

Article

Insights into Ontogenetic Niche Changes in Bluegill, *Lepomis macrochirus*, Applying Combined Analyses of Stomach Content and Stable Isotopes

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Abstract: We integrated stomach content analysis (SCA) and stable isotope analysis (SIA) to understand ontogenetic niche shifts in the invasive freshwater fish, bluegill, *Lepomis macrochirus*, inhabiting the Yedang Reservoir in Korea. Based on the total length (TL), we classified *L. macrochirus* as small (23–57 mm), medium (61–99 mm), or large (100–163 mm). Across all study sites, the index of relative importance (IRI) of zooplankton was high for small individuals, whereas those of benthic macroinvertebrates were high for the medium and large groups. Isotopic niche width estimates based on carbon and nitrogen isotope ratios (δ space) also increased with growth, indicating an ontogenetic niche shift in *L. macrochirus*. In particular, the δ space and diet plasticity of large fish was higher in the littoral population, implying *L. macrochirus* are generalist feeders as adults. Individuals classified as small showed confined and constant δ space, regardless of habitat condition. Thus, together with the results on the significantly high IRI of zooplankton, these individuals seem to have strong specialistic feeding ecology. Our study demonstrates the applicability strength of combining SCA and SIA for ecological niche research by providing clear evidence of an ontogenetic niche shift in *L. macrochirus* and elucidates their feeding ecology.

Keywords: ecological niche; ontogenetic change; SCA; SIA; δ space; generalists; specialists



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1. Introduction

The ecological niche, a fundamental concept involving all the interactions between organisms and the biotic and abiotic environment (such as diet and habitat), is essential for understanding the function and role of species in ecosystems [1]. Ontogenetic niche shifts, i.e., the niche changes within a species with development and growth, are a representative survival strategy of organisms and are derived from changes in diverse ecological conditions, such as the increase in and expansion of their population size and habitat ranges, prey selectivity, and growth [2–4]. Thus, ontogenetic niche shifts are key factors in understanding the structure of communities and their ecological functions [5]. For instance, diet, which is one of the major components in the ecological niche of a species, provides information on interactions occurring in an ecosystem, such as prey–predator–prey interactions and intra- and inter-species competition [6]. Ontogenetic dietary changes are affected by diverse factors, such as environmental conditions, the availability of resources, and physiological conditions. Thus, dietary changes and their driving factors are indispensable for understanding interactions in complex ecosystems [7].

Stomach content analysis (SCA) has traditionally been used to obtain high-taxonomic-resolution dietary information for organisms [8]. Moreover, information on prey organisms from SCA, including the number of individuals, wet weight, and frequency of occurrence, can be used to estimate the index of relative importance (IRI), which allows us to identify major dietary sources for consumers [9]. Contents in a stomach which are partially digested and fragments of organisms sometimes causes bias results between numerical and mass information [10]. Because IRI can reduce these errors in biased data, it has been widely applied in many studies on fish diets [11–13]. In contrast, weak points of SCA samples are that they are temporally limited to “snapshot” information and there is difficulty identifying partially digested contents [14–16].

Stable isotope analysis (SIA) is also a widely applied tool in food web studies, because the isotope signatures assimilated in consumer tissues are derived from their diets [17]. The dietary information from SIA sometimes provides a lower resolution than that from SCA due to isotopic overlap between the primary producers occurring from isotopically homogeneous inorganic resources (i.e., dissolved inorganic carbon and nitrogen) in an ecosystem. However, time-integrated dietary information, as long as the tissue turnover rate of a target organism is obtained from SIA, could supplement snapshot information from SCA [18]. Moreover, SIA provides us with dietary, habitat, and trophic information, due to the different carbon isotopic baselines between habitats and the stepwise trophic enrichment in nitrogen isotope ratios in a food chain [19]. A variety of ecological information from SIA has recently been applied to ecological niche studies. For instance, Newsome et al. [20] suggested the applicability of SIA in niche studies by drawing δ space, which provides both diet and habitat information. Consequently, the Stable Isotope Bayesian Ellipses in R (SIBER) were established by Jackson et al. [21] to estimate the width of δ space and are widely applied in ecological niche studies [22–24]. However, isotopic niche estimation can lead to misunderstandings in ecological interpretation due to properties of isotope signals such as variation in isotopic baselines and diverse trophic discrimination factors [25]. Thus, recently, the combination of SCA and SIA has emerged as an alternative approach in ecology to compensate for the disadvantages of each method (the low resolution in SIA and limited snapshot information from SCA). Therefore, studies on the integrated application of both methods are increasing (e.g., [12,26,27]).

Invasive species, which were mostly introduced in the 20th century, can cause a decrease in native biodiversity during their spread and settlement, particularly in freshwater ecosystems [28–30]. The bluegill *Lepomis macrochirus*, which originated in North America, is a representative invasive species in Korea. The negative effects such as the extinction of native species in freshwater ecosystems caused by the drastic increase in the *L. macrochirus* population have long been noticed [31,32]. *L. macrochirus* is a highly predatory fish with a wide feeding niche, ranging from zooplankton to small vertebrates, with its feeding plasticity depending on its growth stage and the habitat it occupies [33,34]. Diverse studies related to the dietary resources of *L. macrochirus* have been conducted, because its broad dietary spectrum poses a severe threat to ecosystems [14,35–37]. However, most of these studies have focused on investigating the major dietary resources for *L. macrochirus* using a single analytical method, either SCA or SIA. Since information on the diet of a species is strongly connected with that of the niche, the application of combining SCA and SIA to evaluate dietary variation within a species is expected to not only obtain information on patterns in dietary changes, but also in niche changes.

Therefore, the present study applied this integrated approach to clarify the ontogenetic dietary changes and its related isotopic niche variation in *L. macrochirus* inhabiting an artificial reservoir in Korea. We hypothesized that diet consumption and isotopic niches would be varied in accordance with both growth and habitat differences. In order to enhance our understanding of such ontogenetic niche changes, our investigation was performed not only for different size classifications, but also for different habitat conditions such as pelagic and littoral environments.

2. Materials and Methods

2.1. Sampling

Sampling was performed in three different waterfront areas of the Yedang Reservoir, one of the largest artificial reservoirs in the west–central region of the Korean Peninsula, on 13 October 2022. Site 1 and 2 (St. 1 and St. 2) are connected to the inflow stream and are highly vegetated, whereas St. 3 is close to the dam wall and the vegetation density is low (Figure 1). Thus, St. 1 and St. 2 were characterized as littoral areas, whereas St. 3 was considered to be a pelagic habitat. The *L. macrochirus* inhabiting each site were caught using a skimming net (mesh 4×4 mm) and casting net (mesh 6×6 mm), and the total length (TL, mm) of individual specimens was measured. The sample number of individuals for each size class was $n = 94$ for the small, $n = 88$ for the medium, and $n = 33$ for the large group (Table 1). After abdominal incision, the stomach was extracted and stored in 10% formalin. In order to analyze the carbon and nitrogen stable isotope, dorsal muscle tissue was excised, placed in a vial, and stored at -20 °C.

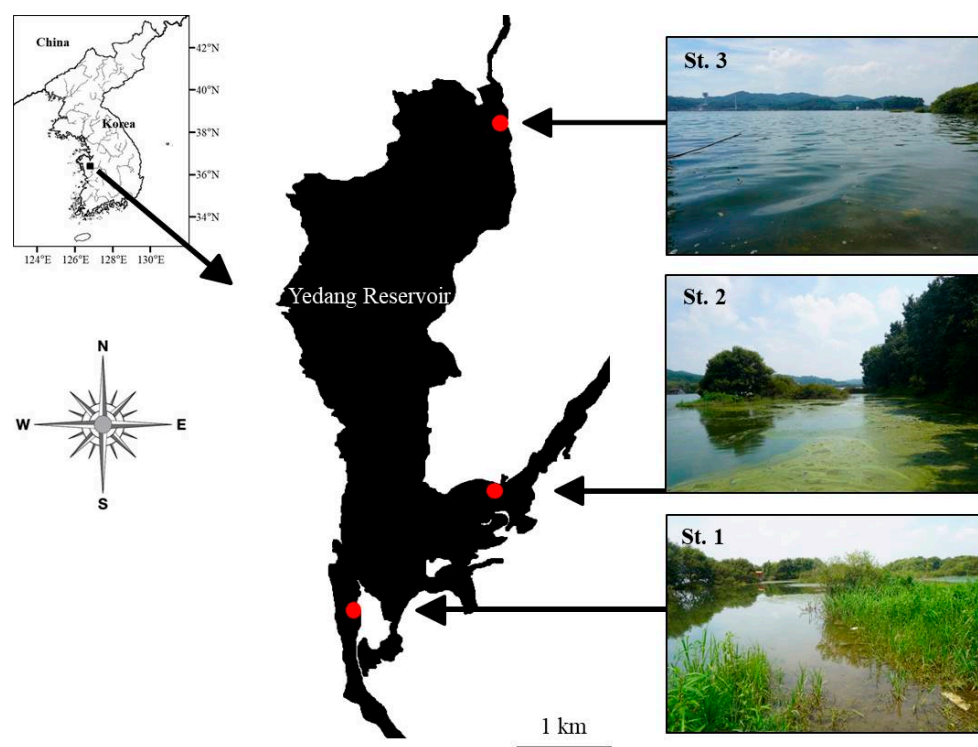


Figure 1. Study sites and photographs of each habitat environment.

Table 1. Information on sample size of *L. macrochirus* for both stomach contents analysis (SCA) and stable isotope analysis (SIA) in the present study.

Size Class (mm)	St. 1	St. 2	St. 3	Total
Small (20–60)	28	42	24	94
Medium (61–99)	28	26	34	88
Large (100–163)	17	9	7	33
Total	73	77	65	215

2.2. Stomach Contents Analysis (SCA)

Stomach contents were observed using a stereoscopic microscope, and dietary items that were partially or wholly digested and morphologically difficult to recognize were identified to the lowest taxonomic level by referring to illustrated books [38–40]. Dietary items that were partially or wholly digested and morphologically difficult to recognize

were identified at the family level. The identified prey organisms were counted, and their wet weight was measured to the nearest 0.001 mg. The index of relative importance of each prey organism was expressed as a percentage estimated by the number of individuals (%N), wet weight (%W), and frequency of occurrence (%F), as established by Pinkas et al. [41].

$$\%F = A_i / N \times 100 \quad (1)$$

$$\%N = N_i / N_{\text{total}} \times 100 \quad (2)$$

$$\%W = W_i / W_{\text{total}} \times 100 \quad (3)$$

$$\text{IRI} = (\%N + \%W) \times \%F \quad (4)$$

$$\%\text{IRI} = \text{IRI}_i / \text{IRI}_{\text{total}} \times 100 \quad (5)$$

where A_i and N indicate the number of stomachs containing a particular prey taxon and the total number of stomachs containing any prey, respectively; N_i and W_i are the total number and weight of a particular prey taxon in the stomach, respectively; and N_{total} and W_{total} are the total number and weight of all identified prey, respectively.

2.3. Stable Isotope Analysis (SIA)

Dissected dorsal muscle tissues were freeze-dried and homogenized using a mortar and pestle. Approximately 1 mg of ground muscle sample was weighed and sealed in tin capsules. Carbon and nitrogen stable isotope analyses were performed using an isotope ratio mass spectrometer linked to an elemental analyzer (EA-IRMS, Isoprime, Manchester, UK). Each isotope ratio was expressed with the conventional delta (δ) notation in parts per thousand (‰) using the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 1000 (\text{‰}) \quad (6)$$

where R_{sample} and $R_{\text{reference}}$ are $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ for the sample and reference materials, respectively. Reference materials for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were vPDB (Vienna Pee Dee Belemnite, IAEA) and atmospheric N_2 gas, respectively. Every ten samples run, CH-3 (IAEA, $\delta^{13}\text{C} = -24.72 \pm 0.1 \text{‰}$) and N-1 ($\delta^{15}\text{N} = 0.4 \pm 0.1 \text{‰}$, IAEA) were analyzed as standard materials.

2.4. Statistical Analysis

Differences in carbon and nitrogen isotopes for size classes of *L. macrochirus* were evaluated using one-way ANOVA, and Bonferroni correction was applied for post hoc testing. All statistical analyses were conducted with R (ver. 4.2.0). We applied Isotope Bayesian Ellipses in R (R package SIBER, [21]) to evaluate δ space. Based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, δ space was demonstrated and the standard ellipse area (SEA) was estimated as the width of δ space from SIBER.

3. Results

3.1. Size Distribution

A total of 235 *L. macrochirus* individuals were collected at the three sites, and the TL distribution ranged from 23 to 163 mm, with an average TL of 67.13 ± 27.64 mm. Based on the length–frequency distribution [42], *L. macrochirus* were classified into three groups: small (23–57 mm), medium (61–99 mm), and large (100–163 mm) (Figure 2). The sample number of individuals for each size class was $n = 94$ for small, $n = 88$ for medium, and $n = 33$ for the large group, respectively.

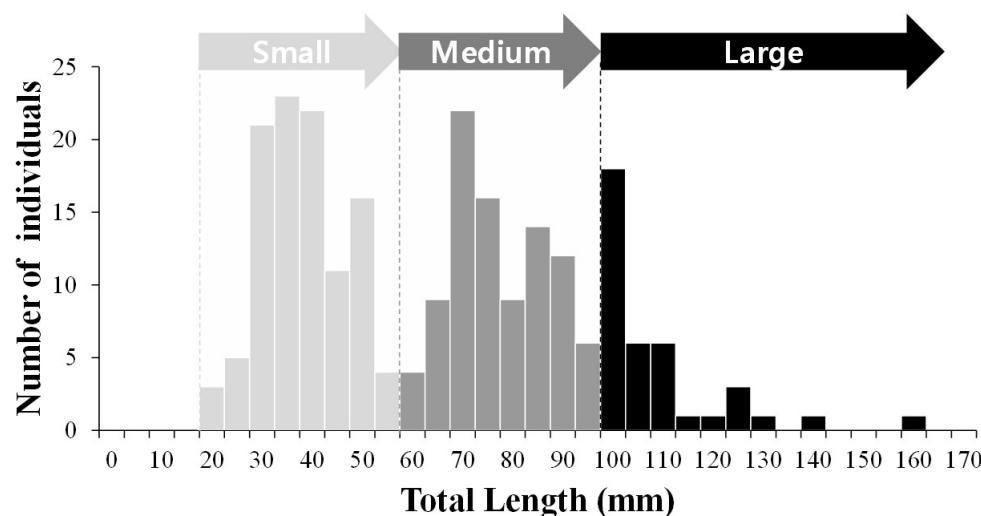


Figure 2. Total length distribution of *Lepomis macrochirus* sampled in the Yedang reservoir. The range of each TL base classification is shown by arrows. Small, medium, and large groups ranged from 20 to 60 mm, 61 to 99 mm, and 100 to 163 mm, respectively.

3.2. Stomach Contents Analysis (SCA)

Stomach contents were examined in all 235 individuals of *L. macrochirus*, but the stomachs were empty in 19 individuals (8.1%). The most important prey groups for *L. macrochirus* was zooplankton, with 55.84% of IRI, followed by benthic macroinvertebrates (44.11%) and fishes (0.05%). The zooplankton *Diaphanosoma brachyurum* and *Sinodiaptomus* sp. were numerically dominant, with correspondingly high importance values, while benthic macroinvertebrates in the family Chironomidae contributed the most to the total mass and had the highest importance value (Table 2). These taxa were also the most frequently consumed by *L. macrochirus*. The smaller zooplankton were most numerous but contributed less to the total mass than relatively larger chironomids which, in turn, contributed less to the total number. Except for these three taxonomic groups, the other prey items contributed very little to the number, mass, and frequency, hence indicating negligible importance values.

Table 2. Prey items in stomach of *Lepomis macrochirus* in the present study. The number of individuals (%N), wet weight (%W), frequency of occurrence (%F), and index of relative importance (IRI) are estimated according to Pinkas et al. [41].

Prey Organisms in Gut (Group/Taxa)		%N	%W	%F	%IRI
Zooplankton	<i>Diaphanosoma brachyurum</i>	49.21	13.82	54.42	31.51
	<i>Sinodiaptomus</i> sp.	32.61	10.86	60.93	24.33
	<i>Physa acuta</i>	0.05	4.18	1.86	0.07
Benthic macroinvertebrates	<i>Gyraulus convexiusculus</i>	0.10	4.64	2.79	0.12
	<i>Parafossarulus manchouricus</i>	0.03	2.62	0.93	0.02
	Coenagrionidae	0.01	0.46	0.47	+
	Corduliidae	0.01	0.60	0.47	+
	<i>Ecnomus tenellus</i>	0.04	0.67	1.40	0.01
	<i>Tetragrathia</i> sp.	0.01	0.92	0.47	+
	<i>Macrobrachium nipponense</i>	0.01	2.42	0.47	0.01
	Chironomidae	17.90	47.07	73.49	43.86
	Fishes				
	<i>Pseudorasbora parva</i>	0.01	11.74	0.46	0.05

Note: + indicates < 0.01.

The most important diet for the small group was zooplankton (IRI = 83.75%), followed by benthic macroinvertebrates (16.25%) as the second most important prey group (Figure 3).

In the medium group, benthic macroinvertebrates had the highest IRI (65.97%), followed by zooplankton (34.03%), indicating an ontogenetic dietary shift. In the large group, the IRI proportion of benthic macroinvertebrates (84.49%) increased compared to that in the medium group, and that of zooplankton was even smaller (14.40%). Additionally, the IRI proportion of fish increased to 1.12% for large *L. macrochirus*. While zooplankton were the most important prey organisms for small individuals in all of the study sites, with an average IRI of $83.31 \pm 15.24\%$, benthic macroinvertebrates were the most important prey organisms in the medium group at St. 1 and 2, with an average IRI% of 80.18 ± 24.56 . In contrast, the IRI of benthic macroinvertebrates in the medium group at St. 3 increased to 24.19% but was lower than that of zooplankton (75.81%). Similar to the medium group, large individuals at St. 1 and 2 had a benthic macroinvertebrate IRI of $98.34 \pm 2.34\%$, and that of fishes (1.53 ± 2.17) became noticeable. The large group at St. 3 showed an increased benthic macroinvertebrate IRI (31.20%) compared to the medium group, but that of zooplankton fell to 68.80%. Each size group classified between St. 1 and 2 showed similar changes in prey organisms. However, St. 3 showed a similar tendency for benthic macroinvertebrates to increase and zooplankton to decrease as the body size increased from small to large, but medium to large fish did not exhibit the level of prey shifting observed at the other sites.

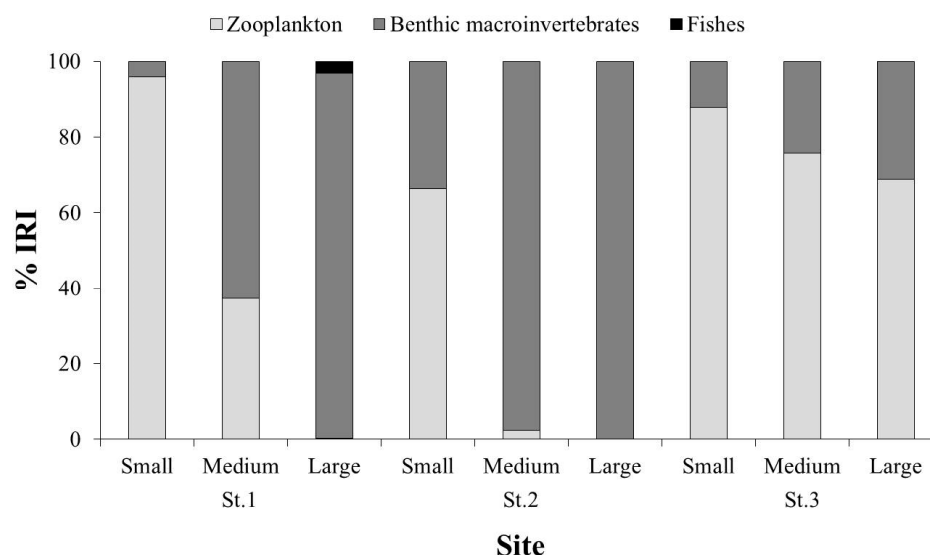


Figure 3. Index of relative importance (IRI) of each diet item in taxonomic level for each classified size of *Lepomis macrochirus*.

3.3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values and δ Space for TL-Classified *L. macrochirus*

Among the size classes of *L. macrochirus*, the large group showed relatively wider ranges in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than the medium group at St. 1 (Table 3). However, no significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges between the two groups were found at the other sites. The $\delta^{13}\text{C}$ range in the small group was similar at St. 1 and St. 2, whereas a relatively larger range was observed at St. 3. In contrast, $\delta^{15}\text{N}$ values in the small individuals were consistently lower than those in other TL groups at all sites, except for St. 1.

The estimated width of standard ellipse area (SEA) of *L. macrochirus* based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranged from 1.0 to 2.7‰^2 , 0.9 to 3.8‰^2 , and 0.7 to 9.6‰^2 for the small, medium, and large groups, respectively (Figure 4). In particular, the large group at St. 1 showed the largest SEA (9.6‰^2) among all size-classified groups at all of the study sites, while the smallest SEA was shown by the large group at St. 3 (0.7‰^2). The SEA of the small groups were smaller at St. 1 and St. 2 (1.0‰^2) than at St. 3 (2.8‰^2), while the medium (1.0‰^2) and large groups (0.8‰^2) at St. 3 had the smallest δ space among the same groups from the other sites. The overlapped area of SEA between the small and medium group was 0.82‰^2 and 0.21‰^2 between small and large group at St. 1. On the other hand, the

overlapped SEA of the small group with other size classes in St. 2 and St. 3 was less than $0.001\% ^2$, indicating a clearly separated isotopic niche. In comparison, the δ space between the medium and large groups within sampling sites partially overlapped ($0.61\% ^2$ at St. 1, $0.64\% ^2$ at St. 2, and $0.25\% ^2$ at St. 3, respectively), indicating niche competition between two of the TL groups.

Table 3. Ranges in carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios (‰) of TL-classified *L. macrochirus*. The letters indicate significant differences among size classes at each site ($p < 0.05$).

Sampling Sites TL-Classes		St. 1			St. 2			St. 3		
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
$\delta^{13}\text{C}$	min	−26.6	−27.4	−29.5	−26.8	−27.3	−27.2	−28.2	−26.4	−25.1
	max	−22.8	−23.6	−23.3	−23.2	−24.5	−25.5	−21.4	−23.1	−22.8
	mean ($\pm$ SD)	−24.9 ^a (± 0.9)	−25.3 ^{ab} (± 0.9)	−26.1 ^b (± 1.7)	−25.1 ^a (± 0.9)	−25.7 ^b (± 0.8)	−26.4 ^b (± 0.6)	−24.3 ^a (± 1.9)	−24.6 ^a (± 0.8)	−23.7 ^a (± 0.7)
$\delta^{15}\text{N}$	min	14.7	13.7	10.2	15.0	16.1	16.3	14.9	17.2	17.9
	max	16.3	18.2	17.3	16.5	18.4	18.3	16.9	18.9	18.8
	mean $\pm$ SD	15.5 ^a (± 0.4)	16.3 ^a (± 1.4)	14.2 ^b (± 1.9)	15.9 ^b (± 0.3)	17.1 ^a (± 0.6)	17.3 ^a (± 0.7)	15.9 ^b (± 0.5)	18.1 ^a (± 0.4)	18.3 ^a (± 0.3)

Note: Small = 23–57 mm, Medium = 61–99 mm, and Large = 100–163 mm.

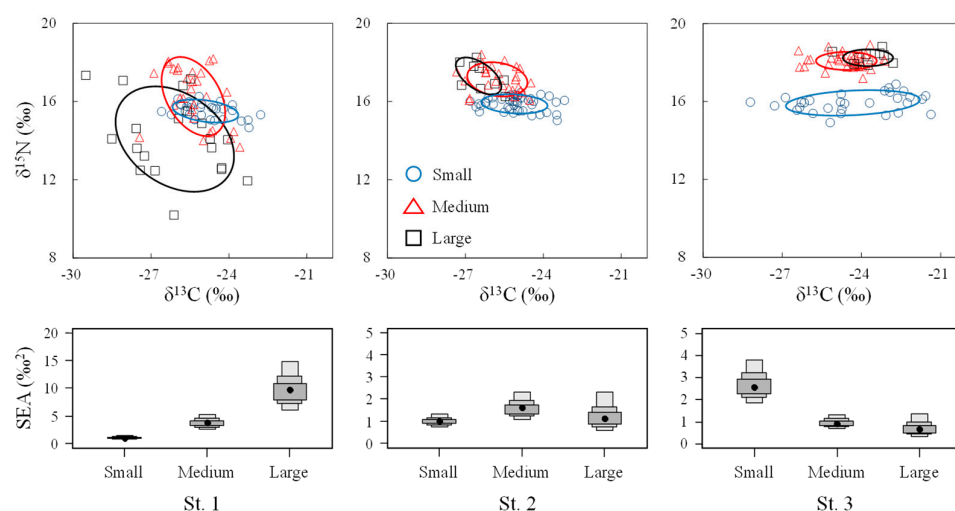


Figure 4. Dual plot with carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios and estimated standard ellipse area (SEA) of each classified size of *L. macrochirus* at each site. The ellipses in the dual plots indicate the δ spaces for each size groups.

4. Discussion

Our size classification based on TL is consistent with the previously reported annual growth of this species, likely reflecting differences in birth year [43]. Thus, the differences in the IRI of dietary items between each TL group distinctively demonstrated an ontogenetic dietary shift from zooplankton to benthic macroinvertebrates with *L. macrochirus* growth. Showalter et al. [44] suggested that a dietary shift in *L. macrochirus* occurred at around 25 mm TL, owing to differences in the elemental requirements. Although the threshold TL size during the dietary shift was slightly different, our results strongly support the previous suggestion of an ontogenetic dietary shift in this species at a small size. However, intraspecific diet changes seem to be affected not only by growth, but also by habitat properties. Indeed, based on the IRI, benthic macroinvertebrates were the main dietary resource for *L. macrochirus* larger than 60 mm at St. 1 and St. 2, whereas the IRI for zooplankton was higher for all sizes of *L. macrochirus* at St. 3. Our sampling sites were divided into littoral (St. 1 and St. 2) and pelagic (St. 3) environments, based on their geographical and vegetation properties. Gerry et al. [45] reported differences in major dietary resources and in morphology according to the habitat of *L. macrochirus* and even

larger average TLs (18.61 ± 1.72 cm) than the present study. In addition, a greater diversity in dietary items in littoral habitats than in pelagic habitats has been reported previously [46]. Thus, the exceptionally high IRI of zooplankton for large (>60 mm) *L. macrochirus* at St. 3 reflects habitat traits.

Similar to the SCA results, the variable δ space, the standard ellipse area estimates based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, between *L. macrochirus* size classes and habitats also reinforced the ontogenetic niche shift in this species. The largely overlapped δ space between the medium and large groups is consistent with SCA results of similar dietary compositions between these two groups at each site, and the distinctive δ space in the small group indicates a separate dietary composition in this size class compared to the others. Moreover, the relatively consistent δ space among the small individuals of all the sampling sites can be interpreted as a consequence of a strong selective feeding strategy, as shown by the dominant IRI of zooplankton from SCA. In comparison, variation in the width and position of δ space shown in the medium and large size classes contrasts with their IRI in benthic macroinvertebrates. For example, the large group of *L. macrochirus* at St. 1 and St. 2, adjacent to the inflow stream, showed different patterns of δ space despite similar dietary proportions. This offset between the SCA and SIA results may have been derived from variable isotope ratios in benthic macroinvertebrates, particularly in chironomid larvae, which were the dominant benthic dietary items in our SCA results. Unfortunately, we did not include $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses for diet items, but small spatial variation in chironomid larvae due to its short turnover rate and diverse larval stage has been reported [47,48]. In addition, variable isotopic signatures in basal resources (e.g., primary producers) due to the inflow creek may have resulted in the wide δ space at St. 1. The difference in the timescale recorded in the SCA and SIA could also have led to this offset. In general, it is known that SIA provides time-integrated information, whereas snapshot information is obtained from SCA. Thus, dietary information generated by either approach causes some degree of bias. Nevertheless, conspicuous δ space variation in *L. macrochirus* coincided with changes in the IRI of diet-evident changes in the isotopic niche with TL growth.

Based on their feeding behavior, consumers are generally classified as specialists with a strong diet selection and generalists that consume a broad range of dietary items. Accordingly, the ecological niche width of specialist populations is comparatively smaller than that of generalist populations [49]. The stationery and regular δ space in small individuals of *L. macrochirus* resemble general niche properties in specialists, whereas the expanded and spatially variable δ spaces in medium and large individuals are close to that of generalists. Previous studies on the diet of adult *L. macrochirus* have reported diverse dietary items, including zooplankton, benthic macroinvertebrates, and small fish [50,51]. Our SCA results also demonstrated variable dietary importance depending on habitat and ontogeny. Thus, the wide prey spectrum and its relevant broad niche width indicate that *L. macrochirus* can be considered a generalist species in the adult stage. Furthermore, our findings on the high IRI of zooplankton for all small *L. macrochirus* provide clear evidence of a narrow prey spectrum in its early stages, indicative of a specialist species. Therefore, changes in the IRI of each dietary item and in δ space with growth and habitat suggest an ontogenetic niche shift from specialist to generalist in *L. macrochirus*.

5. Conclusions

The present study provides clear evidence of an ontogenetically expanded niche in *L. macrochirus*, from planktivoric specialists to generalists, consuming both benthic and pelagic macroinvertebrates. Moreover, our results demonstrate that the habitat affects major diet selection in the adult stage of *L. macrochirus*, highlighting the importance of the ecological environment, such as habitat and size, in understanding their feeding ecology. The niche of organisms comprises diverse factors, including environmental habitat conditions and biological conditions, such as consumer size, population, and diet composition. The SIA technique is widely applied in niche studies because the position of the space and its width display a complex relationship among the components of the isotopic niche.

Furthermore, our study suggests that the detailed information provided by SCA can enhance the comprehension of ontogenetic niche shifts. Studies on isotopic niches and their ontogenetic variations provide the principal information necessary for ecosystem management. SCA and SIA are traditional and widely applied approaches in this scientific field, and their combined application complements the weaknesses of each approach and promises to enhance ecological knowledge.

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