Size-related changes in food of dwarf loach, Kichulchoia brevifasciata (Kim & Lee, 1995)

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Size-related changes in food of dwarf loach, *Kichulchoia brevifasciata* (Kim & Lee, 1995)

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Abstract. Size-related changes in feeding habits of the dwarf loach *Kichulchoia brevifasciata* were studied by assessing the gut contents in 43 specimens collected between March 2006 and April 2007. The food items showed rapid changes when the fish reached approximately 35-40 mm of standard length. On the basis of the index of the relative importance of the gut contents (IRI), we identified that small individuals mainly fed on the amoebozoa *Difflugia* and larvae of the aquatic insect Ephemeroptera, while large individuals mainly fed on the diatom algae *Navicula* and larvae of the insect groups Chironomidae and Trichoptera. The results indicate that a suitable habitat for this highly endangered species has to contain a very diverse community of small benthic invertebrates to allow recruitment across all ontogenetic stages.

Key words: ecology, feeding, growth, gut content

Introduction

The dwarf loach *Kichulchoia brevifasciata* is an endemic fish species found in only a few locations of southwestern Korea (Kim & Kim 2008), and is likely to be one of the most endangered cobitid loaches in the world. Despite its endangered status, the fish has not yet been designated as an endangered conservation species in Korea (Ministry of Environment 2008). Reports on its distribution (Kim 1997, Chae & Yoon 2007), chromosome analysis (Kim & Kim 2008), and phylogenetic position among Cobitidae fishes (Šlechtová et al. 2008) have been published. However, to understand the ecological requirements of this declining species, in order to provide the necessary background knowledge for a future conservation program, studies on its life history, reproduction, feeding, and ecological differences between ontogenetic stages are urgently required.

Cobitid loaches are generally known as feeding specialists. They suck up fine bottom material with the sucking force of the expanding gill basket, and physically sort the inorganic material from the organic material, on which they feed (Robotham 1982). Their feeding method leads to a non-selective filter feeding mode. On the one hand, this mode results in the uptake of very small food items even in relatively large specimens (Ritterbusch & Bohlen 2000). On the other hand, with increasing size, large specimens gain the potential to handle and swallow even large items. As a result, the food items of previously studied *Cobitis* and *Sabanejewia* species contained a variety of items ranging from small detritus to insect larvae (Robotham 1977, Ritterbusch & Bohlen 2000, Soriguer et al. 2000, Marszaĺ et al. 2003, Valladolid & Przybylski 2003, Mičetić et al. 2008). However, in some cases, significant differences in food choice have been reported between smaller and larger specimens, indicating that an ontogenetic shift in food might be part of the ecology of at least some cobitid loaches (Lee 1984, Chong 1986, Soriguer et al. 2000, Kim et al. 2006). Adults of *K. brevifasciata* have been shown to eat mainly insect larvae, some algae, and vegetal remains (Kim & Lee 1995, Kim 1997), but there have been no reports on the detailed contents of the gut and changes in feeding habits according to the size of the fish. Furthermore, there have been

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no ecological studies on *K. brevifasciata*. The present study describes a detailed analysis of *K. brevifasciata* food items, and considers potential changes in food during ontogenetic development.

Material and Methods

Fish were collected using a hand net (mesh size, 1 mm) at 34°28′ N and 127°13′ E in a stream in Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea between March 2006 and April 2007. The sampling site was situated 3-4 km upstream from the river mouth, and was mainly covered with pebbles, but contained some sand and cobbles. This stretch of the stream was 1-2 m wide and less than 100 cm deep. The major habitat of this study site was characterized by narrow water width (0.5-3.5 m), a very low water depth (5-18 cm), low water velocity (0-0.037 m/sec), and an Aa stream form, following Kani's classification (Kim 2008, unpublished data). Capturing was conducted for 3 hours in the middle of the stream between 13:00 and 16:00 by three persons. Due to the extremely small population size of *K. brevifasciata*, only up to 17 specimens could be collected per day, and 43 specimens were collected in total. None of the specimens were collected during winter months.

The collected specimens were placed into 10 % formaldehyde. In the laboratory, fish were measured (standard length, SL), weighed (body weight, BW), and dissected. After dissection, the gonads were removed and weighed for determination of gonadosomatic index (gonad wt/body weight × 100). Based on the developmental stage of the gonads, 16 individuals between 10 and 35 mm SL were classified as juveniles and 27 individuals between 35 and 60 mm SL as adults (Table 1), because females and males can be distinguished once individuals reach sizes approximately 35 mm SL (Fig. 1). The alimentary tract of each specimen was removed, the wet weight of the gut content (GW) was measured, and the first half of the digestive tract, which contains mostly undigested food, was examined under an optical microscope. The food items were identified according to Won et al. (2005), Jo (1993), and Jung (1993). For algal food, the number of cells on the microscopic slide was counted for different diet components, and the volume of the different items in the gut contents was visually estimated using a microscopic slide grid. The feeding habits were estimated according to (a) the gut content index (GCI = gut content weight/body weight × 100), which is an indicator of gut content abundance; (b) the food frequency, (%F = the number of specimens containing a particular food item/(the total number of specimens minus the number of specimens with an empty gut) × 100), which is the percentage of species containing a particular food item from the total number of guts investigated; (c) the number of food items, (%N = the number of individuals with a particular food item in the gut/the total number of specimens with food items in the gut × 100), which is the percentage of a particular food item from the total number of confirmed food items; (d) the volume of food items, which is the volume of a particular food item in relation to the total volume of food items in the gut contents (%V = the volume of a particular food item in the gut of the fish/the total volume of the food items in the gut of the fish × 100); (e) for the each food category the index of the relative importance of the gut contents (IRI = (%N + %V) × %F) was calculated (Yodo & Kimura 1998, Takahashi et al. 1999).

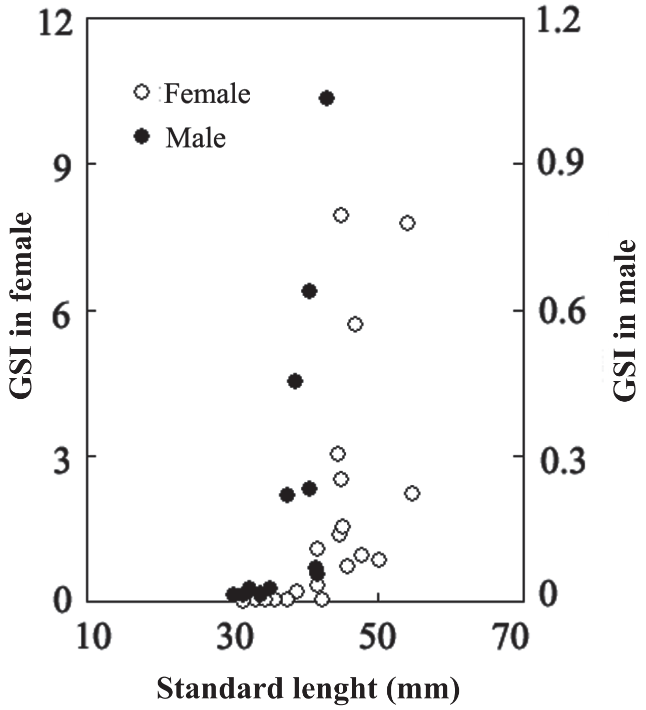

***Fig. 1.** Size-related changes in the gonadosomatic index of female and male of* Kichulchoia brevifasciata *from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

Relationships between the gut contents index (GCI), the number of food items, and average volume of food items along fish size were estimated by the Spearman rank correlation tests. The composition of animal and plant food was compared between juveniles and adults using a Chi-square test on the basis of the total number and volume of the plant and animal prey data.

Table 1. *Standard length and numbers of* Kichulchoia brevifasciata*, from Geougeum-myeon, Goheung-gun, Jeollanam-do, used to investigate gut contents.*

	Range of SL	2006							2007
	(mm)	Mar.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Apr.
Juvenile	10-35	3	1	1	1	10	0	0	0
Adult	35-60	2	1	1	5	7	4	4	3

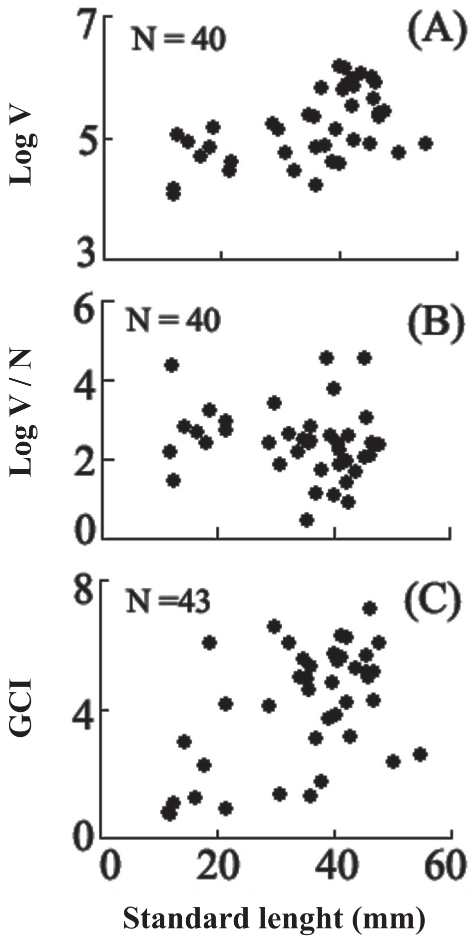

Fig. 2. *Relationships of the number (A), average volume (B) on a log scale, and gut content index (GCI) (C) of food items in the guts against standard length of* Kichulchoia brevifasciata *from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

Results

Relationships between body size and GCI, and the number, and volume of food items

The relationships between SL and GCI, SL and the number of food items, and SL and the average volume of food items in each individual are shown in Fig. 2. The GCI ranged from 0.71 to 7.11 (4.02 ± 1.85, mean ± standard deviation) and was positively correlated with SL (Spearman's rank correlation, $rs = 0.419$, $p < 0.01$). The number of food items ranged from 1.32 to 3.98 (2.72 ± 0.73) on a log scale, and was also positively correlated with SL ($rs = 0.419$, $p < 0.01$). The average volume of food items ranged from 0.44 to 4.57 (2.42 ± 0.9) on a log scale, and was not significantly correlated with SL ($rs = -0.240$, $p > 0.05$).

Size-related changes in IRI

The relative values of %N, %V, %F, and IRI based on the major gut contents of *K. berevifasciata* are summarized in Table 2. In 43 specimens, the number of major food items was limited but a total of 60 species of food items were identified, including algae, microzoobenthon, aquatic insects, and some detritus. In the analysis of each specimen, class Bacillariophycea, *Cymbella* (0.6 < IRI ≦ 121), *Navicula* (4.8 < IRI ≦ 572.6), and *Fragilaria* (0 < RI ≦ 92) were the dominant representatives of the algae. The amoebozoa, *Difflugia* (0 < IRI ≦ 1071.5) and larvae of the aquatic insect groups Chironomidae (0 < IRI ≦ 211.6), Ephemeroptera (0 < IRI ≦ 158.8), and Trichoptera (0 < IRI ≦ 187.4) were the main food items of animal origin in the gut contents.

The relationship between the main food items and SL is presented in Fig. 3. *Navicula* was the major plant food item especially in fish over 35 mm SL, but *Cymbella* was also an important food item in individuals > 40 mm SL. Although *Difflugia* was the main animal prey of juvenile specimens that was still found in specimens > 40 mm SL. Chironomidae larvae were found only in specimens that were 35-36 mm in SL, and Trichoptera was increasingly found in specimens measuring around 40 mm in SL. These results suggest that the food items showed rapid changes when the fish reached approximately 35-40 mm of SL.

Comparison of vegetable and animal prey

A comparison between %N and %V of plant and animal prey found in juvenile and adult *K. brevifasciata* specimens are shown in Fig. 4. In juveniles, animal prey as the major element of food accounted for 23.5 % of the number and 88.3 % of the volume of the total

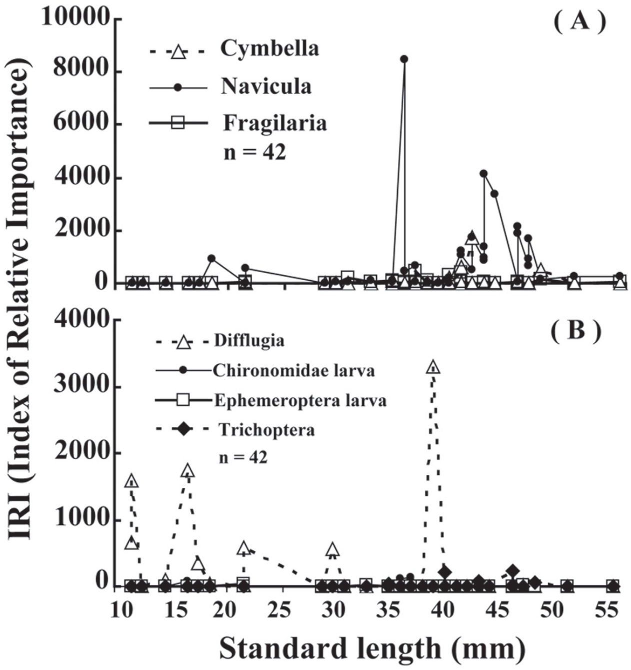

Fig. 3. *Size-related changes in food content and the index of relative importance (IRI) for major vegetable (A) and animal prey (B) of* Kichulchoia brevifasciata *in each specimen from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

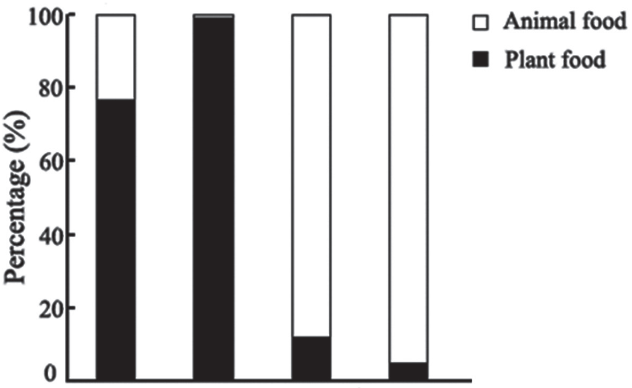

Fig. 4. *Comparison of the number (%N) and volume (%V) of vegetable and animal prey items in juvenile (< 35 mm standard length) and adult (> 35 mm standard length)* Kichulchoia brevifasciata *from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

gut contents. Plant prey accounted for 76.5 % of the number and 11.7 % of the volume. In adults, animal prey was observed to be inversely proportional to plant prey, accounting for 1.2 % of the number and 95.4 % of the volume of the total gut contents, while plant prey accounted for 98.8 % of the number and 4.6 % of the volume. The total numbers and volumes of plant and animal prey differed significantly between juveniles and adults in Chi-squared test (number: $\chi^2 = 10946$, $p < 0.01$; volume: $\chi^2 = 270192$, $p < 0.01$).

Discussion

K. brevifasciata inhabits the pebble-covered substratum of small, shallow streams (Kim & Lee 1995). Benthivorous fish are known to commonly feed on both animal and vegetable food items (Vadas 1990), and also spined loaches have been shown to generally feed on such a mixture of items (Robotham 1977, Ritterbusch & Bohlen 2000, Soriguer et al. 2000, MarszaĺI et al. 2003, Valladolid & Przybylski 2003, Mičetić et al. 2008). On the basis of our IRI results (Table 2), the main prey of *K. brevifasciata* included Trichoptera, aquatic insect larvae, *Difflugia* of zoobenthon, and diatoms such as *Navicula* and *Cymbella* are periphyton as the omnivorous. However, there were some differences in food preference depending on the SL of the fish. This observation in not entirely consistent with the assumption of a non-selective filter feeding mode, as was found in *Cobitis taenia* (Robotham 1977, 1982, Ritterbusch & Bohlen 2000).

In other cobitid fish that have been shown to change their dietary preferences during growth, the dominant prey of juveniles included Arcellidae, Ploima, and Branchioda in *C. tetralineata* (Kim et al. 2006), Diptera in *Iksookimia pumila* (Lee 1984), and copepods in *C. paludica* (Soriguer et al. 2000), while no difference was observed in *K. multifasciata* over time (Chong 1986). In all species except for *K. multifasciata*, animal prey was shown to increase and Ploima (*Difflugia*) was shown to decrease as the fish developed.

In this study, it was possible to distinguish between two groups of fish that objectively indicate a rapid switch in the major food contents during growth, namely, small versus larger fish. On the basis of the index of the relative importance of the gut contents (IRI), we confirmed that 11 juveniles mainly fed on the amoebozoa *Difflugia* especially (Fig. 3 and Table 2). In order to assess whether the dominance of *Difflugia* in juveniles is caused by the seasonal availability of this food item, we compared the gut contents of the juvenile and adult specimens that were collected in August 2006 from the same environment (Table 3). The results confirmed that *Difflugia* is the most dominant food item in juveniles and declines in adults regardless of the season. We, therefore, conclude that the selection of *Difflugia* by juveniles is not an artifact of its seasonal abundance, but reflects the food preferences of different size classes of *K. brevifasciata*.

Table 2. *Major gut contents analysis for the relative values of the food number, volume, and frequency, and the index of the relative importance of the gut contents for different standard length (mm) classes of* Kichulchoia brevifasciata *from the Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

		IRI[a] in each class of standard length (mm)						%N[b]	%V[c]	%F[d]
		10 ~ 20	20 ~ 30	30 ~ 40	40 ~ 50	50 ~ 60	Total			
Phytoplankton										
Bacillariophyceae	*Cymbella*	0.01	1.36	4.27	282.73	6.50	31.39	6.65	0.50	4.39
	Navicula	0.70	37.67	511.10	838.44	129.68	386.05	75.41	0.98	5.05
	Fragilaria	0.00	4.53	4.73	85.88	19.10	11.37	2.27	0.40	4.26
Zooplankton										
Trochelminthes										
Rhizopodea	*Difflugia*	673.46	175.96	147.87	5.18	0.00	58.47	0.34	14.82	3.59
Arthrophoda										
Chironomidae	Chironomidae larva	9.62	168.51	6.43	258.34	8.25	52.39	0.13	20.61	2.93
Ephemeroptera	Ephemeroptera larva	74.06	3.86	1.91	17.29	0.00	27.15	0.01	9.27	2.53
	Trichoptera	0.00	0.00	24.26	320.92	0.00	91.80	0.06	36.27	1.20

IRI[a], index of relative importance; %N[b], percentage of the number of the prey item; %V[c], percentage of the wet volume of the prey item; %F[d], percentage of the frequency of occurrence of the prey item.

Table 3. *Major gut contents analysis for the relative values of the food number, volume, and frequency, and the index of the relative importance of the gut contents in August 2006 between juveniles and adults of* Kichulchoia brevifasciata *from the Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.*

		IRI[a]	%N[b]	%V[c]	%F[d]	IRI[a]	%N[b]	%V[c]	%F[d]
		Juveniles				Adults			
Phytoplankton									
Bacillariophyceae	*Cymbella*	2.22	0.57	0.01	3.81	6.13	0.31	0.80	5.56
	Navicula	99.28	17.3	0.10	5.71	763.75	96.51	19.33	6.59
	Fragilaria	0.00	0.44	0.039	0.00	12.15	0.25	1.59	6.59
Zooplankton									
Trochelminthes									
Rhizopodea	*Difflugia*	337.36	14.40	24.95	8.57	12.57	0.04	2.82	4.39
Arthrophoda									
Chironomidae	Chironomidae larva	103.78	2.95	15.21	5.71	78.70	0.08	35.72	2.20
Ephemeroptera	Ephemeroptera larva	51.71	0.13	27.01	1.90	0.00	0.00	11.64	0.00
	Trichoptera	0.00	0.00	5.64	0.00	32.82	0.02	9.94	3.30

IRI[a], index of relative importance; %N[b], percentage of the number of the prey item; %V[c], percentage of the wet volume of the prey item; %F[d], percentage of the frequency of occurrence of the prey item.

There are two possible reasons for the ontogenetic shifts in diet as the fish grow: larger individuals may be able to consume larger prey due to mouth morphology, and/or different sized specimens may occupy different habitats where they live (Robotham 1982, Bohlen 2003). However, in contrast to the latter possibility, *K. brevifasciata* juveniles and adults differed in food preferences, but occupied the same habitats in this study. Furthermore, we confirmed that the number of food items was positively correlated with SL, but that the volume of food items was not (Fig. 2), suggesting that a greater number of food items can be selected with increased growth without the sizes of food items changing. It is most likely that in *K. brevifasciata* the food shift goes along with an improvement in capture abilities rather than a habitat selection of the size classes.

The results show that *K. brevifasciata* generally feeds on a broad range of food items, but that dependence upon certain items changes during the ontogenetic development. Certain habitat requirements for this highly endangered species are highlighted from these results. For instance, the stream for *K. brevifasciata* has to contain a broad diversity of benthic invertebrates, and include a broad variety of plants and animals ranging in size from unicellular organisms to insect larvae. Moreover, if the habitat of one of the dominant prey items of *K. brevifasciata* is altered, e.g., by the construction of artificial river systems, there will be insufficient food available for the fish, in habitats, which will impact the growth of *K. brevifasciata.*

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