

# Ontogenetic dietary shifts of largemouth bass do not increase trophic position in a shallow eutrophic lake in Japan

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**Abstract** – The trophic positions of top predators can provide useful information for estimating the length of food chains and for assessing the impacts of invasive species or the bioaccumulation of harmful compounds. In aquatic ecosystems, large carnivorous fishes may change their trophic positions ontogenetically. We used stable carbon and nitrogen isotope ratios to test the hypothesis that the trophic positions of largemouth bass, top predators in Lake Izunuma, Japan, would increase with total length (TL) as a result of ontogenetic shifts in diet. Results from a stable isotope analysis in R mixing model indicated that largemouth bass < 100 mm TL fed mostly on zooplankton and small omnivorous fishes, while individuals from 100 to 199 mm TL size class relied more heavily on small omnivorous fishes. Red swamp crayfish *Procambarus clarkii* were a major food source for largemouth bass ≥ 200 mm TL. However, the trophic positions of largemouth bass did not increase with the dietary shift from zooplanktivory to piscivory, and actually decreased when larger fish shifted towards feeding on red swamp crayfish. In Lake Izunuma, the trophic positions of small omnivorous fishes and red swamp crayfish were not higher than those of zooplankton. This explains why the largest largemouth bass occupied lower trophic positions than smaller individuals. Our results suggest that the body size of carnivorous fishes should be taken into consideration when using their trophic positions as top predators to evaluate aquatic systems.

**Key words:** Stable isotope / invasive species / SIAR / piscivory / crayfish

## Introduction

Food-web analysis is commonly used to study the complex trophic interactions between organisms and organic matter flow within ecosystems (Hairston and Hairston, 1993). Determining the trophic positions of organisms (which are continuous variables) and the position they occupy in food chains is a critical step for investigating these interactions (e.g., Post, 2002a, 2002b; Vander Zanden and Vadeboncoeur, 2002). In aquatic ecosystems, the trophic positions of fish species can be used to assess the negative impacts of invasive species on native aquatic communities (Vander Zanden *et al.*, 1998),

to measure contaminant bioaccumulation (Rasmussen *et al.*, 1990) and to improve fishery productivity (Pauly and Christensen, 1995).

Stable carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) have been widely used to investigate food-web structure in a range of aquatic ecosystems (e.g., Vander Zanden and Vadeboncoeur, 2002; Jones and Waldron, 2003; Vander Zanden *et al.*, 2011) because the respective ratios in an organism reflect its diet, providing insight into its feeding history in the long term. Trophic positions of consumers can be estimated from their  $\delta^{15}\text{N}$  values, which are typically enriched by 3–4‰ through trophic transfer (Post, 2002b; Vanderklift and Ponsard, 2003).  $\delta^{13}\text{C}$  values can provide information on an organism's primary carbon source because these values change little through trophic transfer (DeNiro and Epstein, 1978; Fry and Sherr, 1984; Post, 2002b).

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Body size has been recognized as a key trait of aquatic consumers since it influences predator–prey interactions (Persaud *et al.*, 2012). Previous studies in fishes have reported an increase in trophic position with increased body size as a result of ontogenetic dietary shifts (Kline *et al.*, 1998; Vander Zanden *et al.*, 1999; Jennings *et al.*, 2001). Because larger carnivorous fish have correspondingly larger gape sizes and can thus capture larger prey (Shelton *et al.*, 1979), large individuals of piscivorous fish species are often recognized as top predators in aquatic ecosystems (Vander Zanden and Vadeboncoeur, 2002; Vander Zanden *et al.*, 2004; Doi *et al.*, 2009). However, some studies have reported that the trophic positions of fishes do not always increase with body size (Wang *et al.*, 2011; Persaud *et al.*, 2012).

The largemouth bass *Micropterus salmoides* is a piscivorous fish native to North America. Largemouth bass have been introduced outside of their native range for sport fishing (Hickley *et al.*, 1994; García-Berthou, 2002; Yonekura *et al.*, 2004; Jang *et al.*, 2006; Magoro *et al.*, 2015) and have greatly altered native fish communities through predation (Azuma and Motomura, 1998; Whittier and Kincaid, 1999; Takahashi *et al.*, 2001; Yonekura *et al.*, 2004; Jang *et al.*, 2006). Consequently, largemouth bass have become top predators in many bodies of water in Japan (Yonekura *et al.*, 2004; Maezono *et al.*, 2005). Estimation of the trophic position of invasive piscivores is crucial to assessing their negative impacts, including predation and competition with native communities (Vander Zanden *et al.*, 1999; Correa *et al.*, 2012; Feiner *et al.*, 2013). Different size classes of largemouth bass could have different effects on native aquatic communities due to ontogenetic diet shifts. However, the relationship between the trophic position and body size of largemouth bass in Japan is still unclear. In addition, the contamination of freshwater carnivorous fishes with radioactive cesium has been investigated in the wake of the serious accident at the Fukushima Dai-ichi Nuclear Power Plant (Arita *et al.*, 2015; Matsuda *et al.*, 2015). Freshwater carnivores show higher radioactive cesium levels than do omnivores and herbivores, likely due to bioaccumulation through the food chain (Mizuno and Kubo, 2013). Therefore, the relationship between trophic positions and body size of largemouth bass could provide useful information in assessing the bioaccumulation of radioactive cesium.

This study was conducted at Lake Izunuma, which is shallow and relatively small, and representative of the bodies of water into which largemouth bass are frequently introduced. Our objectives were (1) to estimate trophic positions, (2) to determine the major prey organisms of largemouth bass in each body size class, and (3) to establish the relationship between trophic positions and body size using stable carbon and nitrogen isotope ratios. We hypothesized that the trophic positions of largemouth bass, the top predators in Lake Izunuma, Japan, would increase with total length as a result of ontogenetic shifts in diet.

## Materials and methods

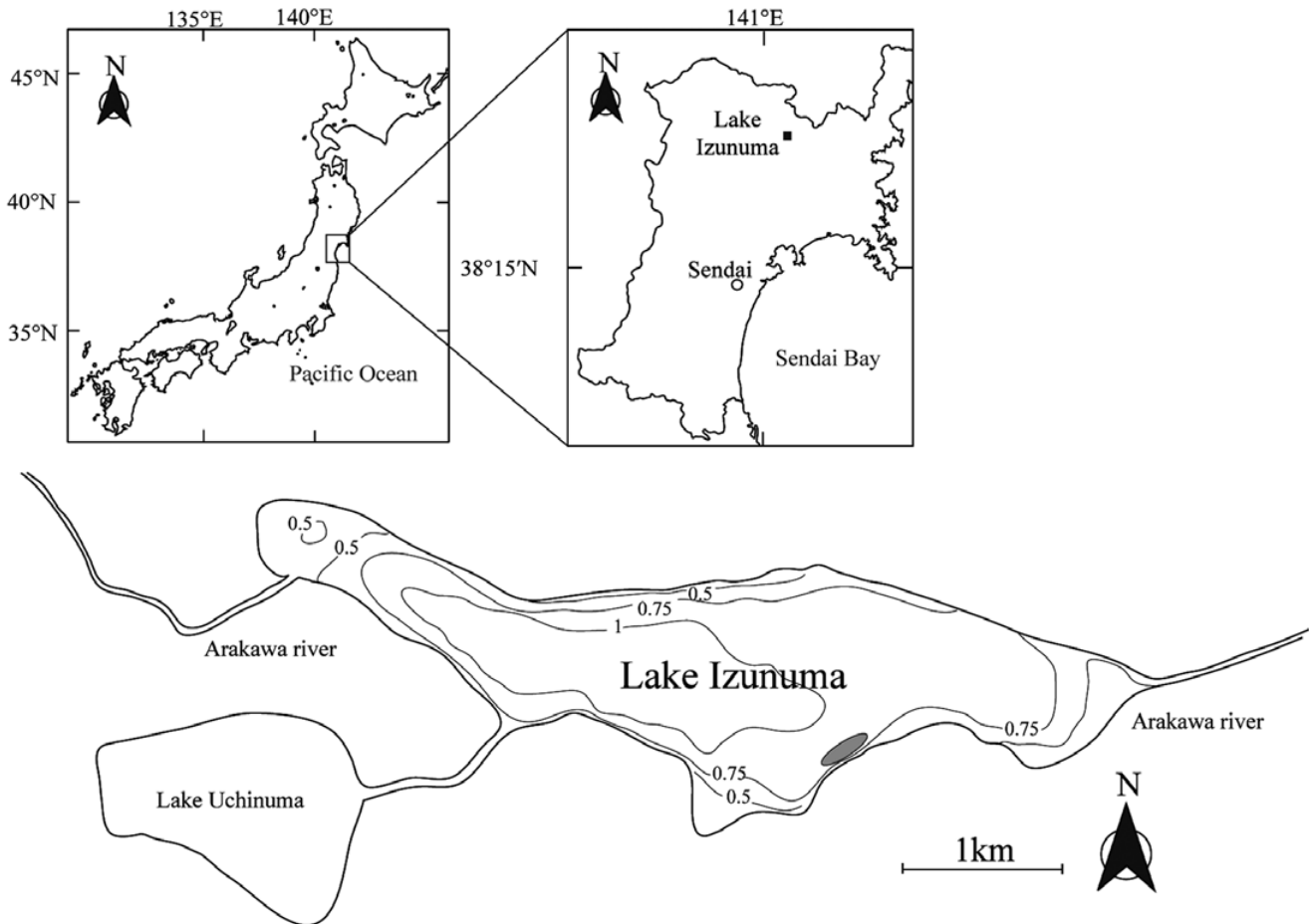
### Study area

Lake Izunuma in temperate northeastern Honshu, Japan (Fig. 1) is eutrophic (Yasuno *et al.*, 2009), polymictic and shallow (Table 1) (Shidara, 1992). The lake is largely dominated by macrophytes (mainly lotus, *Nelumbo nucifera* and other floating, leaved macrophytes). Benthic algae and submerged plants have restricted distributions, likely due to high water turbidity. Largemouth bass were introduced to Lake Izunuma for angling and were first caught in 1996; they subsequently became the top predator species in the ecosystem (Takahashi *et al.*, 2001). Until 1995, the annual harvest of native fish in Lake Izunuma exceeded 30 tons and consisted primarily of cyprinids (Takahashi *et al.*, 2001). Since then, the number of largemouth bass has increased, while other fish species have decreased sharply. The fish community is still dominated by small cyprinid species, mainly *Gnathopogon elongates* and *Pseudorasbora parva*. These two species of cyprinids make up 70% of the fish community in the lake, while largemouth bass account for 8.8% (Fujimoto *et al.*, 2008).

### Sample collection

We collected largemouth bass in May 2006, using gill nets, and in December 2006 using fyke nets near the south shore (Fig. 1). Because largemouth bass spawned by the south shore, we were easily able to catch adult individuals (> 300 mm TL) there using gill nets. From May–December 2006, we also used fyke nets to catch small omnivorous fishes and red swamp crayfish *Procambarus clarkii*, which are potential prey for largemouth bass. A fyke net is a type of fixed net that is dustpan-shaped and resembles a trawl net (mesh size: 5 mm, length: 5 m, diameter: 0.6 m) with long wings (mesh size: 10 mm, length: 30 m, depth: 1.8 m). Fyke nets were set for 24 h. We measured the TL of largemouth bass and potential omnivorous prey fishes < 200 mm TL to the nearest 1 mm, and measured individuals > 200 mm TL to the nearest 10 mm in the laboratory. We then dissected each fish and removed a sample of dorsal muscle tissue for freeze-drying and subsequent stable isotope analysis. Two species of dragonfly (*Ephthalma elegans* and *Sinictinogomphus clavatus*) and a species of snail (*Radix auricularia japonica*) were captured using dip nets near the south shore in June 2006. We used the whole bodies of dragonflies, while we removed muscle tissue samples of snails and red swamp crayfish for freeze-drying and subsequent stable isotope analysis.

To determine stable isotope ratios, we ground freeze-dried samples of all invertebrates and fishes, homogenized them in a pre-combusted (500 °C, 2 h) agate mortar and pestle, and then treated them with a chloroform:methanol mixture (2:1, v/v) to remove lipids. The samples were concentrated onto Whatman GF/C glass filters



**Fig. 1.** Sampling locations in Lake Izunuma. Solid lines indicate the isobath in meters. We collected fishes within the gray area by setting two or three gillnets or fyke nets.

**Table 1.** Characteristics of Lake Izunuma.

Location	38°43'N, 141°06'E
Altitude (m above sea level)	6 <sup>a</sup>
Area (km <sup>2</sup> )	3.69 <sup>a</sup>
Max depth (m)	1.6 <sup>a</sup>
Chl. <i>a</i> (μg.L <sup>-1</sup> )	36.1 <sup>b</sup>
Secchi depth (m)	0.6 <sup>b</sup>
pH	7.5 <sup>c</sup>
Conductivity (μS.cm <sup>-1</sup> )	40.4 <sup>c</sup>

<sup>a</sup>Shidara (1992).

<sup>b</sup>Yasuno *et al.* (2009).

<sup>c</sup>Yasuno unpublished data (from July 2006 to June 2007).

(pre-combusted at 500 °C for 2 h), which were then freeze-dried.

### Stable isotope analyses

The stable isotope ratios of the samples were measured with a Finnigan MAT Delta-plus mass spectrometer (Thermo Finnigan, San Diego, CA, USA) connected to an NA2500 elemental analyzer (CE Instruments, Wigan,

UK). Stable isotope ratios were presented using the standard delta notation:

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

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . We report isotope ratio values relative to the following standards: Vienna Pee-Dee belemnite for  $\delta^{13}\text{C}$  and atmospheric nitrogen gas for  $\delta^{15}\text{N}$ . The analytical error of the analyzer was within  $\pm 0.1\text{‰}$  for carbon and  $\pm 0.2\text{‰}$  for nitrogen.

### Diet assessment

#### Model inputs

We used the isotopic data of largemouth bass collected in this study and cited the data of age-0 + largemouth bass from Yasuno *et al.* (2012); then, we divided largemouth bass into four size classes (TL < 100, 100–199, 200–299 and  $\geq 300$  mm). Given that largemouth bass forage on various organisms such as fishes, crustaceans, aquatic insects and zooplankton (Keast and Eadie, 1985; Olson, 1996), we assumed four end members of their potential food sources: eight species of small omnivorous fishes, red

swamp crayfish *P. clarkii*, benthic invertebrates (two species of dragonfly and larval chironomids) and zooplankton. We collected three species of small omnivorous fishes, red swamp crayfish and the two species of dragonfly used in this study. We used reference isotopic data for the other five species of small omnivorous fishes and zooplankton from Yasuno *et al.* (2016) and for larval chironomids from Yasuno *et al.* (2013). Largemouth bass generally forage on zooplankton during their early life-history (< ca. 100 mm TL) (Keast and Eadie, 1985; Olson, 1996; Takahashi *et al.*, 2001) and zooplanktivorous individuals are often < ca. 200 mm long (Ha *et al.*, 2015). In contrast, red swamp crayfish are typically too large for small individuals (< 200 mm TL) to capture (Schramm and Maceina, 1986; Hickley *et al.*, 1994; García-Berthou, 2002; Wheeler and Allen, 2003; Torigoe and Shiraiwa, 2010). In our own experience, we have often observed red swamp crayfish in the stomachs of largemouth bass > 200 mm TL in Lake Izunuma, but we have never found them in the stomachs of smaller individuals (N. Yasuno, personal observation). Therefore, small benthic invertebrates, zooplankton and small omnivorous fishes were assumed to be potential food for largemouth bass < 200 mm TL, while benthic invertebrates, small omnivorous fishes and red swamp crayfish were assumed to be prey for individuals > 200 mm TL.

### Bayesian mixing model

The relative contributions of these potential food sources to the diets of largemouth bass were estimated using SIAR (Stable Isotope Analysis in R; R Development Core Team, 2015). Conventional isotope mixing models can calculate relative dietary contributions from the mean isotopic values of food sources and isotopic discrimination through trophic transfer (Phillips and Gregg, 2001). However, uncertainties such as variation around the mean isotope values of food sources (prey) and fractionation factors may affect the solution of the model. The SIAR isotope mixing model uses Bayesian inference and has the advantage of allowing variation and uncertainties to propagate throughout the model, with the output better reflecting the natural variability within a system. The SIAR model was fit using Markov chain Monte Carlo methods, which used a Dirichlet prior distribution to simulate dietary proportions of food sources that were consistent with the data (Parnell *et al.*, 2010). We assumed that the isotopic discrimination for nitrogen and carbon were  $3.23 \pm 0.41$  and  $0.94 \pm 1.13\%$ , respectively (Vander Zanden and Rasmussen, 2001). The SIAR mixing model ran 500 000 iterations, discarding the first 50 000 samples.

### Estimation of trophic positions

The trophic positions of consumers can be estimated from their  $\delta^{15}\text{N}$  values and a baseline (typically from a primary producer or consumer). Lake food-webs are typically supported by phytoplankton in the pelagic zone,

and macrophytes and their epiphytic algae in the littoral zone (Scheffer, 1998). Because the isotopic baselines of primary producers may differ, it is important to consider the contribution of each food chain pathway to consumers in order to more accurately estimate their trophic positions. Therefore, we used cladoceran zooplankton and snails as pelagic and littoral isotopic baselines, respectively, to estimate the trophic levels of consumers. Long-lived primary consumers, such as filter-feeding mussels (pelagic consumers) and snails (littoral consumers), are often used as isotopic baselines because they integrate the temporal fluctuations of primary producers (Cabana and Rasmussen, 1996; Post, 2002b). However, the unionid mussel *Cristaria plicata* in Lake Izunuma has been shown to ontogenetically increase its stable nitrogen isotope ratios, indicating that large individuals could transition to secondary consumers (Yasuno *et al.*, 2014). Therefore, we used  $\delta^{15}\text{N}$  values from filter-feeding cladoceran zooplankton as a pelagic baseline instead of mussels. We estimated the trophic positions of primary consumers, largemouth bass and their potential prey species, including copepod zooplankton, eight species of small omnivorous fishes and red swamp crayfish, using the following formulas:

Trophic position

$$= (\delta^{15}\text{N}_{\text{consumer}} - [a \times \delta^{15}\text{N}_{\text{snail}} + (1-a) \times \delta^{15}\text{N}_{\text{zp}}]) / \Delta N + 2$$

$$a = (\delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{zp}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{zp}})$$

where  $\Delta N$  represents the isotopic discrimination of  $^{15}\text{N}$  (assumed value previously mentioned). The subscripts “consumer,” “snail” and “zp” represent consumers (largemouth bass or their potential prey species), littoral isotopic baseline species (*R. auricularia japonica*) and pelagic isotopic baseline species (cladoceran zooplankton), respectively. We rounded “a” to 1 or 0 if the values were slightly > 1 or < 0, respectively. Differences in mean trophic positions between size classes of largemouth bass were evaluated using the Kruskal–Wallis test. *Post hoc* analysis of differences between size classes was conducted using the Steel–Dwass test.

### Relationships between isotopic ratios, trophic positions and body size

We evaluated the relationships between the  $\delta^{13}\text{C}$  of largemouth bass and their TL using Spearman’s correlation coefficient. We also tested the relationship between their trophic position and TL using the same method.

## Results

### Stable isotope ratios of largemouth bass and their potential prey

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of largemouth bass varied considerably among individuals, ranging from  $-32.2$  to

**Table 2.** Stable isotope values of largemouth bass.

	Sampling season	Total length mean (range) TL (mm)	<i>n</i>	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
				Mean	SD	Mean	SD
< 100 mm	Jul–Sep, Nov 2006 <sup>a</sup>	73 (35–99)	82	–29.7	1.2	16.2	0.8
100–199 mm	Aug, Sep, Nov, 2006 <sup>a</sup> Dec 2006	142 (103–185)	28	–28.4	0.8	16.7	1.2
200–299 mm	Dec 2006	250 (234–274)	7	–27.7	1.1	17.6	0.8
≥ 300 mm	May, Dec 2006	330 (300–385)	16	–25.4	1.4	16.5	1.5

<sup>a</sup>Data from Yasuno *et al.* (2012).

**Table 3.** Stable isotope values of potential prey for largemouth bass.

Samples	Sampling season	Size range (mm TL)	<i>n</i>	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
				Mean	SD	Mean	SD
Snail							
<i>Radix auricularia japonica</i>	Jun 2006		3	–26.1	1.1	12.6	0.4
Zooplankton <sup>a</sup>			17	–31.5	1.2	12.1	2.1
Copepod zooplankton			9	–31.9	1.1	13.8	1
<i>Eodiaptomus japonicus</i>	Jun–Sep 2006		4	–31.2	0.8	13.3	0.9
<i>Eodiaptomus japonicus</i> + <i>Cyclops</i> sp.	Oct 2006		1	–32.7		13.7	
<i>Cyclops</i> sp.	Nov, Dec 2006		4	–32.3	1.2	14.3	1.0
Cladoceran zooplankton			8	–31.1	1.2	10.1	1.1
<i>Sida crystalline</i>	Jun, Jul, Sep 2006		5	–31.0	0.7	10.0	1.1
<i>Sida crystalline</i> + <i>Diaphanosoma brachyurum</i>	Jul, Aug 2006		2	–30.7		11	
<i>Diaphanosoma brachyurum</i>	Sep 2006		1	–32.4		9.1	
Benthic invertebrates			46	–35.2	6.1	10.3	2.5
<i>Chironomus plumosus</i> <sup>b</sup>	May–Dec 2006		41	–35.8	6.2	10.2	2.6
<i>Epophthalmia elegans</i>	Jun 2006		4	–29.5	2.4	12.6	0.4
<i>Sinictinogomphus clavatus</i>	Jun 2006		1	–35.5		10.5	
Red swamp crayfish							
<i>Procambarus clarkia</i>	May–Jul 2006		6	–26.5	1.0	13.6	0.9
Small omnivorous fishes			74	–27.8	1.8	13.6	1.5
<i>Gnathopogon elongatus elongatus</i> <sup>a</sup>	Jul–Sep 2006	43–80	15	–29.0	0.5	14.8	1.0
<i>Pseudorasbora parva</i> <sup>a</sup>	Jul–Sep 2006	34–102	15	–28.6	0.8	14.9	0.8
<i>Biwia zezera</i> <sup>a</sup>	Jul–Sep 2006	32–62	8	–28.3	1.0	13.1	0.8
<i>Acheilognathus rhombeus</i> <sup>a</sup>	Jul–Sep 2006	34–85	20	–26.4	2.0	12.0	1.0
<i>Tridentiger obscurus</i> <sup>a</sup>	Jul 2006	31–35	3	–28.3	0.7	14.0	0.5
<i>Sarcocheilichthys variegatus microoculus</i>	Sep 2006	68–93	5	–26.3	3.1	13.3	1.6
<i>Carassius</i> sp.	Jul 2006	44–48	4	–28.7	1.5	13.7	1.2
<i>Opsariichthys platypus</i>	Jul 2006	54–70	4	–26.4	1.0	12.4	0.6

<sup>a</sup>Data from Yasuno *et al.* (2016).

<sup>b</sup>Data from Yasuno *et al.* (2013).

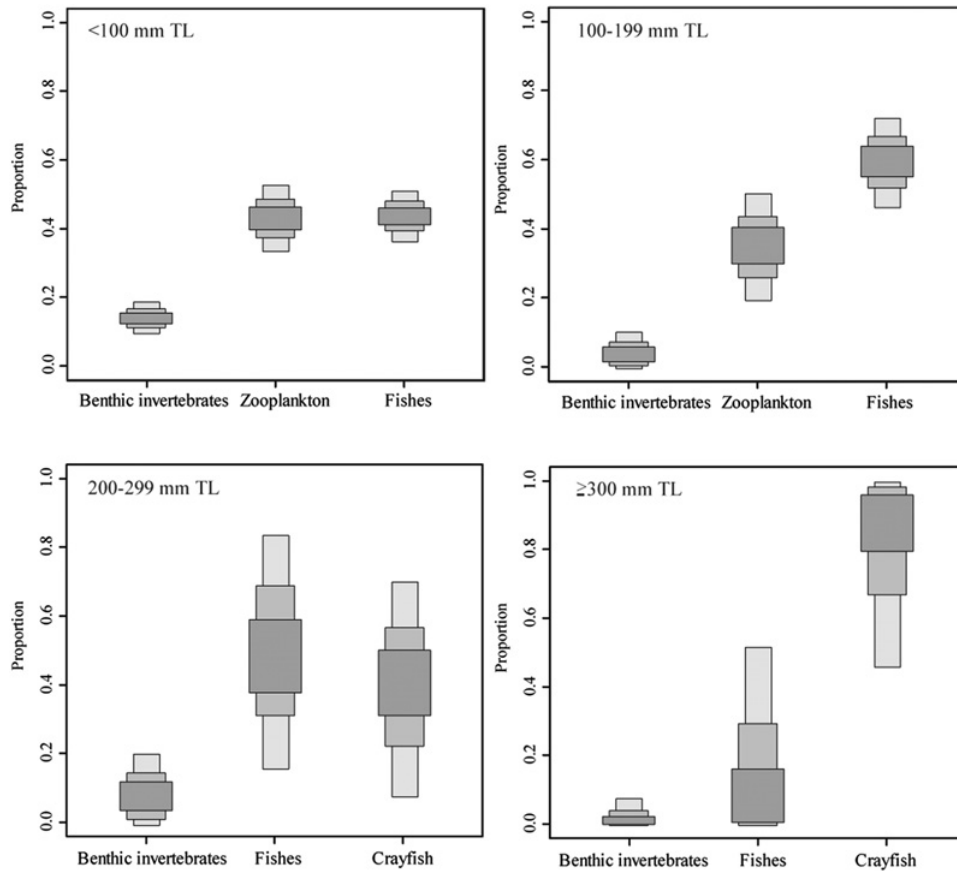
– 22.0‰ and from 14.0 to 18.9‰, respectively. Bass that were > 300 mm TL were the most  $\delta^{13}\text{C}$ -enriched, while bass < 100 mm TL were the most  $\delta^{13}\text{C}$ -depleted (Table 2). The 200–299 mm TL size class was more enriched in  $\delta^{15}\text{N}$  than any other size class (Table 2).

The potential prey of largemouth bass (small omnivorous fishes, red swamp crayfish, benthic invertebrates and zooplankton) had distinctive mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in Lake Izunuma (Table 3). The mean  $\delta^{13}\text{C}$  value of red swamp crayfish was more enriched than those of other food sources and similar to that of grazing snails *R. auricularia japonica*. By contrast, the mean  $\delta^{13}\text{C}$  value of benthic invertebrates was the most depleted among potential prey species. The mean  $\delta^{13}\text{C}$  values of small omnivorous fishes and zooplankton had intermediate values among the potential prey species, although the mean value of small omnivorous fishes indicated more

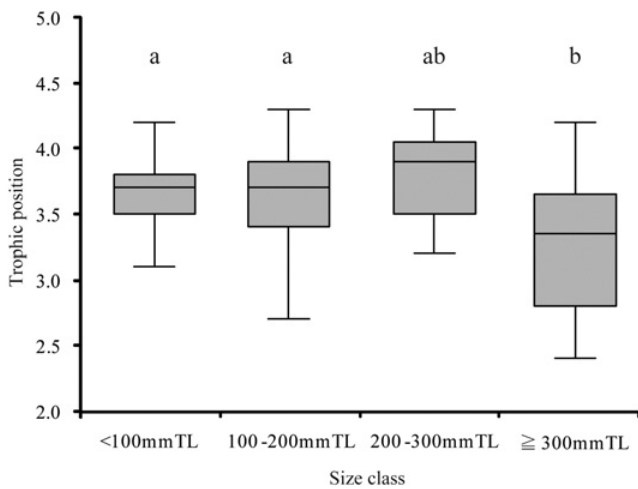
enrichment than that of zooplankton. The mean  $\delta^{15}\text{N}$  values of small omnivorous fishes and red swamp crayfish were more enriched than those of zooplankton and benthic invertebrates. Among zooplankton, the mean  $\delta^{15}\text{N}$  values of copepods and cladocerans were the most enriched and the most depleted values, respectively, among potential prey species.

#### Fractional dietary contribution of food sources

The SIAR mixing model showed the dietary differences among size classes of largemouth bass (Fig. 2). Among the potential prey species, zooplankton contributed more to the diet of the smallest size class (< 100 mm TL; 95% credibility interval, 33–52%) than to fish 100–199 mm TL (20–51%) (Fig. 3), while small omnivorous fishes were the



**Fig. 2.** Boxplots derived from the stable isotope analysis in R mixing model showing the contribution of different potential food sources to the diets of four largemouth bass size classes using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Fishes = small omnivorous fishes; crayfish = red swamp crayfish. The proportions show credibility intervals plotted at 95, 75 and 25% credibility intervals.



**Fig. 3.** Trophic positions of four largemouth bass size classes. Shared lower-case letters indicate that no significant differences were found in the trophic positions at  $P < 0.05$ . Different letters indicate significant differences.

most important prey items for both of these size classes (36–51 and 47–72% for fish < 100 mm TL and 100–199 mm TL, respectively). These results were consistent with previous reports that largemouth bass

consume zooplankton during their early life-history (< ca. 100 mm long) (Keast and Eadie, 1985; Olson, 1996).

We found that red swamp crayfish did contribute more to the diet of the largest size class ( $\geq 300$  mm TL; 46–100%) than small omnivorous fishes (0–51%). The 200–299 mm TL size class depended both on small omnivorous fishes (16–84%) and red swamp crayfish (8–71%). Benthic invertebrates rarely contributed to the diets of any size classes.

**The trophic positions of largemouth bass and their potential prey**

Largemouth bass occupied higher trophic positions than other organisms (Fig. 3, Table 4). The trophic positions of largemouth bass  $\geq 300$  mm TL were significantly lower than those of largemouth bass < 100 mm TL and 100–199 mm TL ( $P < 0.05$ , Fig. 3).

Among the potential prey species of bass, copepod zooplankton, which was the dominant zooplankton taxon and small omnivorous fishes (*G. elongates elongates*, *P. parva* and *T. obscurus*) occupied a trophic position of approximately 3, suggesting that they are secondary consumers (Table 4). The trophic positions of *A. rhombeus*

**Table 4.** Trophic positions of potential prey for largemouth bass.

Samples	n	Trophic position	
		Mean	SD
Snail			
<i>Radix auricularia japonica</i>	3	2.0 <sup>a</sup>	–
Zooplankton	17	2.6	0.6
Copepod zooplankton	9	3.1	0.3
Cladoceran zooplankton	8	2.0 <sup>a</sup>	–
Red swamp crayfish			
<i>Procambarus clarkii</i>	6	2.5	0.3
Small omnivorous fishes	74	2.6	0.6
<i>Gnathopogon elongatus elongatus</i>	15	3.1	0.3
<i>Pseudorasbora parva</i>	15	3.1	0.3
<i>Biwa zezera</i>	8	2.5	0.3
<i>Acheilognathus rhombeus</i>	20	2.0	0.5
<i>Tridentiger obscurus</i>	3	2.8	0.3
<i>Sarcocheilichthys variegatus microoculus</i>	5	2.4	0.4
<i>Carassius</i> sp.	4	2.7	0.6
<i>Opsariichthys platypus</i>	4	2.0	0.2

<sup>a</sup>Trophic positions of snail and cladoceran zooplankton were assumed at 2.0.

and *Opsariichthys platypus* were approximately 2, suggesting that they are primary consumers (herbivores). Other small omnivorous fishes and red swamp crayfish had intermediate trophic levels between 2 and 3. Small omnivorous fishes (mean  $\pm$  1 SD;  $2.6 \pm 0.6$ ) and zooplankton ( $2.6 \pm 0.6$ ) as a whole had intermediate trophic positions that were similar to that of red swamp crayfish ( $2.5 \pm 0.3$ ).

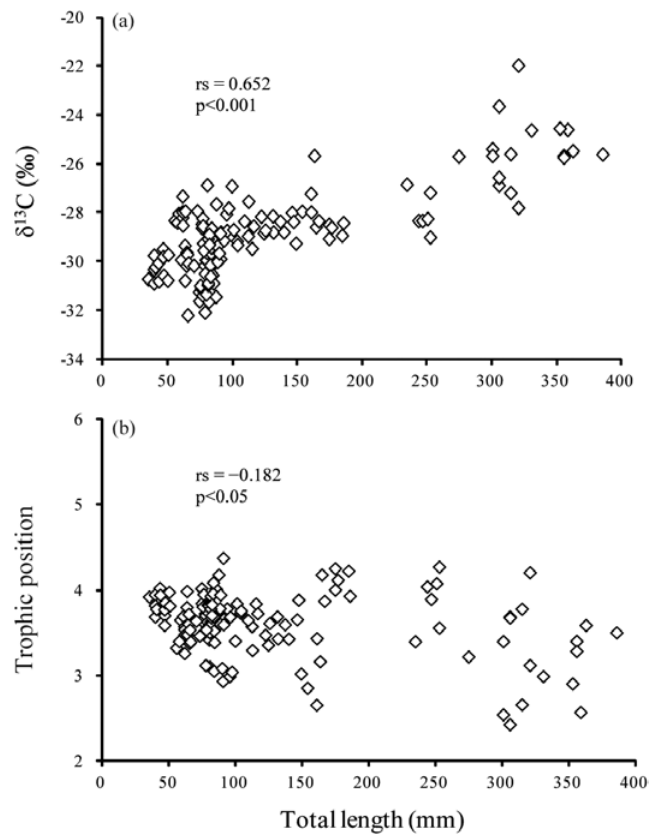
#### Relationships between isotopic ratios, trophic positions and body size

The  $\delta^{13}\text{C}$  values of largemouth bass correlated significantly with TL ( $r_s = 0.652$ ,  $P < 0.001$ , Fig. 4(a)), indicating ontogenetic dietary shifts toward more  $\delta^{13}\text{C}$ -enriched food sources. Their trophic positions correlated negatively with TL ( $r_s = -0.182$ ,  $P < 0.05$ , Fig. 4(b)).

## Discussion

#### Dietary analyses of largemouth bass using stable isotopes

Piscivorous fishes typically experience ontogenetic diet shifts, either moving directly from zooplanktivory to piscivory, or including a period of insectivory (Keast, 1985; Olson, 1996). Previous studies have reported that largemouth bass forage on aquatic insects (e.g., Hickley *et al.*, 1994; Garcia-Berthou, 2002). In Lake Izunuma, however, benthic insects (larval chironomids and dragonflies) were rarely consumed by any size class of largemouth bass (Fig. 3). In fact, Takahashi *et al.* (2001) reported that the stomach contents of juvenile largemouth bass contained  $< 5\%$  aquatic insects. Because largemouth bass are opportunistic feeders (Schindler *et al.*, 1997; Wasserman *et al.*, 2011; Magoro *et al.*, 2015) and find prey visually



**Fig. 4.** Correlations between (a)  $\delta^{13}\text{C}$  values and (b) trophic positions of largemouth bass and total length. 'rs' represents Spearman's correlation coefficient.

(Crowl, 1989), they may only rarely forage on larval chironomids and dragonflies, which inhabit lake bottoms. We found a positive correlation between  $\delta^{13}\text{C}$  values and TL in the largemouth bass population (Fig. 2(a)). In Lake Izunuma, pelagic producers (mainly phytoplankton) had lower  $\delta^{13}\text{C}$  values than did littoral producers (epiphytes

and aquatic plants) (Yasuno *et al.*, 2014; Yasuno *et al.*, 2016). This suggests that the contributions of the pelagic food chain to the diet of largemouth bass decreased, while those of the littoral food chain increased as the bass became larger. Using an SIAR mixing model, we determined the possible contribution of four potential prey sources to largemouth bass tissues. The results showed that the smallest size class (< 100 mm TL) relied on both zooplankton and small omnivorous fishes, the 100–199 mm TL size class relied mostly on fishes, and the largest size class ( $\geq$  300 mm TL) foraged mostly on red swamp crayfish (Fig. 3). Largemouth bass generally shift from zooplanktivory to piscivory during their first year (Keast, 1985; Olson, 1996). Our previous study (Yasuno *et al.*, 2012) in Lake Izunuma, based on stable carbon isotope analyses, showed that the shift to piscivory in the largemouth bass population began at approximately 60–90 mm TL, and the threshold size for piscivory became larger as the season progressed because of the increasing sizes of prey fishes. Therefore, the main diet of bass in Lake Izunuma appears to shift from zooplankton to small fishes and finally to red swamp crayfish.

### Trophic positions of largemouth bass

Ontogenetic diet shifts towards piscivory are generally considered to increase the trophic position of piscivores (Persaud *et al.*, 2012). In Lake Izunuma, the smallest size class (< 100 mm TL) relied on both zooplankton and small omnivorous fishes, while the 100–199 mm TL size class relied mostly on fishes (Fig. 3). However, there was no significant difference in trophic positions between these size classes (Fig. 4). Our previous study (Yasuno *et al.*, 2012) showed that their  $\delta^{15}\text{N}$  values did not clearly increase with ontogenetic shifts to piscivory. Thus, dietary shifts from zooplanktivory to piscivory do not always increase trophic positions. Our results showed that the trophic positions of small omnivorous fishes were as low as, or lower than, those of copepod zooplankton, although the mean trophic levels of omnivorous fishes and zooplankton were similar (Table 4). In Lake Izunuma, small omnivorous fishes forage on epiphytes (producer; lower trophic level) as well as zooplankton (Yasuno *et al.*, 2016). Foraging on epiphytes may explain the lower trophic positions of small omnivorous fishes. As a result, ontogenetic dietary shifts to piscivory did not increase the trophic position of largemouth bass in Lake Izunuma.

Our results also showed that the mean trophic position of the largest size class of bass ( $\geq$  300 mm TL) was significantly lower than those of smaller size classes (Fig. 4). The largest size class foraged mostly on red swamp crayfish (Fig. 3), which feed on various food sources, including plants and invertebrates (Smart *et al.*, 2002) and thus can function as both primary and secondary consumers. The trophic position of red swamp crayfish was lower than those of the dominant fishes in Lake Izunuma (*G. elongates elongates* and *P. parva*), although it was similar to the mean trophic positions of small

omnivorous fishes. Therefore, ontogenetic shifts from foraging on fishes to red swamp crayfish could actually decrease the trophic position of largemouth bass.

We showed that the trophic position of largemouth bass did not increase with TL in a temperate, eutrophic, shallow lake and the mean trophic position of the largest class size was significantly lower than those of the smaller size classes. Red swamp crayfish, the main food source for bass  $\geq$  300 mm TL, had a slightly lower trophic position than small omnivorous fishes and zooplankton. The feeding strategies of consumers are often flexible, being able to switch to lower, more productive trophic levels (Pimm, 1982). In Lake Izunuma, abundant primary producers, such as epiphytes and aquatic plants, are widely available for omnivores, allowing omnivorous fishes and red swamp crayfish to heavily rely on littoral production. Red swamp crayfish are, in fact, known to rely heavily on litter derived from terrestrial plants in Japanese farm ponds (Kobayashi *et al.*, 2011). The positive correlation between  $\delta^{13}\text{C}$  and TL of largemouth bass (Fig. 2(a)) suggests that the contributions of littoral rather than pelagic production increased with growth. Consequently, the trophic position of largemouth bass did not increase with the ontogenetic shift from zooplanktivory to piscivory. Furthermore, the trophic position of the largest individuals decreased following their shift to foraging on red swamp crayfish. Our results suggest that the trophic position of largemouth bass should be calculated with caution and their body size should be considered when estimating food chain length or assessing contaminant bioaccumulation. Further clarification of the trophic positions of large piscivores as they relate to body size will greatly improve studies focusing on food-web structures, especially in shallow eutrophic lakes with wide littoral habitats.

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