/0.01	100/0	0.12/0	0.1/0	43/1.4	0.01/0.2	0/0.1	66/20	0.05/0.1	0.1/0.1
Gastropoda	100/36	7.61/01	7.61/0.01	100/0	5.19/0	5.7/0	64/2.8	1/8.2	1.3/0.1	93/8	3.4/0.2	6.7/0.1
Izopoda	0/21	0/6.9	0/2.08	0/12	0/27.6	0/9.5	39/1.6	41.9/8.2	34/0.4	36/36	36.7/17.4	27.7/14.6
Mysida	0/7	0/6.0	0/0.61	80/18	41.4/34	36.1/18.3	0/0	0/0	0/0	6/36	29.7/10.7	3.7/7.1
Cumacea	0/0	0/0	0/0	0/0	0/0	0/0	0/63	0/6.8	0/14.5	0/3	0/3.3	0/0.3
Tanaidacea	0/7	0/6.9	0/0.7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Decapoda	0/7	0/7.1	0/0.7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Polychaeta	0/0	0/0	0/0	0/3	0/8.1	0/7.9	0/4.3	0/6.7	0/0.93	0/16	0/2.2	0/0.9

F, frequency of occurrence; *P*, weight proportion; *IR*, index of relative significance; *n*, sample size.
*Here and below: values before slash are for young-of-the-year; after slash, for adults.

ance ($p \leq 0.05$). To visualize trophic relationships, ellipses were constructed using the SIBER package [31]. The standard ellipse areas (SEA) were corrected for a small sample size (SEAc), because, as previously shown, the total projection area of isotope niche (TA) depends on sample size [31], while the size of SEAc (SEA for small samples) reconstructed by the Bayesian method is not sensitive to sample size. Calculations and data visualization were carried out using the Statistica 10 (StatSoft) software package and the R software environment [41].

RESULTS

Trophic Relationships Inferred from Stomach Content Analysis

During the observation period, the lowest stomach fullness index (SFI) of 43‰ was recorded for the white-nosed gunnel. In the ocellated blenny and legless gunnel, SFI amounted to, respectively, 67 and 68‰, or 3 points on the Lebedev scale [5]. The proportion of empty stomachs varied from 9% in ocellated blenny to 19% in white-nosed gunnel.

The most important component in the diet of YOY seaweed gunnel was amphipods ($F = 100\%$, $IR = 92.24\%$) (Table 1), mainly the amphipod *Crassicorophium bonellii* (Table 2). Harpacticoids and ostracods were frequently found in stomachs ($F = 100\%$), but they were a less important food ($IR = 7.61$ and 0.15%, respectively). The role of amphipods remained in the lead in the diet of adult fish as well (Table 1). Out of the six amphipods species identified in seaweed gunnel stomachs, *C. bonellii* was the most common one. Among ostracods, adult seaweed gunnel preferred *Xestoleberis hanaii* and *Boreostoma coniforme*. The gastropod mollusk *Lottia angusta* proved to be a spe-

cific food item for this Opisthocentridae species. The presence of the cumacean *Alamprops quadriplicatus* in the diet of seaweed gunnel is another feature of its feeding habits.

Amphipods also played a leading role in the diet of YOY ocellated blenny ($F = 100\%$, $IR = 58.1\%$). Mainly such species as *C. bonellii*, *Pontogeneia intermedia*, and *Caprella cristibranchium* were found in fish stomachs (Table 2). The secondary food item for ocellated blenny was isopods ($IR = 36.1\%$). Harpacticoids ($F = 100\%$, $IR = 5.7\%$), which were used as food by all YOY, belonged mainly to the genus *Parathalestris*. Ostracods ($F = 100\%$, $IR = 0.1\%$) were represented by only two species, of which the most abundant one was *X. hanaii*. The diet range of adult fish of this species proved to be much narrower. Amphipods remained the major food item ($IR = 71.43\%$); of these, *Atylus collingi*, *P. intermedia*, and *Caprella bispinosa* dominated. It should be noted that the amphipods *Anisogammarus locustoides*, *Parapleustes derzhavini*, and *Ischyrocerus elongatus* were recorded only from ocellated blenny stomachs. The isopod *Idotea ochotensis* and the gastropod *Siphonacmea oblongata* were secondary diet components. Harpacticoids and ostracods were not found in adult ocellated blenny stomachs.

Along with the amphipod *C. cristibrachium*, the major food item of YOY white-nosed gunnel was the gastropod *S. oblongata* ($IR = 58.2$ and 34%, respectively). Ostracods and harpacticoids occurred more commonly compared to other food items in fish stomachs ($F = 43$ and 64%, respectively); however, due to their low weight, they were not an important component in the diet ($IR = 0.1$ and 1.3%, respectively). The diet range of adult fish was mainly represented by amphipods, among which the most important species were *Ischyrocerus anguipes* and *Caprella eximia* (Table 2). The second

Table 2. Species composition of food items of Opisthocentridae fishes from coastal waters of the Sea of Japan as inferred from the fish stomach content analysis

Taxon/species	*Opisthocentrus zonope*	*Opisthocentrus ocellatus*	*Opisthocentrus tenuis*	*Pholidapus dybowskii*
Amphipoda				
Crassicorophium bonellii	+*/+	+*/+	–/–	+/+
Atylus collingi	–/+	–/+*	–/–	–/+*
Atylus ekmani	–/–	–/–	–/+	–/+
Pontogeneia intermedia	–/+	+/+	–/+	–/+
Ampithoe dyakonovi	+/+*	+/–	–/–	+/+
Ampithoe lacertosa	–/–	–/+	–/–	+/+
Sunamphithoe mea	–/–	–/+	–/–	+/+
Caprella cristibrachium	–/–	+/+	+*/–	+/+
Caprella bispinosa	–/–	–/+	–/+	–/–
Caprella eximia	–/–	–/+	–/+	–/+
Caprella penantis	–/–	–/–	–/+	–/–
Caprella acanthogaster	–/–	–/–	–/–	–/+
Accedomoera melanophthalma	–/–	–/–	–/–	–/+
Anisogammarus locustoides	–/–	–/+	–/–	–/–
Anisogammarus pugettensis	–/–	–/+	–/–	–/+*
Parapleustes derzhavini	–/–	–/+	–/–	–/–
Ischyrocerus elongatus	–/–	–/+	–/–	–/–
Ischyrocerus anguipes	–/–	–/+	–/+*	–/–
Protomedeia fasciatoides	–/–	–/–	–/–	–/+
Vonimetopa zernovi	–/+	–/–	–/–	–/–
Pareurystheus gurjanovae	–/+	–/–	–/–	–/–
Aoroides sp.	–/–	–/–	–/–	+/–
Photis sp.	–/–	+/–	–/–	+/–
Parhyale zibellina	–/–	–/–	+/–	–/–
Mysida				
Neomysis mirabilis	–/–	–/–	–/+*	–/+
Cumacea				
Alamprops quadriplicatus	–/+	–/–	–/–	–/–
Tanaidacea				
Pseudotanais sp.	–/+	–/–	–/–	–/–
Decapoda				
Pandalus latirostris	–/–	–/+	–/+	–/+
Heptacarpus longirostris	–/–	–/–	–/–	–/+
Eualus leptognathus	–/–	–/–	–/–	–/+
Ostracoda				
Xestoleberis hanai	+/+	+/–	+/+	+/+
Hemicytherura sp.	–/–	–/–	–/–	+/+
Boreostoma coniforme	–/+	–/–	–/–	–/–
Boreostoma ussuricum	–/–	–/–	–/–	–/+
Cythere nishinipponica	–/–	–/–	–/–	–/+

Table 2. (Contd.)

Taxon/species	Opisthocentrus zonope	Opisthocentrus ocellatus	Opisthocentrus tenuis	Pholidapus dybowskii
Harpacticoida				
Parathalestris sp.	–/–	+/–	–/+	+/–
Scutellidium sp.	+/+	+/–	+/–	+/+*
Paramenophia platysoma	–/–	–/–	–/–	+/–
Zaus sp.	–/–	+/–	+/–	+/–
Harpacticoida gen. sp. 1	–/–	+/–	–/–	–/–
Harpacticoida gen. sp 2	–/–	+/–	–/–	–/–
Gastropoda				
Siphonacmea oblongata	–/–	+/**+**	+*/+	+/+*
Pusillina plicose	–/–	–/–	–/–	–/+
Lottia angusta	–/+	–/–	–/–	–/–
Isopoda				
Idotea ochotensis	–/–	–/+*	–/–	–/+
Syniodotea brashnikovi	–/+	–/–	–/–	–/+
Idoteidae gen. sp.	–/–	–/–	–/–	–/+
Sphaeromatidae gen. sp.	–/–	–/–	–/–	–/+
Isopoda spp.	+/–	+/–	–/–	+/–
Polychaeta spp.				
	–/–	–/–	+/+	+/+
Pisces spp.				
	–/–	–/–	–/+	–/–

*The taxa commonly represented in the sample of this species and age category.

most important diet component was the mysid *Neomysis mirabilis* (IR = 14.05%).

The major diet component of YOY legless gunnel were amphipods and gastropods (IR = 60.7%, IR = 27.7%) (Table 1), and minor components were represented by harpacticoids (IR = 6.7%) and ostracods such as mainly *X. hanaii* and rarely *Hemicytherura* sp. (Table 2). In adult fish, amphipods (IR = 73.13%) and gastropods (IR = 14.62%) also constituted the major part of the diet. Isopods and ostracods were of secondary importance. The species composition of ostracods in food bolus was extended due to preying also on *Boreostoma ussuricum* and *Cythere nishinipponica*, recorded only from the diet structure of legless gunnel. Mysids and decapods were randomly found diet components. Legless gunnel was characterized by the widest range of food items, which included 29 invertebrate species.

A comparison between the YOY diet ranges of all the studied fish species by the ANOSIM analysis showed no significant differences in the percentage of food items at the level of high taxa (R = 0.06, P = 0.063). Significant differences were found only by pairwise comparison of the diet ranges in white-nosed and legless gunnels (R = 0.12, P = 0.002) and also in ocellated blenny and seaweed gunnel (R = 0.34, P = 0.01). According to the results of the SIMPER analysis, ocellated blenny and white-nosed gunnel differed in the abundance proportion of gastropods in stomach contents; ocellated blenny and seaweed gunnel, in the proportion of harpacticoids; ocellated blenny and legless gunnel differed in the consumption of ostracods. The white-nosed gunnel diet range was characterized by a high frequency of occurrence of gastropods (Table 1).

The study of adult fish trophic relationships using the ANOSIM analysis showed that the white-nosed gunnel sample significantly differed from the samples of other Opisthocentridae species (R = 0.2, P = 0.001). According to data obtained by the SIMPER analysis, the presence of mysids was a distinguishing feature of the white-nosed gunnel diet. Based on the results of this analysis, significant differences were also found between ocellated blenny and legless gunnel. The calculated Schoener's pairwise similarity indices [45] did not reveal any competitive relationships (Fig. 1). The similarity between the YOY diet ranges of the species under study was found to be higher than in adult fish: the similarity index value varied from 10 to 37% in YOY and from 4 to 12% in adults, except the pair seaweed/legless gunnels with their similarity index of 34%.
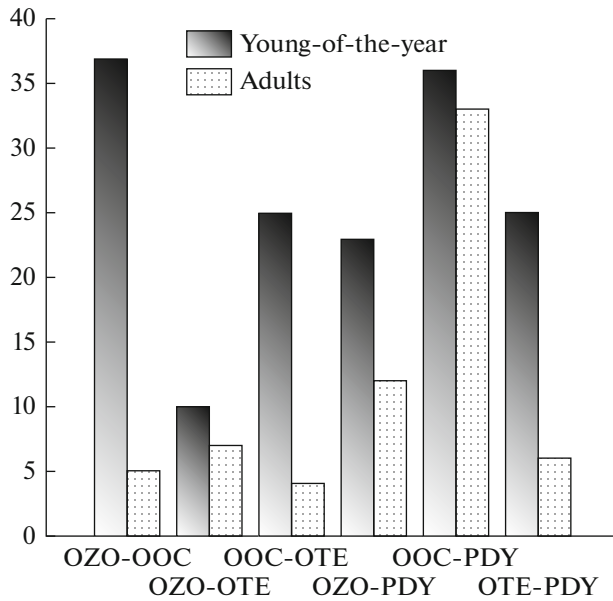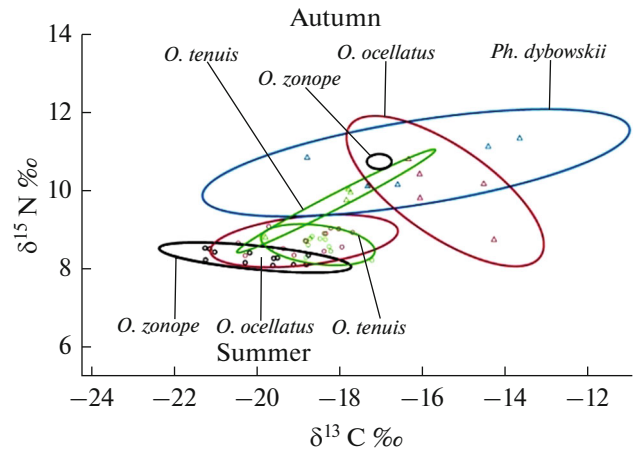
Fig. 1. Schoener's pairwise index of similarity between the diets of the four Opisthocentridae species. The acronyms are as follows: OZO, *Opisthocentrus zonope*; OOC, *O. ocellatus*; OTE, *O. tenuis*; PDY, *Pholidapus dybowskii*.

Fig. 2. Isotope niches (SEAc) of the four Opisthocentridae species from the Sea of Japan in the summer and autumn seasons.

Feeding Relationships Inferred from Stable Isotope Analysis

The analyzed fish specimens showed similar nitrogen isotope signatures. In the summer, the range of interspecific variations in δ^{15}N were 0.43‰, while δ^{13}C was 2.64‰. The lowest values for the heavy carbon isotope (-20.04 ± 0.95) were recorded for seaweed gunnel, which significantly differed ($H = 19.66290$, $p = 0.0014$) from the other fish species in both parameters. In the autumn season, the stable isotope ratios significantly differed from the summer data. The maximum value of δ^{15}N was recorded for legless gunnel (10.71 ± 0.55), and the minimum value for white-nosed gunnel (9.74 ± 0.54). The autumn samples were characterized by a greater heterogeneity of nitrogen isotope signatures than the summer ones, with the range of variations being 0.97‰. The lowest δ^{13}C values were recorded for white-nosed gunnel (-18.08 ± 0.98).

In the summer season, the isotope niche area of ocellated blenny corrected for small sample (SEAc) was 0.812, which is more than twice as large as the values of this parameter for white-nosed (SEAc = 0.396) and seaweed (SEAc = 0.407) gunnels. In the autumn season, the legless gunnel occupied the widest niche (SEAc = 0.3902). The isotope niche area of white-nosed gunnel (SEAc = 0.3648), which was reduced compared to that in the summer, whereas the niche area of the ocellated blenny in the autumn was, in contrast, larger than in the summer (Fig. 2).

DISCUSSION

The analysis based on two complementary methods has shown significant species-specific differences in the diet ranges of the four Opisthocentridae species. The legless gunnel dominates in the diversity of food components: YOY and adult fish of this species show the widest diet range including 14 and 29 taxa, respectively. Other species are characterized by narrower trophic niches. According to the results of our study, the white-nosed gunnel has proven to be the most specialized species, with the smallest isotope niche, which decreased with growth of fish (Fig. 2).

It has been shown that the isotope composition of fish body is formed in the period of intensive somatic growth and then changes during ontogeny [3]. According to our results, the significant variation in the δ^{15}N values in juvenile ocellated blenny and white-nosed gunnel for three months (from July to October) indicates their one-level move along the food chain and a high rate of metabolic processes leading to the accumulation of heavy isotopes.

It was observed that different Opisthocentridae species, after making a transition from a pelagic to demersal lifestyle, stay in the water column near margins of brown algae beds [2] or man-made structures covered by fouling (personal observations), form common schools, and have similar feeding habits, which is confirmed by the overlap of their isotope niches revealed by the isotope analysis. However, interspecific differences in primary carbon sources have also been found. The shift of the isotope niche towards the accumulation of heavy carbon isotope indicates an increase in the proportion of benthic organisms in the diet.

A significant difference in primary carbon sources was found between the most phylogenetically close species, ocellated blenny and white-nosed gunnel.

The lower $\delta^{13}C$ values in tissues of white-nosed gunnel are explained by a marked contribution of pelagic organisms to its food, which was confirmed by a significant number of individuals of the mysid *Neomysis mirabilis* ($F = 62\%$, $IR = 14.05\%$) in the structure of bolus. A similar differentiated aquatic habitat use was reported earlier for other sympatric fish species [25, 44].

The use of amphipods as the major food supply by the fish species under study suggests the probability of competitive feeding relationships, which is confirmed by the significant overlap of their isotope niches (Fig. 2). For a more accurate understanding of the mechanism of food resource partitioning, taxa of food items should be considered at a lower systematic level, up to the species one [18]. The Schoener's index, calculated on the basis of the relative abundance of food items identified to species, has indicated a negligible level of food competition that even decreased with age and growth of fish (Fig. 1). The similar isotope profiles and also the high probability of overlap of isotope and trophic niches, as inferred from a more detailed analysis of food items, have shown that Opisthocentridae fishes demonstrate food selectivity on a species level. Amphipods constitute the major part of the diet of the fish species under study. However, for the ocellated blenny, the most commonly encountered amphipod is *Atylus collingi*; for the seaweed gunnel, *Crassicorophium bonellii*; for the white-nosed gunnel, *Ischyrocerus anguipes*; for the legless gunnel, *Atylus collingi* and *Anisogammarus pugettensis*. Preying on these crustaceans results in similar isotope signatures, which, with the lack of data on food species composition in stomachs, can be interpreted as a competitive relationship.

The study of feeding relationships between the sympatric Opisthocentridae fish species through analyzing the contents of their stomachs, as well as the analysis of the ratios of stable nitrogen and carbon isotopes in soft tissues, has revealed the mechanisms of trophic niche partitioning. Amphipods are the major food item for all the fish species under study, which is clearly demonstrated by the overlap of their isotope niches. The trophic niche partitioning is explained by the selective consumption of prey crustaceans that are specific to each fish species, and also by the use of aquatic invertebrates from different taxonomic groups, that is, Gastropoda, Polychaeta, Decapoda, etc., as an additional food supply.

Legless gunnel dominated in the diversity of food items and also showed the widest diet range. According to the results of our study, the white-nosed gunnel has proven to be the most specialized species. The Schoener's index, calculated on the basis of the relative abundance of food items identified to species in fish stomach contents, indicated the lack of competitive relationships between the studied fish species; the minimum values of this index were recorded for the most phylogenetically close species, *O. ocellatus* and *O. tenuis*.

It should also be noted in conclusion that the combined use of the two methods provides an integral view of the trophoecological relationships between the species under study, which allows clear representation of trophic niches and their overlaps, while avoiding probable misinterpretation of trophic relationships.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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