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Trophic polymorphism in bluegill sunfish (*Lepomis macrochirus*) introduced into Lake Biwa: evidence from stable isotope analysis

Abstract Trophic polymorphism was recently reported in introduced bluegill (*Lepomis macrochirus*) in Lake Biwa, Japan, where three morphs are specialized in benthic invertebrates (benthivorous type), submerged aquatic plants (herbivorous type), and zooplankton (planktivorous type). We evaluated the long-term effects of food resource utilization by these trophic morphs using stable isotope ratios, $\delta^{15}N$ and $\delta^{13}C$. A significant difference in $\delta^{15}N$ was found between the benthivorous and planktivorous types. The planktivorous type had the higher $\delta^{15}N$ value, which corresponded with the value expected from its prey, zooplankton. The lower $\delta^{15}N$ value of the benthivorous type would be derived from the lower $\delta^{15}N$ values of benthic prey organisms compared to zooplankton. These results support previous findings that the benthivorous and planktivorous types have different food resource utilization. In contrast, the $\delta^{15}N$ and $\delta^{13}C$ values of the herbivorous type were distinctly different from the expected values, indicating that this type was unlikely to utilize aquatic plants substantially, contradicting the results of the dietary analysis.

Key words Trophic polymorphism · Introduced bluegill · Food resource utilization · Stable isotope

Introduction

Bluegill (*Lepomis macrochirus*), a freshwater fish native to North America, is now one of the most prosperous exotic species in Japanese freshwater ecosystems. Eighteen blue-

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gills captured in the Mississippi River at Guttenberg, Iowa (U.S.A.), were first imported to Japan in 1960, and then rapidly spread throughout the country. PCR–RFLP (polymerase chain reaction–restricted fragment length polymorphism) comparisons of mitochondrial DNA between introduced populations in Japan and native populations indicate that today's Japanese populations originated from a small number of fish from the Guttenberg population (Kawamura et al. 2006). The establishment of this species in Lake Biwa, the biggest and oldest lake in Japan containing many endemic fish species, was confirmed in the mid-1960s, and its population dramatically increased in the 1980s (Nakai 1999).

The bluegill is known as a generalist feeder, and a study of an introduced population in Japan showed that it consumes a variety of items, such as zooplankton, aquatic insects, benthic fish, and aquatic plants (Azuma 1992). In some native populations of this fish, trophic polymorphism has been observed, which consists of two morphs (Ehlinger and Wilson 1988; Ehlinger 1990; Wilson 1998). One morph has an enlarged body depth, long pectoral fins, and specializes in slow searching for benthic prey in vegetation (littoral morph), whereas the other has a slender body and short pectoral fins, which allows efficient swimming while searching for zooplankton in open water (open-water morph). In Lake Biwa, it was reported that the bluegill mainly feed on shrimp in the 1970s, just after its establishment (Terashima 1980). However, Yonekura et al. (2002) found a trophic polymorphism in the current population, consisting of three trophic morphs with different diets and morphological characteristics. These three morphs were 56–131 mm in standard length and were not yet showing secondary sexual traits. They differed especially in body depth and had different stomach content compositions, preferentially feeding on three distinct food resources (benthic invertebrates, submerged aquatic plants, or zooplankton). This is a unique case in that the trophic polymorphism occurred in the introduced population on a short timescale.

Stable isotope analysis is a powerful tool with which to evaluate consumer–diet relationships. It has the advantage of detecting the long-term record of food assimilated, which

cannot be determined using stomach content analysis, as it gives a short-term record of the food ingested. Nitrogen isotope ratios (δ^{15}N) increase by 3–4‰ with each increasing trophic level, thereby allowing the estimation of trophic position (DeNiro and Epstein 1981; Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). Carbon isotope ratios (δ^{13}C) increase only slightly (<1‰) between consumers and their diet (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001); thus, the carbon source can be traced to a primary food source. This isotopic information allows the trophic position of focal species in food webs to be estimated. In this study, we used stable isotope analysis to examine the long-term effects of food resource utilization by the trophic morphs of introduced bluegill in Lake Biwa.

Methods

Sample collection and preparation

Bluegill (*Lepomis macrochirus*) were collected from the north basin of Lake Biwa, Japan, at adjacent two sites, Sugaura (35°27′ N, 136°07′ E) and Shiotsu (35°30′ N, 136°09′ E), in September 2005. Sugaura had a mixture of three structurally different environments: a rocky substratum with no aquatic macrophytes (<2 m deep), an environment with aquatic macrophytes (1–4 m deep), and an open water zone (>4 m deep). Shiotsu comprised a rocky substratum and a macrophyte zone (<2.5 m deep). Bluegill were caught by fishing, immediately killed by pithing, placed on ice, and transferred to the laboratory within 12 h. In the laboratory, the fish were individually labeled, and their standard length and three morphological characteristics (body depth, pectoral fin length on the left side, and mouth width) were measured to the nearest 0.01 mm using a digimatic caliper (Mitutoyo, Kanagawa, Japan). After measurement, they were dissected to collect the stomach contents for dietary analysis. Bluegill were further used for stable isotope analysis; the muscle tissue was excised from the dorsal part of their lateral body and kept at −20°C until analysis.

Potential prey organisms for each feeding type of bluegill were also collected for stable isotope analysis. As main prey items for the benthivorous type, three taxonomic groups of benthic invertebrates, i.e., chironomid larvae, trichopteran larvae, and gastropods, which were often found in the stomach contents of this type in previous studies (Yonekura 2002; Yonekura et al. 2002), were collected between May and August 2005 from the north basin of Lake Biwa [$n = 5$ for Chironomidae larva, $n = 3$ for Trichoptera larva, $n = 2$ for Lymnaeidae (Basommatophora), and $n = 8$ for Pleuroceridae (Mesogastropoda)]. The main prey item of the herbivorous type, the dominant submerged aquatic plant species *Elodea nuttallii* (Yonekura, personal communication), was sampled at Sugaura in September 2005. Zooplankton was collected using a plankton net (300-μm mesh) at a depth of 7–8 m at Sugaura in October 2005. In the laboratory, the zooplankton was washed with distilled water and used in bulk for stable isotope analysis. The zooplankton was composed of approximately 60% Copepoda and 40% *Daphnia* spp. in number.

Stomach content analysis

The feeding types of the bluegill were classified according to Uchii et al. (2006). The stomach contents were sorted into five prey items, i.e., benthic invertebrates, aquatic plants, zooplankton, terrestrial insects, and other, under a binocular microscope. Stomach contents that could not be identified were regarded as unidentified matter. For each fish, the proportions of these prey items were estimated by the points method (Swynnerton and Worthington 1940; Hynes 1950). Ward's linkage cluster analysis using squared Euclidean distances was performed to classify individual fish into several clusters based on prey composition using the statistical package SPSS 12.0 (SPSS, Chicago, IL, USA).

Morphological analysis

Standard length and the three morphological measurements, i.e., body depth, pectoral fin length, and mouth width, were log-transformed before statistical analysis. Residuals of each morphological measurement from linear regressions with standard length were analyzed using canonical discriminant analysis to test for morphological differences associated with feeding type. To examine the overall morphological difference pairwise between feeding types, a set of resulting canonical scores were compared using multivariate analysis of variance (MANOVA). Differences in single morphological characteristics were analyzed using analysis of covariance (ANCOVA), with feeding type as a fixed factor and standard length as a covariate, after checking for homogeneity of slopes. Sex was not incorporated as an independent variable because individuals showing secondary sexual traits were not collected.

Stable isotope analysis

The samples for stable isotope analysis were dried at 60°C for 48 h, and then ground to a fine powder. Animal samples (bluegill, benthic invertebrates, and zooplankton) were further placed in chloroform–methanol (2:1) solution to remove lipids and re-dried. The samples were wrapped in tin cups for combustion. Stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) were measured using a mass spectrometer delta-S (Finnigan MAT, Bremen, Germany). The δ^{13}C or δ^{15}N is expressed as the ratio (‰) of ^{13}C/^{12}C or ^{15}N/^{14}N relative to the standard as follows:

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

where R is ^{13}C/^{12}C or ^{15}N/^{14}N. Pee Dee Belemnite (PDB) and atmospheric nitrogen were used as the standard for carbon and nitrogen, respectively. The standard deviations of the working standard (DL-alanine) were less than 0.1‰ for both δ^{13}C and δ^{15}N in all runs. One-way analysis of variance (ANOVA) using a post hoc Bonferroni test was

Table 1. Mean proportion and occurrence rate (in parentheses) of each prey item for three bluegill clusters, determined using Ward's linkage cluster analysis, and the mean standard length of bluegill in each cluster

Cluster	n	Mean standard length ± SD (mm)	Prey items					
			Benthic invertebrates	Aquatic plants	Zooplankton	Terrestrial insects	Other organisms	Unidentified matter
I	10	101.80 ± 11.16	0.46 (1)	0.21 (0.6)	0	0.08 (0.2)	0	0.25
II	8	98.57 ± 5.03	0.03 (0.25)	0.14 (0.5)	0.75 (1)	0	0.08	0
III	15	105.47 ± 8.63	0.05 (0.2)	0.91 (1)	0	0.02 (0.13)	0	0.02

n, number of individuals
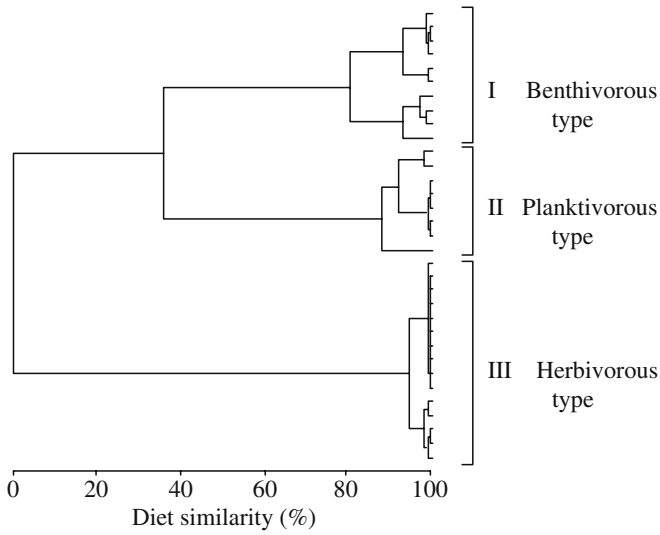
Fig. 1. Cluster dendrogram using Ward's method for bluegill individual prey composition

Fig. 2. Plot of canonical discriminant scores for the three feeding types: ■, benthivorous type; ×, herbivorous type; ○, planktivorous type

performed to detect differences in $\delta^{13}C$ and $\delta^{15}N$ among the feeding types.

Results and discussion

Classification of feeding types

Benthic invertebrates found in the bluegill stomachs were mainly Chironomidae larvae, Trichoptera larvae, and Lymnaeidae; the aquatic plant species was mainly *E. nuttallii*; and zooplankton mainly consisted of *Daphnia* spp. Ward's linkage cluster analysis classified individual bluegill into three clusters (Fig. 1). We found no significant difference in the standard length among the three clusters (one-way ANOVA, $P > 0.2$). In the prey composition of the three clusters (Table 1), cluster I had a higher proportion of benthic invertebrates (46% on average) than the other two clusters. Cluster II had a higher proportion of zooplankton (75% on average), and cluster III had a higher proportion of aquatic plants (91% on average), compared to the other two clusters. No clear differences were observed in the proportions of terrestrial insects and other prey organisms
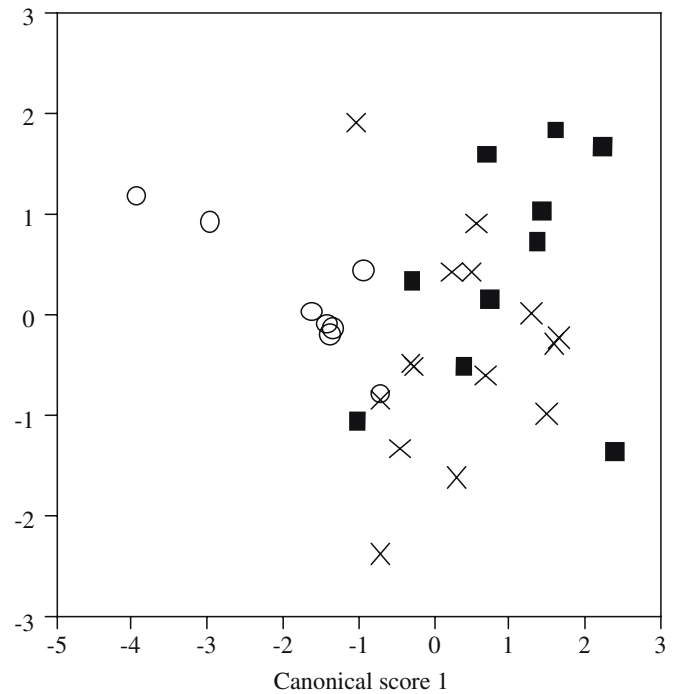
among the three clusters. Based on these results, we defined individuals of cluster I as the benthivorous type, those of cluster II as the planktivorous type, and those of cluster III as the herbivorous type.

Morphological difference among feeding types

The canonical discriminant analysis found an overall significant morphological difference among the three feeding types (Wilks' $\lambda = 0.480$, $\chi^2 = 21.27$, $P < 0.01$). The plot of canonical scores showed a distinct distribution between the benthivorous and planktivorous types (Fig. 2). Pairwise comparisons using MANOVA on the first and second canonical scores detected significant differences between the planktivorous type and the other two types (vs. benthivorous type: Wilks' $\lambda = 0.448$, $F_{2,15} = 9.24$, $P < 0.01$; vs. herbivorous type: Wilks' $\lambda = 0.531$, $F_{2,20} = 8.85$, $P < 0.01$), but no difference between the benthivorous and herbivorous types

Table 2. Adjusted means of log-transformed morphological measurements for three bluegill feeding types, adjusted for log-transformed standard length through analysis of covariance (ANCOVA)

Morphological characteristic	P (all three)	Adjusted mean (log-transformed)		
		Benthivorous type	Herbivorous type	Planktivorous type
Body depth	0.18	3.865[a]	3.856[a]	3.834[a]
Pectoral fin length	0.40	3.451[a]	3.475[a]	3.466[a]
Mouth width	<0.001	2.487[a]	2.479[a]	2.372[b]

Values with the same letter within rows are not significantly different ($P > 0.05$) after Bonferroni correction

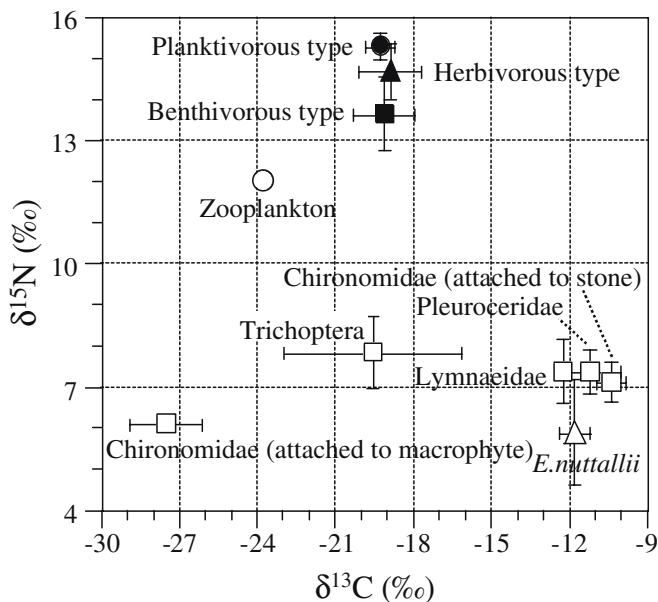**Fig. 3.** $\delta^{13}C$ and $\delta^{15}N$ means of three bluegill feeding types and their potential prey. *Vertical and horizontal bars* indicate standard deviations

(Wilks' $\lambda = 0.861$, $F_{2,22} = 1.78$, $P = 0.19$). In the ANCOVA on each of the three morphological characteristics (body depth, pectoral fin length, and mouth width), the regression slopes did not differ among the feeding types for each morphological characteristic. Mouth width differed significantly among the three feeding types (ANCOVA, $P < 0.001$; Table 2), and subsequent pairwise comparisons found significantly wider mouths of the benthivorous and herbivorous types than the planktivorous type after Bonferroni correction ($P < 0.05$; Table 2). In threespine sticklebacks (*Gasterosteus aculeatus*), it has been reported that benthic species with wider mouths have greater success in foraging benthic prey than limnetic species, and vice versa in foraging planktonic prey (Schluter and McPhail 1992; Schluter 1993, 1995). Also in bluegill, the wider mouth of the benthivorous type may be a consequence of adaptation to the benthic feeding. The body depth and pectoral fin length showed no clear differences among the feeding types.

Evaluation of food utilization by feeding types based on stable isotopic signatures

The mean isotopic values, $\delta^{15}N$ and $\delta^{13}C$, of the three feeding types of the bluegill and the potential prey organisms were plotted on a $\delta^{13}C$–$\delta^{15}N$ map (Fig. 3). A significant difference was found in $\delta^{15}N$ among the three feeding types (one-way ANOVA, $F_{2,30} = 13.15$, $P < 0.001$). The post hoc Bonferroni test showed that the benthivorous type had significantly lower $\delta^{15}N$ than the herbivorous type ($P < 0.01$) and the planktivorous type ($P < 0.001$), indicating different food use between the benthivorous type and the other two types. The nitrogen isotopic difference between the herbivorous and planktivorous types was not statistically significant ($P = 0.17$). No significant difference was found in $\delta^{13}C$ among the three types (one-way ANOVA, $P > 0.7$).

The benthivorous type (13.64‰) had distinctly higher $\delta^{15}N$ values than the planktivorous type (15.29‰) (Fig. 3). This difference well corresponded to previous observations on $\delta^{15}N$ of other fish species in Lake Biwa (Yamada et al. 1998; Maruyama et al. 2001; Ogawa et al. 2001), where benthic fish feeding on mainly zoobenthos have lower values (13‰ for gudgeon, *Sarcocheilichthys variegatus*, and 13.3‰ for goby, *Rhinogobius* sp.), and pelagic fish feeding on mainly zooplankton have higher values (15‰–18‰ for Japanese trout, *Plecoglossus altivelis altivelis*, 16‰ for smelt, *Hypomesus transpacificus*, and 15.2‰–16.7‰ for goby, *Chaenogobius isaza*). In bluegill, the different $\delta^{15}N$ values between the benthivorous and planktivorous types may reflect a difference in $\delta^{15}N$ between their prey organisms. Benthic invertebrates (chironomid larvae, trichopteran larvae, and gastropods), which were the main prey organisms for the benthivorous type, showed lower $\delta^{15}N$ values (6.10‰–7.84‰) than zooplankton (12.05‰) (see Fig. 3). The $\delta^{15}N$ mean of the planktivorous type was 3.24‰ higher than that of zooplankton, which corresponds with the average ^{15}N enrichment per trophic level (3‰–4‰) based on empirical data (DeNiro and Epstein 1981; Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). However, the $\delta^{15}N$ mean of the benthivorous type was more than 5‰ higher than those of their prey organisms. Although we cannot determine the isotopic fractionation of nitrogen between the benthivorous type and its prey, the lower $\delta^{15}N$ of the benthivorous type would be partly explained by the lower $\delta^{15}N$ of benthic prey organisms.

In contrast, $\delta^{13}C$ signatures could not account for differences in food resource utilization between the benthivorous and planktivorous types. The benthivorous type showed large individual variation in $\delta^{13}C$, which might have derived from a variety of benthic prey organisms whose $\delta^{13}C$ values were also widely distributed. The difference in $\delta^{13}C$ between zooplankton and the planktivorous type (4.6‰) was much greater than the average ^{13}C enrichment (<1‰) between the diet and consumers estimated in empirical studies (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001). This discrepancy may have been caused by a greater seasonal change in $\delta^{13}C$ of zooplankton, which have rapid growth rates than fish (Yoshioka et al. 1994; Zohary et al. 1994). For example, the half-change period of muscle $\delta^{13}C$ is estimated to be more than 20 days for rapidly growing juvenile fish (Suzuki et al. 2005) and more than 1 year for slow-growing salmonid fish (Hesslein et al. 1993). In Lake Biwa, the $\delta^{13}C$ of small particulate organic matter (POM; 2.7–$40\,\mu m$), which is equivalent to phytoplankton in the diet of zooplankton, increases up to -14‰ in summer, and drastically declines to -24‰ in autumn (Yamada et al. 1998). In the present study, such a temporal change in $\delta^{13}C$ in the pelagic food web and the great interspecific variation in $\delta^{13}C$ among the benthic prey organisms make it difficult to estimate the food resources of each feeding type using carbon isotope ratios.

For the herbivorous type, both the $\delta^{13}C$ and $\delta^{15}N$ values were vastly different from those expected from its staple food, *E. nuttallii* (7.10‰ lower for $\delta^{13}C$ and 8.79‰ higher for $\delta^{15}N$). This result suggests that this type would not successfully utilize *E. nuttallii*, in contradiction to the dietary analysis. Unclear distinction of the herbivorous type from the other two types in morphology and isotopic compositions also suggests incompleteness of this type as a "herbivorous" trophic morph. As shown in the present study, stomach content analysis alone can lead to erroneous estimation of food resource use. In contrast, stable isotope analysis provides integrated information of assimilated food over time (Bearhop et al. 2004). When studying the trophic polymorphism in fish population, the stable isotope analysis enables us to make more accurate evaluation of food segregation by trophic morphs.

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