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Physical structure of habitat network differently affects migration patterns of native and invasive fishes in Lake Biwa and its tributary lagoons: stable isotope approach

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Abstract A good understanding of how migratory animals use their habitat network is expected to provide important insights for the prediction of population dynamics at both local and regional scales. We focused on how the physical structure of a habitat network could affect fish migration between Lake Biwa and its tributary lagoons. Although the lagoons provide suitable breeding and nursery grounds for native fishes, it is a matter of concern that they can also be a hotbed of invasive exotic fishes. Here, we assessed the migration patterns of native crucian carps (*Carassius* spp.) and exotic largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) using their carbon and nitrogen stable isotopes as migration tracers. As there were marked differences in the carbon isotope ratios of basal food webs between the main lake and its tributary lagoons, stable isotopic signatures of individual fishes collected from each lagoon enabled us to judge whether they were residents of the lagoon or recent immigrants from the main lake. The analysis revealed that native and invasive fishes showed different migration patterns across a variety of lagoons. Exotic fishes frequently immigrated from the main lake to the lagoon as the distance of the channel connecting these two habitats was short. For native crucian carps, in contrast, their migrations were unaffected by the channel distance but were promoted by narrow channels. Physical barriers of weirs and dense vegetation within the channel obstructed their migrations. Such ecological information on migration behavior will be vital to plan designs for habitat restoration to conserve native fishes.

Keywords Bluegill · Channel width · Crucian carp · Distance · Largemouth bass · Physical barrier

Introduction

For animals, migration is an important life history trait. They migrate among different habitats diurnally for feeding and predation avoidance, seasonally for reproduction, or at a given stage of their lives according to ontogenetic changes in their resource requirements (Aidley 1981; Ohman 1990). Their migrations greatly affect not only individual fitness consequences but also their population structure. The lack of habitat connectivity can affect genetic population structure, often decreasing genetic diversity in a local population (e.g., Matsubara et al. 2001; Yamamoto et al. 2004). The low genetic diversity may decrease population viability because of inbreeding depression and population fragility under fluctuating environments (Primack 1993). For such reasons, conservation biologists have made much effort to assess how the physical destruction of habitat networks or habitat fragmentation accelerates the extinction risk of wildlife populations, using the population viability analysis (PVA) model (Ferreras et al. 2001; Akçakaya et al. 2004; Hakoyama and Iwasa 2005).

In spite of the importance of field data of migration patterns in animal conservation, we often have limited access to them because it is difficult to track the migration

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routes of such animals as birds and fishes that migrate long distances or underwater. Stable isotope analysis is a powerful tool to infer animal migration routes (reviewed by Hobson 1999). Carbon isotope ratios of animals provide temporal-integrated information of basal food web, which reflects the isotopic signature of primary producers (DeNiro and Epstein 1978; Fry and Sherr 1984), of their origin for the past several weeks to months depending on metabolic turnover or growth rate (Hobson 1999). Aquatic ecosystems can show great spatial heterogeneity of the stable isotope ratios of basal food webs among habitats via biogeochemical processes depending on physical and chemical environments of each habitat (Fry 2006). Thus, when animals migrate between patchy habitats within the ecosystem, isotopic information of their previous habitat is recorded in their tissues (Peterson and Fry 1987). Based on such a property, stable isotope analysis has been used to estimate recent migration routes of wild animals (Kennedy et al. 2002; Rubenstein et al. 2002; Fukumori et al. 2008).

Our study site, Lake Biwa, is an ancient lake, the third oldest in the world, harboring over 1,000 species including 58 endemics (Nishino and Watanabe 2000; Nishino 2003). Because of its biological uniqueness, this lake is considered to be a biodiversity hotspot (Wildlife survey group in Shiga Prefecture 2006). Lake Biwa has also a remarkable geographic feature: there are hundreds of tributary rivers in its watershed whose area is equivalent to that of Shiga Prefecture, forming a huge habitat network for aquatic organisms. At present, it is reported that there exist more than 60 native freshwater fishes including 15 endemics in its watershed. In this watershed, all fishes, even if they are pelagic species of the main lake, spawn in its shallow coastal areas, tributary rivers, and temporary waters, such as paddy fields, reed areas, and flood plains. Particularly for many cyprinid fishes that show the highest species richness among fish taxa, tributary lagoons, called "Naiko" in Japanese, provide suitable spawning grounds because they are calm, highly productive, and rich in reeds, so that fish larvae and juveniles can use them for resting, feeding, and refuge (Hosoya 2005).

The tributary lagoons have played host to a myriad of spawning migrations by native fishes from the main lake (Miura et al. 1966). However, most of them are now highly fragmentated and degraded, and some have already disappeared due to reclamation for agricultural land use. All around the lake coasts, roads have been constructed across water channels connecting with the adjacent lagoons and many agricultural weirs have been built within the channels. Such physical barriers may prevent native fish from spawning migration to the tributary lagoons, and may have contributed to the recent decline in native fish populations.

Regarding the biological aspects of native fish conservation, another matter of concern is the introduction of exotic invasive species, such as largemouth bass [*Micropterus salmoides* (Lacépède)] and bluegill sunfish [*Lepomis macrochirus* (Rafinesque)]. They have great ecological impact on the native fishes through predation or competition for food (Findlay et al. 2000; Maezono and Miyashita 2003). These predatory fishes predominate in number in some tributary lagoons (Minobe and Kuwamura 2001), suggesting that the lagoons may function as a hotbed of exotic fishes or as source for their dispersal, rather than as sanctuaries for the native fishes. If this were so, connectivity between the main lake and its tributary lagoons would stabilize the metapopulation dynamics of exotic fishes and destabilize that of native fishes. To design a conservation plan for habitat restoration of native fishes, we need to know how native and exotic fishes use habitat networks in the watershed of Lake Biwa.

In the present study, we aimed (1) to infer how many fishes migrate from Lake Biwa to its tributary lagoons, and (2) to provide some insights into effects of the physical structure of habitat network on fish migration, using stable isotope analysis for lagoon fishes. We focused on two major fish groups, native crucian carps (*Carassius* spp.) and exotic centrarchid fishes. We also discuss effective management plans for the conservation of native fishes and the removal of exotic invasive fishes in the watershed of Lake Biwa.

Materials and methods

Stable isotope estimation of migration route

We first explain the methodology to estimate how fishes migrate between patchy habitats, using stable isotope analysis. Isotopic signatures of food webs can vary spatially based on several possible biogeochemical processes (Fry 2006) and the signatures of consumers reflect the characteristic isotopic signatures of food web in the habitat where the consumers grew up (DeNiro and Epstein 1978). Therefore, we can infer origin of animals by comparing the signatures of the animals and geographical distribution of isotopic characteristics of each habitat (reviewed by Hobson 1999). We will gain the best estimation of animal origin when we can know isotope signatures of all food items used by individuals. However, it is very hard to investigate the isotopic signatures of all food items in many habitats in detail, especially for focal species having a high degree of omnivory. Thus, we assessed differences in isotopic signatures among habits based on isotopic range of each food web, which could include all consumers feeding in each habitat. In lake ecosystems, there are two primary producers, phytoplankton and periphyton, which form the base of pelagic and benthic food chains, respectively.

These two producers show a marked difference in isotopic signatures, with enrichment of carbon isotope ratios for the periphyton (Fig. 1; France 1995a, b; Brönmark and Hansson 2005; Fry 2006). For consumers, their carbon isotope ratios depend on that of basal food, with a trophic enrichment factor of 0.8‰ (Fig. 1; Vander Zanden and Rasmussen 2001). The consumers also enrich their nitrogen isotope ratios by 3.4‰ on average per trophic level (Minagawa and Wada 1984; Vander Zanden and Rasmussen 1999). As a result, the pure pelagic and benthic food chains form two lines that run parallel to each other but have different carbon isotope ratios on the C–N map (Fig. 1). For virtually all consumers, which are usually omnivores themselves or feed on omnivorous prey, their stable isotopic signatures are usually located between these two lines if they are embedded within a focal food web (e.g., Yamada et al. 1998).

For example, there exist two adjacent lacustrine habitats that are channeled to each other but have sufficiently different isotopic boundaries, depicted by Habitats A and B in Fig. 1. Fishes A1 and A2 were collected from Habitat A, and fishes B1 from Habitat B. After performing the stable isotope analysis for these fishes, we found that the stable isotopic signatures of A1 were characteristic of Habitat A (shown in black), and those of B1 and A2 were characteristic of Habitat B (shown in white). Judging from their isotopic distributions, we can conclude that A2 recently migrated from Habitat B to A and that A1 and B1 are residents of Habitat A or B, respectively.

In order to demonstrate the utility of this method in Lake Biwa and its tributary lagoons systems, we conducted a preliminarily study in a tributary lagoon, Nodanuma (site no. 12 in Fig. 2), and the main lake coast adjacent to it, which were connected by a channel of 93 m. We collected bivalves (*Unio douglasiae biwae* Kobelt), snails (*Sinotaia quadrata histrica* Gould), shrimps (*Palaemon paucidens* De Haan), and exotic invasive fishes, bluegill sunfish and largemouth bass, from these two habitats and then measured their stable isotope ratios (see below for details of analysis). It has been suggested that isotopic signatures of long-lived primary consumers are good proxies of those of basal food webs (Vander Zanden and Rasmussen 1999; Mckinney et al. 2002; Vander Zanden et al. 2005), because aquatic primary producers often show high degree of temporal variation in their stable isotope ratios but long-lived consumers provide constant results as integrated values of the variable isotopic signatures of primary producers (Cabana and Rasmussen 1996). We used the bivalves as an indicator of the pelagic food chain because they feed mainly on plankton (e.g., Vander Zanden and Rasmussen 1999; Gustafson et al. 2007). Although Vander Zanden et al. (2005) used snails to estimate the benthic food chain, we used the shrimps instead (Vander Zanden
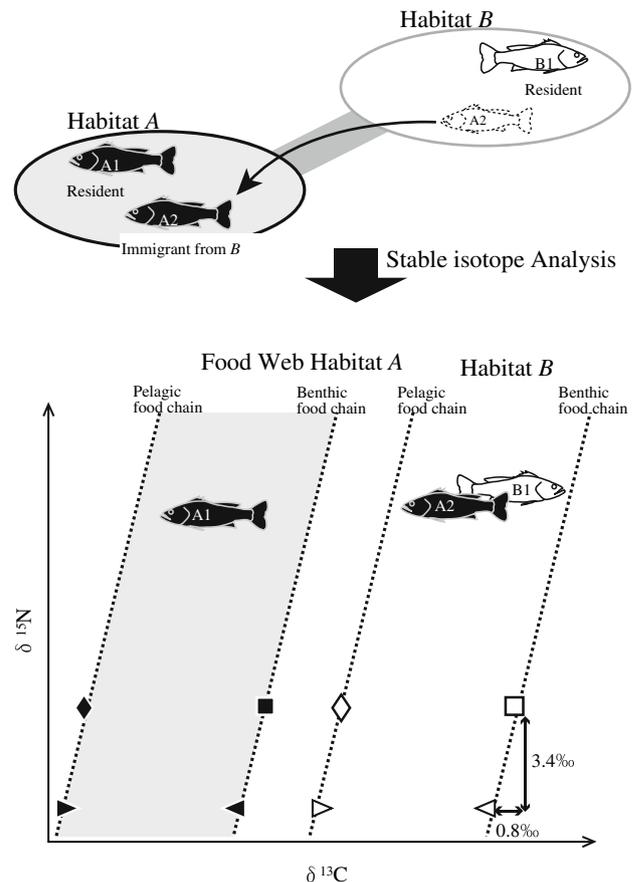

Fig. 1 A conceptual schema of stable isotope approach to estimate fish migration between habitats. Lake food webs consist of pelagic and benthic food chains which are isotopically different from each other on the carbon and nitrogen isotope (C–N) map. The pelagic food chain starts from phytoplankton (*right-pointed filled triangle*, *right-pointed open triangle*), being trophically transferred to phytoplankton feeders (*filled diamond, open diamond*). The benthic food chain starts from periphyton (*left-pointed filled triangle*, *left-pointed open triangle*), being trophically transferred to benthic algal grazers (*filled square*, *open square*). Trophic enrichment factors of these two food chains are 0.8‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$. Food webs in *Habitat A* and *B* have discrete isotopic boundaries encompassed by *black* and *white* marks, respectively. Fish *A1* and *A2* (*black*) were collected from *Habitat A* and fish *B1* (*white*) from *Habitat B*. Residents *A1* and *B1* show isotopic signatures of each collected habitat, but immigrant *A2* shows isotopic signatures of origin (*Habitat B*). See text for further explanation

and Hulshof 1998), because the shrimps showed greater enrichment of carbon isotope ratios ($\delta^{13}C = -23.83 \pm 2.45$, $n = 18$) than the snails (-28.44 ± 3.16, $n = 18$; paired t test, $df = 17$, $t = -6.34$, $P < 0.0001$), suggesting that the former were algal grazers and the latter were opportunistic omnivores in these habitats. We confirmed that there was little variation in the carbon isotope ratio of these two invertebrates (bivalves: -28.84 ± 0.33 in June 2006, -28.28 ± 0.22 in November 2006, -29.00 ± 0.12 in June 2007; shrimps: -22.75 ± 1.05 in June 2006,
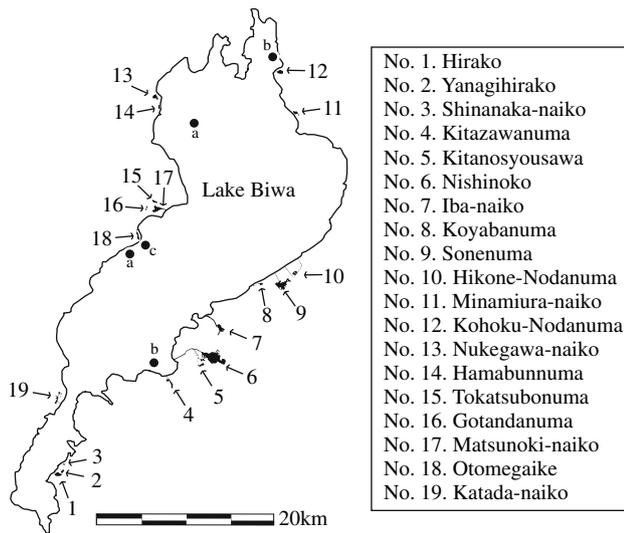
Fig. 2 Location of study sites, Lake Biwa and its tributary lagoons. *Filled dots* are sampling sites of the main lake fishes of past studies (*a* Yamada et al. 1998; *b* Takai and Sakamoto 1999; *c* Maruyama et al. 2001a)

No. 1. Hirako
No. 2. Yanagihirako
No. 3. Shinanaka-naiko
No. 4. Kitazawanuma
No. 5. Kitanosyousawa
No. 6. Nishinoko
No. 7. Iba-naiko
No. 8. Koyabanuma
No. 9. Sonenuma
No. 10. Hikone-Nodanuma
No. 11. Minamiura-naiko
No. 12. Kohoku-Nodanuma
No. 13. Nukegawa-naiko
No. 14. Hamabunnuma
No. 15. Tokatsubonuma
No. 16. Gotandanuma
No. 17. Matsunoki-naiko
No. 18. Otomegaike
No. 19. Katada-naiko

Fig. 3 The C–N map of food webs in the main lake and its adjacent lagoon, Nodanuma (site no. 12). Bivalves collected from main lake (*open diamond*) and from lagoon (*filled diamond*) indicate the base of pelagic food chain and shrimp collected from main lake (*open square*) and from lagoon (*filled square*) the base of benthic food chain. *Horizontal* and *vertical bars* are standard deviations. Bluegill sunfish (*Lepomis macrochirus*) collected from main lake (*open circle*) and from lagoon (*filled circle*) and largemouth bass (*Micropterus salmoides*) collected from main lake (*open triangle*) and from lagoon (*filled triangle*) are higher consumers in these two food webs. Hypothetical food chains have the same trophic enrichment factors as those in Fig. 1

−22.69 ± 2.52 in November 2006, −22.92 ± 1.54 in June 2007). Terrestrial insects may be confounding factors for estimation of the isotopic signatures of limnetic food webs as important allochthonous organic sources for fishes. However, the terrestrial insects make few contributions to fishes inhabiting around Lake Biwa (Yodo and Kimura 1998; Nakao et al. 2002). Therefore, we omitted the terrestrial insects from consideration of the isotopic signatures of food webs.

The isotopic signatures of individual fishes collected from the two habitats (main lake and its tributary lagoon) and their pelagic and benthic food chains are plotted on the C–N map shown in Fig. 3. The two habitats had discrete isotopic food webs, with the main lake food web showing more enriched carbon isotope ratio. All largemouth basses and bluegills collected from the main lake showed similar carbon isotope ratios that overlapped or were close to the main lake food web, suggesting they were residents of the main lake. By contrast, of the fishes collected from the tributary lagoon, three bluegills showed depleted carbon isotope ratios that were within the isotopic boundary of the tributary lagoon food web, whereas two bluegills and all largemouth basses showed enriched carbon isotope ratios that deviated from the lagoon food web isotopic boundary and were similar to a member of the main lake food web. We therefore conclude that the latter two bluegills and all largemouth basses possibly migrated from the main lake to its tributary lagoon in the recent past. For the remaining one bluegill, its isotopic signature was located between the isotopic boundaries of the two food webs, without belonging to either of them. One possibility is that it might
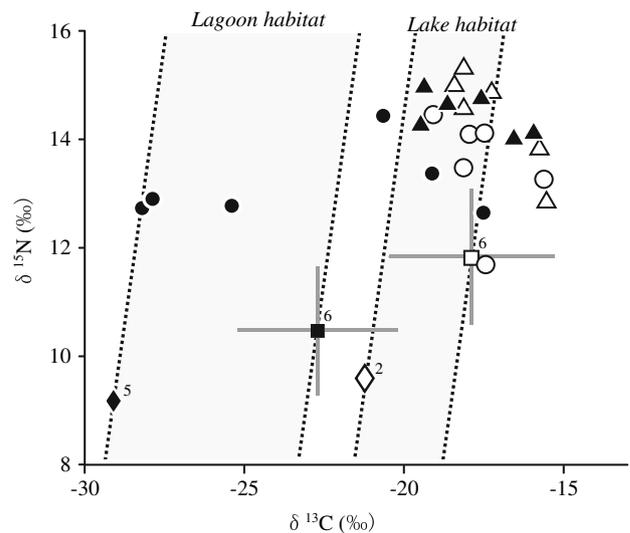
have migrated from the main lake long ago and then its isotope ratios have been gradually replaced by that of prey from the lagoon food webs through its tissue's metabolic turnover. In such a way, we estimated the migration history of each fish collected from each tributary lagoon, based on the results of stable isotope analysis (see below).

Sample collection

We applied this stable isotope approach to estimate the migration histories of fishes collected from 19 tributary lagoons around Lake Biwa (Fig. 2). We focused on two fish groups: one is native crucian carps and the other is exotic invasive centrarchids, largemouth bass and bluegill. The former consists of three closely related species *Carassius cuvier* (Temminck and Schlegel)*, C. auratus grandoculis* (Temminck and Schlegel), and *C. a. langsdorfii* (Cuvier and Valenciennes). It has been reported that all three species have the habit of spawning migration to temporary waters, such as reeds, paddy fields, and lagoons, from spring to rainy season (Nakamura 1969; Hosoya 2005). As the sample size was not large for each species collected from each lagoon and they show similar migration behavior, we pooled these data for convenience. We collected the subject fishes from the 19 tributary lagoons using

seine and gill nets and basket traps, from late May to early July 2007.

As indicators for the basal food chains, we also collected bivalves and shrimps from all lagoons except two (site nos. 3 and 4) where we failed to collect bivalves. These sample collections were conducted in June and September 2006 and from May to July 2007. To estimate the base of the main lake pelagic food chain, we also used bivalves collected from the coastal areas of the main lake for another study in November 2005 and February 2006. We selected bivalve specimens collected from the coastal areas where each study lagoon was located in closest proximity. We did not use shrimps collected from the main lake coasts for the stable isotope analysis because the base of the benthic food chain, the upper end with more enriched carbon isotope ratio in the main lake food web, was not necessary for estimating fish migration from the main lake to its tributary lagoons (see "Results").

All specimens were preserved at −30°C for the stable isotope analysis.

Stable isotope analysis

For the stable isotope analysis, we dissected muscle tissues of all animal specimens and dried them at 60°C for 24 h. The samples were pulverized and immersed in 2:1 chloroform–methanol solution for 24 h to remove lipids (Bligh and Dyer 1959). Carbon and nitrogen stable isotope ratios were measured with a mass spectrometer (Delta-S; Finnigan Mat, Barkhausenstrasse 2, D-2800, Bremen14, Germany) connected to an elemental analyzer (EA1108; Fisons, Strada Rivoltana-20090, Rodano, Milan, Italy). Isotopic notations of carbon (δ^{13}C) and nitrogen (δ^{15}N) were expressed as per mil deviation from the standards (atmospheric N_2 gas for nitrogen and PeeDee belemnite carbonate for carbon) as defined by the following equation:

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

where R denotes ^{15}N/^{14}N or ^{13}C/^{12}C, respectively. Analytical errors were ±0.1‰ for δ^{15}N and ±0.2‰ for δ^{13}C.

Data analysis

For each fish collected from a tributary lagoon, we judged whether it was a resident of the lagoon or an immigrant from the main lake, based on the results of origin estimation using mean stable isotope ratios of the primary consumers as proxy of basal food web boundaries, as mentioned above. If its isotopic signature was located between the upper end of the carbon isotopic boundary in the lagoon food web and the lower end in the main lake food web, as in the case of one bluegill collected from Nodanuma (Fig. 3), we regarded it as pending, although we speculate that the fish had migrated into the lagoon long ago. Such cases were all excluded from the data analysis to avoid uncertainty of conclusion. In cases in which the isotopic boundaries of two habitats overlapped and focal fishes were located in the overlapped area, we also excluded them from the data analysis to avoid uncertainty because the fish origin could not be judged whether of the main lake or the tributary lagoon. Based on these criteria for the isotope ratio estimation, we examined the proportion of immigrants in each lagoon population, for each of native crucian carps and exotic largemouth bass and bluegill.

We used the likelihood ratio test (LRT) for generalized linear mixed models (GLMMs; see Lindsey 1999) to compare the proportion of immigrants among the three fishes or to examine how the proportion could be affected by the physical characteristics of channels connecting the main lake to its adjacent lagoons. We incorporated the dependent variable "study site" as random term into a binomial regression model to account for the overdispersion in the model (Williams 1982; Schall 1991; McGilchrist 1994). To describe the physical characteristics of each channel, we measured water depth, water velocity, and channel width, and checked the presence of any barriers, such as agricultural weirs and dense vegetation, within the channel. In addition, we measured the channel distance between the main lake coast and its adjacent lagoon, using GIS software (ChizuTaro ver. 4.0, Tokyo Cartographic).

Prior to using the multiple-regression model of the GLMMs, we checked if data transformation [$\ln(x + 1)$] could improve the power of the test for each dependent variable. We performed the analysis of variance inflation factor (VIF) to assess multicollinearity among dependent variables (Massfield and Helms 1982). In our model, variable "channel depth" was excluded from the analysis because it had VIF values larger than 10, the criterion for avoidance of multicollinearity (Aoki 2008). We adopted a model selection method to obtain minimal adequate models by sequentially eliminating the least significant factors (Crawly 2002). The significance of the factor was assessed by the change-in-deviance associated with dropping the effect from the model. Accordingly, we sequentially eliminated the non-significant factors ($P > 0.05$ in LRT) to obtain the minimal adequate models and show the contribution of each factor to the minimal model by corresponding z and p values in the results. All statistical analyses were performed using R (R Developmental Core Team 2007), and GLMMs were conducted using repeated package (Lindsey 1999).

Results

Stable isotope characteristics of lake and lagoon habitats

We compared the stable isotope characteristics of the basal food webs between the main lake and its tributary lagoon habitats (Fig. 4). The isotopic signatures of bivalves, which indicated the base of the pelagic food chain, of the tributary lagoons significantly depleted in their $\delta^{13}C$ by 8.1‰ on average (paired t test, $df = 16$, $t = 7.90$, $P < 0.0001$) but not in their $\delta^{15}N$ (paired t test, $df = 16$, $t = 1.64$, $P = 0.12$). For some lagoons (site nos. 1, 2, and 3), the isotopic signatures of their food webs entirely overlapped with those of coastal food webs in the main lake (Fig. 4). Although slight overlap was observed in some other sites (site nos. 8, 9, 11, 13, and 17), the stable isotopic signatures of food webs were markedly different between the main lake coast and its adjacent lagoons, suggesting that the stable isotope approach could effectively estimate fish migration between these two habitats. To check the validity of method for inferring fish origin using the isotopic signatures of food webs, we analyzed a total of 123 fishes, for which their carbon and nitrogen stable isotope ratios are known from past reports (Yamada et al. 1998 at sites a in Fig. 2 near to site 14 and 18; Takai and Sakamoto 1999 at sites b in Fig. 2 near to site 4 and 12; Maruyama et al. 2001a at site c in Fig. 2 near to site 18), inhabiting the main lake habitat. While only 6.5% of the fishes ($n = 123$) resulted in wrong estimation that the fishes were judged as lagoon fishes though they resided in the main lake, 61.0% of the fishes were correctly classified as the main lake residents.

Interspecific differences in migration patterns

In Fig. 5, number of individuals estimated as immigrants from the main lake and residents of the tributary lagoons is shown for each fish taxa collected from each lagoon. For three lagoons (site nos. 1, 2, and 3) whose isotopic signatures entirely overlapped with those of the main lake, we could not obtain any estimation, and thus we excluded data of the three lagoons from further analyses. The proportion of immigrants of all fish taxa were not significantly different regardless of whether criteria for classification of each fish origin were mean values or extreme values (i.e., minimum carbon isotope ratios of the main lake bivalves and maximum values of the tributary lagoon shrimps, Fig. 4; LRT, $n_{individuals} = 121$, $n_{sites} = 28$, $df = 1$, $\chi^2 = 0.35$, $P = 0.55$ for largemouth bass; $n_{individuals} = 203$, $n_{sites} = 28$, $df = 1$, $\chi^2 = 0.00016$, $P > 0.99$ for bluegill; and $n_{individuals} = 148$, $n_{sites} = 26$, $df = 1$, $\chi^2 = 0.121$, $P = 0.73$ for crucian carps). Native crucian carps showed a
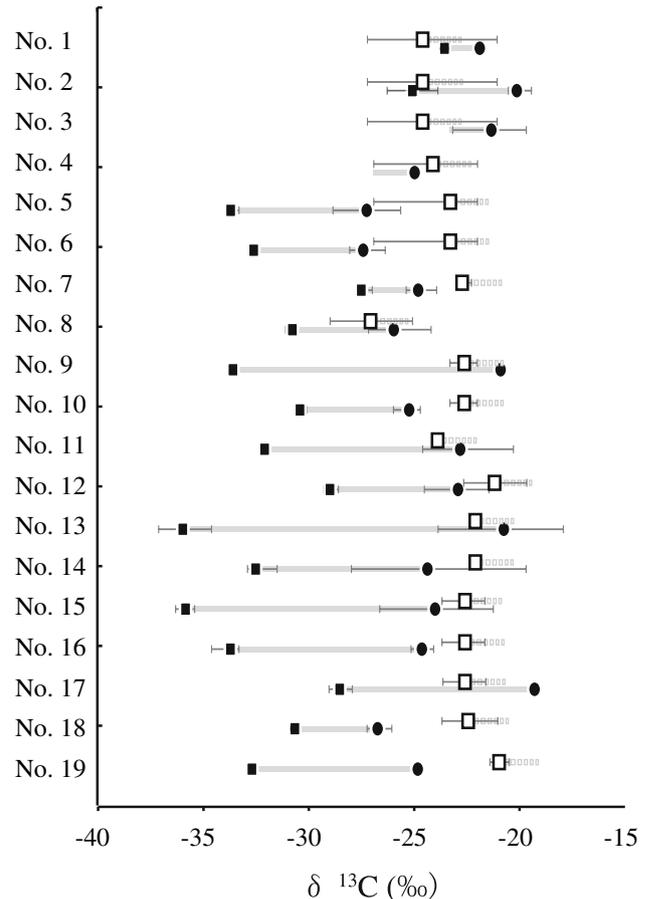

Fig. 4 Carbon isotope boundary of lagoon food webs and its lower end of their adjacent coastal food webs in the main lake. Bivalves from lagoons (*filled square*) and from main lake coasts (*open square*) indicate the base of pelagic food chain and shrimps from lagoons (*filled circle*) the base of benthic food chain. *Horizontal bars* are ranges between minimum and maximum values

significant higher proportion of immigrants (36.2%, $n = 13$) than bluegill (10.3%, $n = 15$) and largemouth bass (14.0%, $n = 13$; LRT, $n_{individuals} = 237$, $n_{sites} = 16$, $df = 2$, $\chi^2 = 11.62$, $P < 0.01$). Among crucian carp species, there were no significant differences in the immigration rate (LRT, $n_{individuals} = 96$, $n_{sites} = 13$, $df = 2$, $\chi^2 = 0.42$, $P = 0.81$).

For each fish group, the proportion of immigrants was also greatly different among lagoons (Fig. 5). Multiple regression analysis showed that there were interspecific differences in the significant effects of the physical characteristics of habitat network on the migration patterns between native crucian carps and exotic centrarchid fishes (Table 1). For largemouth bass and bluegill, the shorter channel distance facilitated their immigration into the lagoons, with no other substantial effects (Fig. 6; Table 1). By contrast, the crucian carps frequently immigrated from the main lake to the lagoons whose channel was narrower and the proportion of immigrants was independent of the

channel distance (Fig. 7; Table 1). Physical barriers, such as agricultural weir and dense vegetation, prevented them from immigrating into the lagoons (Fig. 7; Table 1).
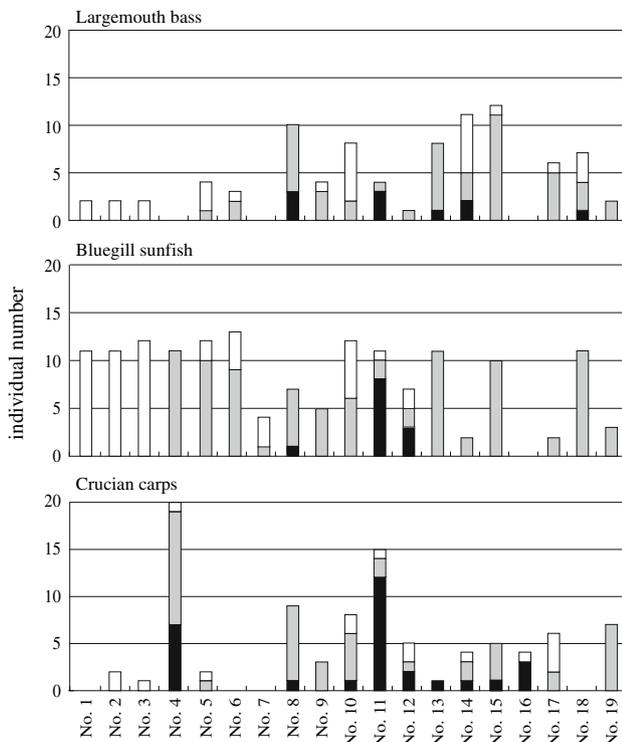

Fig. 5 Number of immigrants from the main lake and lagoon residents of three fishes, crucian carps, bluegill and largemouth bass. Immigrants (*black bars*), lagoon residents (*gray bars*), and individuals (*white bars*) whose migration route could not be identified from the stable isotope analysis (see text)

Discussion

Our study demonstrated that stable isotope analysis was useful for the estimation of fish migration to the tributary lagoons from the main lake in the watershed of Lake Biwa. The stable isotope analysis suggested that the physical structure of habitat network differently affects the migration patterns of native crucian carps and exotic centrarchid fishes. Although it has been reported that the distance between patchy habitats has different effects on the extent of migration of a variety of taxa according to their dispersal ability (Tischendorf and Fahrig 2000), our study provides empirical evidence for how the physical structure of habitat network affects the migration patterns of each species.

Many theoretical studies on metapopulation dynamics assume that the extent of migration between local habitats is negatively affected by the habitat distance (Sjögren 1994; Hanski and Gilpin 1997). In contrast, our study showed that the distance between the main lake and its adjacent lagoon habitats did not apparently affect the immigrations of native crucian carps. Rather, the carps showed a higher migration rate when the channel was narrow, contrary to our expectation that wider channels will facilitate fish migration. Crucian carps have the habit of spawning migration toward the far end of temporary waters, such as paddy fields, reed areas, and flood plains, which are connected by small ditches (Nakamura 1969; Hosoya 2005). Because of this habit, they may prefer to pass though narrow channels for spawning migrations. Since the 1970s, the widening of channels has been promoted as part of agricultural improvement for the purpose

Table 1 Effects of physical characteristics of habitat networks on the proportion of immigrants from the main lake in the tributary lagoon population for each fish

	Largemouth bass	Bluegill sunfish	Crucian carps
Channel distance[a]	-1.26 ± 0.50 (-2.53^*)	-4.61 ± 1.64 (-2.81^{**})	–
Water velocity[a]	–	–	–
Channel width	–	–	-0.37 ± 0.13 (-2.96^{**})
Presence of dense vegetation	–	–	-3.20 ± 1.03 (-3.01^{**})
Presence of weir	–	–	-3.19 ± 0.96 (-3.32^{**})
Intercept	5.00 ± 2.53	18.19 ± 6.14	3.74 ± 1.20
Model P value	0.011	0.0051	0.0042
Null deviance	19.35	51.66	40.24
Residual deviance	11.05	13.86	13.05
Residual df	10	12	8
$n_{individuals}$	58	103	76
n_{sites}	13	15	13

Regression coefficients are given with standard errors and those z values are in parentheses

[a] Data were log transformed [$\ln(x + 1)$]

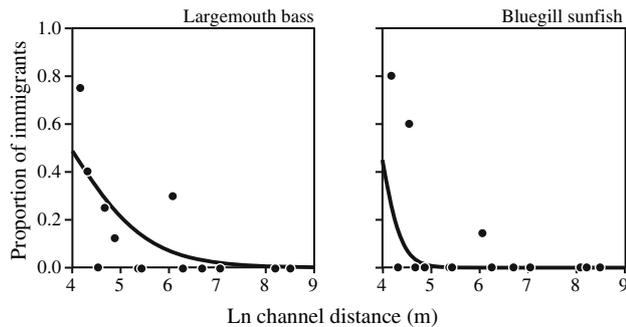* $P < 0.05$

** $P < 0.01$

Fig. 6 Regression curves of proportion of immigrants in the lagoon population against log-transformed channel distance (m) for largemouth bass and bluegill, depicted by the GLMM with binomial errors and logit link function
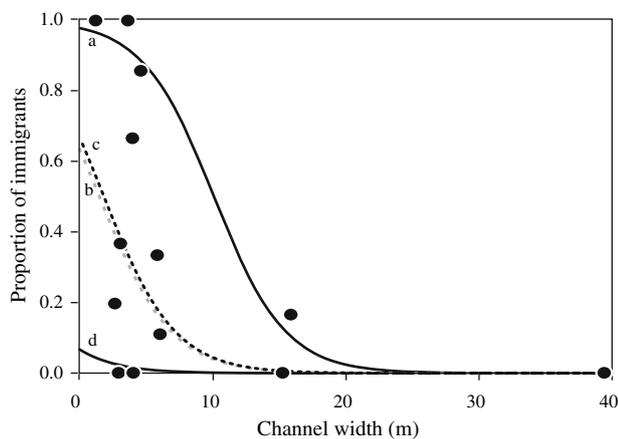

Fig. 7 Regression curves of proportion of immigrants against channel width (m) with four extents (**a**–**d**) of physical barriers within channels for crucian carps, depicted by the GLMM with binomial errors and logit link function. There existed no physical barriers (**a**), only weirs (**b**), only dense vegetation (**c**), and both weirs and dense vegetation (**d**)

of irrigation and flood control in the watershed of Lake Biwa. The present study implies that the channel widening, even if it augments physical habitat connectivity and continuity, does not always increase the extent of fish migration.

Our analysis also revealed that physical barriers within the channels might prevent the crucian carps from immigrating into the lagoons. This is a reasonable and common phenomenon, as reported for many fluvial fishes (Utzinger et al. 1998; Matsubara et al. 2001; Morita and Yamamoto 2002). In the watershed of Lake Biwa, weirs and dense vegetation have recently appeared in different social contexts. The construction of agricultural weirs has progressed as part of agricultural improvement, concurrently with the widening of the channels, since the 1970s. On the other hand, the frequent appearance of dense vegetation within the channels is attributable to the lack of agricultural management by the local community, which is organized

by farmers for the maintenance of irrigation systems. Modern agricultural management and the resultant degeneration of traditional agriculture are likely to have negative effects on the population viability of crucian carps, the representative of agro-dependent species, in the aspect of suitability of habitat network (Katano 2000).

On the other hand, the two exotic centrarchids, largemouth bass and bluegill, showed a high proportion of immigrants when the channel distance was short, in accordance with the general assumption of metapopulation dynamic models. However, none of the physical characteristics of habitat networks had any effect on their migrations. For these two species, it is reported that their upstream migrations are affected by water velocity in the channels (Sakuma et al. 2008). Using artificial channels, Sakuma et al. (2008) experimentally demonstrated that their upstream swimming was inhibited when water velocity was higher than 0.2 m/s and that the proportion of individuals succeeding in the upstream swimming decreased to 60% for the largemouth bass and to 30% for the bluegill when water velocity was 0.5 m/s. In our study sites, by contrast, the water velocities of the channels were usually low, ranging from 0.003 to 0.26 m/s. This is because the tributary lagoons were originally part of the main lake coastal areas, so that their water levels are linked to each other with little water current in the channels (Nishino 2005). The low water velocity may not restrict their migration, resulting in the expansion of their distribution in the watershed of Lake Biwa.

The two centrarchids showed lower proportion of immigrants than the native crucian carps. Conversely, their infrequent migration behavior means that most of the individuals (89.7% for bluegill and 86.0% for largemouth bass) reside in the tributary lagoons. This implies that the lagoons can be a source habitat or a hotbed of exotic invasive centrarchid fishes. The centrarchid fishes could not be only a potential predator of native adult and young fishes but also a potential competitor for zooplankton preys among young (Takamura 2007) and the high residence rate of centrarchids may decrease the persistence of native fish populations.

We often encounter difficulties in conserving native fishes and removing exotic fishes, in terms of time and economic cost. In practical management, it would be more effective to give priority to biological conservation in places where a focal native fish is exposed to the highest extinction risk or where exotic fish has the greatest impact on it. Our stable isotope approach will provide useful information to do so. For example, our efforts to remove exotic fishes should be exerted preferentially on lagoons that accommodate a large number of resident of exotic centrarchids, i.e., that have the potential to be their hotbeds. Another way would be the seasonal opening of agricultural

weirs and the removal of dense vegetation, which would be less costly and more effective in facilitating spawning migration by native crucian carps. To accomplish the adaptive management of native fish populations, we can easily monitor the effects of a specific action on their migration patterns using the stable isotope approach and feedback data that would help improve conservation measures.

Although the origin estimation procedures in this pilot study based on the isotopic signatures of food web provided many useful insights into migration histories of fishes, some individuals were pending estimation of their origin (Fig. 5) and there were some error of estimation of origin for the main lake fishes reported in the past studies (Yamada et al. 1998; Takai and Sakamoto 1999; Maruyama et al. 2001a). In future research, we can improve the robustness and accuracy of results of origin estimation procedure using stable isotope analysis by investigating some aspects of organic sources for fishes in each habitat and retention time of isotopic signatures in the focal fishes. One of the issues is that we have not completely understand how isotopic signatures of primary producers or other organic sources in each food web vary in time and space in the main lake and each lagoon, giving rise to weakness in the confirmation of the criteria for inferring original habitat of fishes. In this study, we used the isotopic signatures of primary consumers as indicators of basal food web in each habitat. The primary consumers can be good proxies of isotopic signatures of primary producers (Vander Zanden and Rasmussen 1999; Mckinney et al. 2002; Vander Zanden et al. 2005), but food items consumed by the primary consumers do not always include all potential food items or organic sources of focal fishes. Thus, understanding isotopic distribution of the primary producers and other organic sources will provide strict isotopic signatures of food web in each habitat and will increase accuracy of estimation of migration history. Another issue is that we do not know for how long large-mouth bass, bluegill and crucian carps, which were investigated in this study, retain the isotopic signatures of their original habitat in their muscles after migration. Following migration, stable isotope ratios of animal tissue gradually change toward the value reflecting the diet in the new habitat. In other fish species, the retention time of isotopic signatures in muscle, which is usually expressed as a half-change period, vary from several weeks to longer than a year depending on growth rate or species-specific metabolic turnover rate (e.g., Hesslein et al. 1993; Maruyama et al. 2001b; Suzuki et al. 2005). Elucidating the retention time of the isotopic signatures of focal species would validate that stable isotope analysis can illuminate past migration history of each fish, and would also provide us with additional suggestions about timing of fish migration (Hobson 1999).

In summary, stable isotope analysis can be a powerful tool to assess how a focal fish uses habitat networks according to its ecological and physiological environmental requirements. Such ecological information on animal migration behavior and habitat use is also helpful in planning designs for habitat restoration to conserve native species, particularly endangered species.

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