Feeding habits of largemouth bass and bluegill estimated based on stomach contents and fecal DNA

Takashi Taguchi, Yoshito Miura, Shozo Sugiura*
The University of Shiga Prefecture, Graduate School of Environmental Sciences, Japan (E-mail: sugiura@ses.usp.ac.jp)

Abstract:

Largemouth bass and bluegill are the dominant invasive fishes in Lake Biwa, the largest lake in Japan. Understanding their predatory and competing behaviors against indigenous species is important to restore diminished populations of indigenous species. We, therefore, studied the feeding habits of these invasive species in Lake Biwa.

The experimental fish were caught between June 17 - Sep.10, 2010 (n=152 fish for largemouth bass, n=74 fish for bluegill) and between June 2 - Sep 1, 2011 (n=173 fish for largemouth bass, n=213 fish for bluegill). All fish were dissected and examined for prey composition. The species of the stomach contents (partially digested) were identified based on the external appearance and the otolith shape. The sizes of prey fishes were measured or estimated from the otolith diameter. In case of crucian carp (*Carassius* spp.), alizarin complexone (ALC) tagging of the otolith was used to distinguish the artificial (stocked) fry from natural fry. Fecal mitochondrial DNA was extracted and analyzed for a 16S rRNA region based on qPCR-SSP using a SYBR Green I-based intercalator method, and clone libraries. For qPCR, universal primers were designed, respectively, for fishes (including amphibians), arthropods, and mollusks. In addition, crucian carp-specific primers were designed to determine the extent of predation. Clone libraries were constructed as follows: fecal DNA was amplified by PCR using universal primers, the PCR products were 3'-blunted, 5'-phososphorylated, concatemerized, ligated into plasmid, transformed into competent cells, and sequenced. The host sequences were removed using a restriction enzyme after PCR. The PCR conditions were optimized to prevent template-switching, heteroduplex formation, and the occurrence of chimera sequences. Based on the data of stomach contents and fecal DNA as well as fish fauna of the studied area, the selectivity index for prey species (E), and the index of relative importance (IRI) were calculated and discussed.

Visual and microscopic examinations of stomach contents, qPCR of fecal DNA, as well as sequencing of fecal DNA clone libraries collectively revealed that largemouth bass tended to prefer larger prey, including ayu (*Plecoglossus altivelis*), than smaller prey, such as crucian carp fry (with the exception of a small freshwater goby, *Rhinogobius* spp.). Bluegill showed algal-omnivorous feeding rather than piscivorous habits. Largemouth bass had a stronger feeding preference for Palaemonid shrimps (*Palaemon paucidens* and *Macrobrachium* spp.) over Atyid shrimps, while bluegill showed the opposite preference.

In largemouth bass, the E values indicated higher preferences for ayu (0.95-0.98) and goby (0.71-0.77) than crucian carp (-0.60- -0.46) for both 2010 and 2011 studies. The IRI values were higher for ayu, goby, and shrimp (*P. paucidens*) than crucian carp and other cyprinids. Besides the kind of prey species, the feeding preference of largemouth bass for indigenous species could depend on the following factors: the size of prey species, the degree of satiation of the predator fish, and the turbidity of the habitat. In bluegill, the IRI values (excluding algal matter) showed higher intakes for snails and chironomids. But, unlike largemouth bass, bluegill also consumed a wide variety of prey, including worms, fish eggs, shrimps, ants, armadillidiums, beetles, leeches, fish larvae, and among other species.

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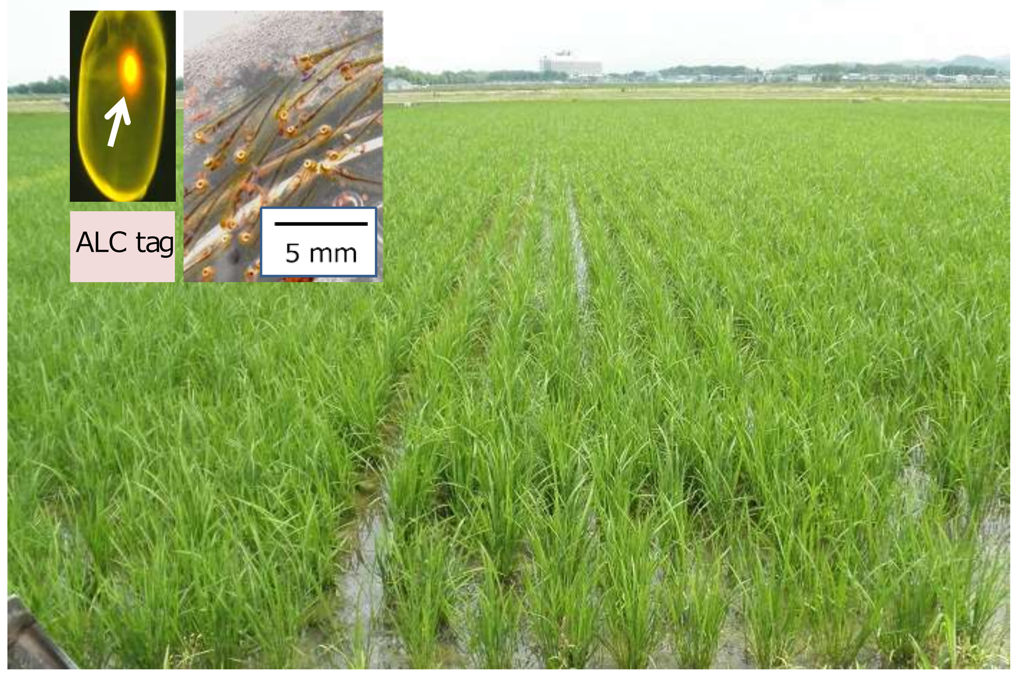
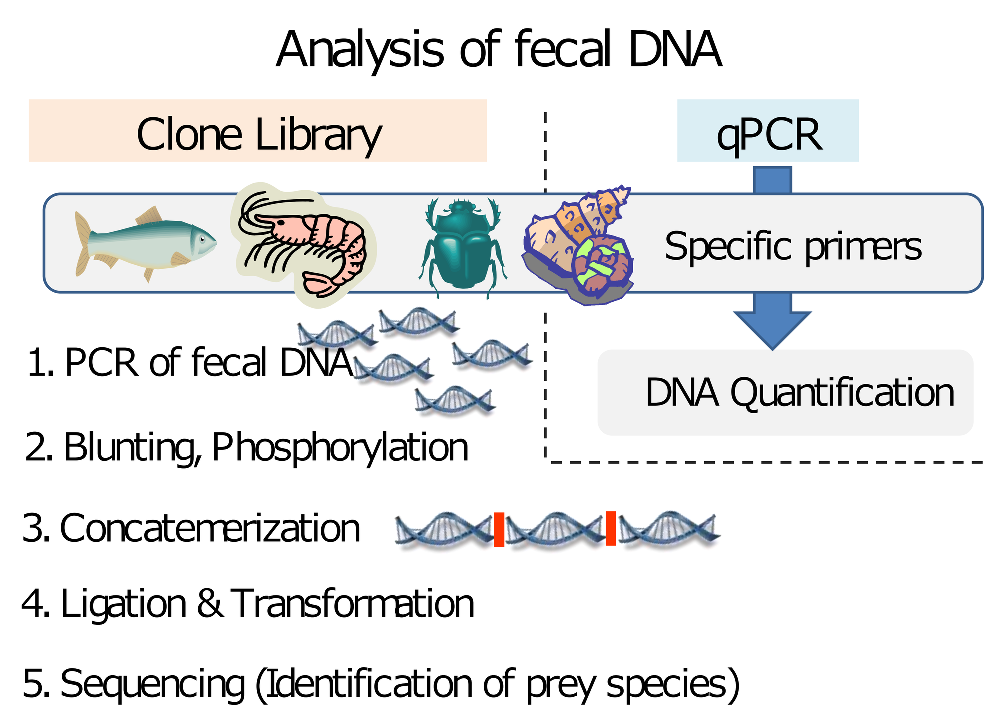


Fingerling production in rice fields

Stocking

How can we minimize the predation of fingerling fish by piscivores after stocking them into natural habitats?



Fish fauna (collected specimen numbers) of surveyed field (2010 data)

Species	6/17-6/30	7/2-7/27	8/14-9/10	Total numbers collected
Hypomesus nipponensis		1 (3)		1
Plecoglossus altivelis	1 (6)	2 (7)		3
Cyprinus carpio	2 (30-45)	1 (4), 1 (15)		4
Carassius langsdorfi	4 (20-25)	5 (15-25)		9
Carassius spp.*	652 (1.7-3)	117 (2-3)	1 (6)	770
Zacco platypus	4 (5-13)	2 (6-8)		6
Ischikauia steenackeri			3 (7)	3
Gnathopogon elongatus	3 (2)	22 (2-3)	1 (5)	26
Squalidus chankaensis biwae	3 (2-2.5), 1 (8)			4
Acheilognathus rhombeus	2 (2-2.5)	21 (2-3)	13 (5-7)	36
Sarcocheilichthys variegatus microoculus	1 (2), 9 (8)	1 (7)		11
Pseudogobio esocinus	1 (5), 1 (15)	1 (15)	3 (10), 1 (5)	7
Misgurnus anguillicaudatus	2 (3)	5 (3-4)	6 (3-4)	13
Silurus asotus	4 (3-6), 1 (30)	1 (35)		6
Oryzias latipes		1 (2)	1 (2)	2
Micropterus salmoides	9 (1.5-2), 90 (9-27)	27 (2-3), 44 (10-42)	4 (3-4), 22 (5-10), 18 (11-28)	214
Lepomis macrochirus	50+ (4-8), 41 (8-13)	50+ (2), 22 (4-5), 23 (10-13)	47 (4-8), 10 (8-13)	243+
Odontobutis obscura	2 (3)	1 (4)		3
Tridentiger brevispinis	1 (5)			1
Rhinogobius spp.	25 (3-5)	30+ (3)	23 (3-4)	78+
Biwia zezera	1 (5)			1

Numbers in parentheses indicate approximate standard length in centimeters.
Dominant species or groups other than fish in the studied area include crustaceans (*Palaemon paucidens*, Atyiid shrimps, *Procambarus clarkii*), amphibians (Frogs), and mollusks (Viviparid snails).
* "*Carassius* spp." were crucian carp fry flown out of rice fields to the studied area, and were mostly *Carassius grandoculis* and *Carassius cuvieri*.

Stomach contents of largemouth bass (2011 data)

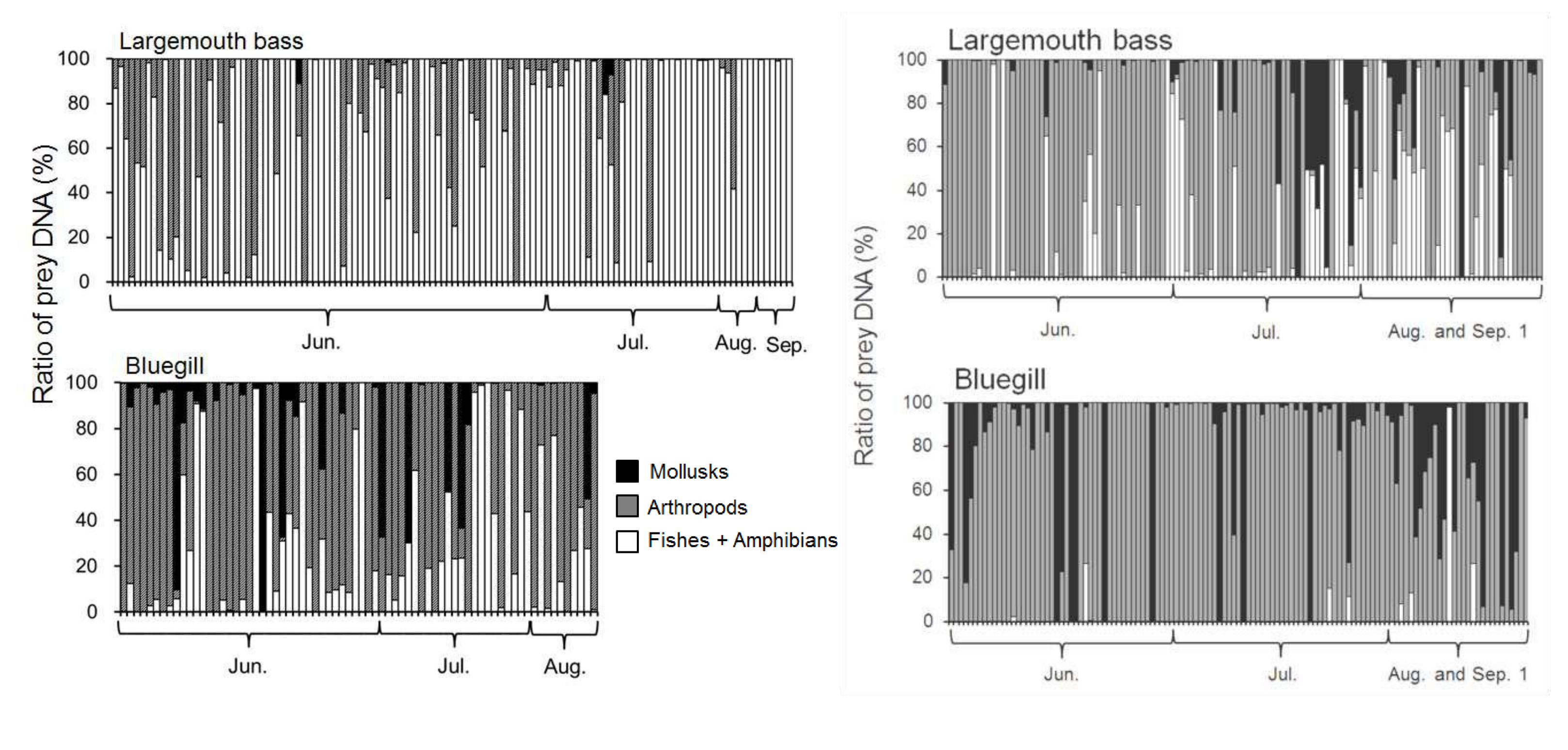
Body length of largemouth bass		below 10 cm	10-15 cm	15-20 cm	over 20 cm
Number of fish dissected		18	41	65	21
Number of fish with empty stomach (% in parentheses)		3 (17)	3 (7)	15 (23)	4 (19)
Prey species		IRI (%IRI)*			
Fishes	*Plecoglossus altivelis*	0 (0)	27 (0)	1260 (33)	3789 (59)
	Carassius spp.	0 (0)	62 (1)	175 (5)	727 (11)
	Cyprinidae	231 (4)	102 (2)	204 (5)	888 (14)
	Channa argus	0 (0)	9 (0)	0 (0)	0 (0)
	Tridentiger brevispinis	0 (0)	0 (0)	4 (0)	0 (0)
	Rhinogobius sp.	5398 (84)	502 (8)	851 (22)	0 (0)
	Gobiidae	0 (0)	39 (1)	20 (1)	0 (0)
	Unidentified spp.	0 (0)	323 (5)	45 (1)	856 (13)
Shrimps	*Palaemon paucidens*	642 (10)	4592 (75)	785 (21)	86 (1)
	Atyid shrimps	0 (0)	2 (0)	0 (0)	0 (0)
	Macrobrachium spp.	0 (0)	16 (0)	177 (5)	0 (0)
	Unidentified spp.	165 (3)	435 (7)	253 (7)	64 (1)
	Procambarus clarkii	0 (0)	3 (0)	12 (0)	0 (0)
Insects	Dragonfly	0 (0)	0 (0)	2 (0)	0 (0)
	Dragonfly larvae	26 (0)	0 (0)	0 (0)	0 (0)
Unidentified species		0 (0)	0 (0)	7 (0)	0 (0)

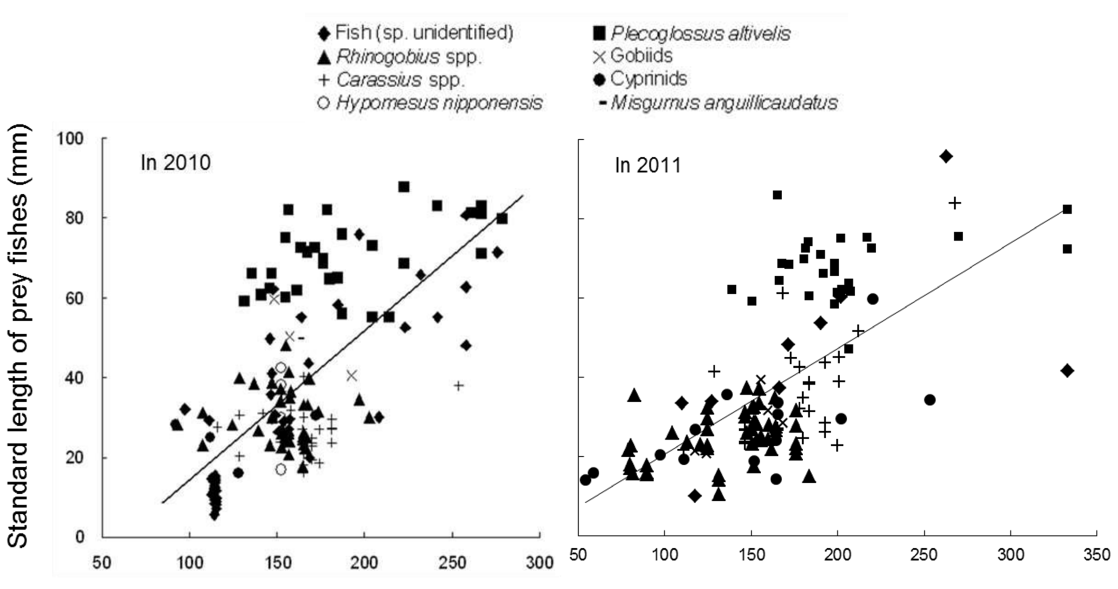
*IRI: Index of relative importance, %IRI: Index of relative importance %

Stomach contents of bluegill (2011 data)

Body length of bluegill		below 10 cm	over 10 cm
Number of fish dissected		67	58
Number of fish with empty stomach (% in parentheses)		3 (4)	4 (7)
Prey species		IRI (%IRI)*	
Fishes	*Carassius* spp.	10 (0)	0 (0)
	Silurus asotus	0 (0)	27 (0)
	Rhinogobius spp.	0 (0)	13 (0)
	Fish eggs	4 (0)	67 (1)
Crustacea	*Palaemon paucidens*	25 (0)	12 (0)
	Atyid shrimps	0 (0)	2 (0)
	Unidentified shrimps	4 (0)	63 (1)
	Procambarus clarkii	0 (0)	3 (0)
	Amphipod shrimps	7 (0)	0 (0)
Insects	Beetles (Coleoptera)	3 (0)	0 (0)
	Beetles (sp. unidentified)	2 (0)	15 (0)
	Dragonfly larvae	11 (0)	3 (0)
	Chironomidae larvae	3705 (41)	536 (5)
	Ants (Formicidae)	71 (1)	2 (0)
	Insect larvae (sp. unidentified)	243 (3)	45 (0)
	Centipede (Myriapoda)	2 (0)	0 (0)
	Spider (Araneae)	7 (0)	0 (0)
	Woodlice (*Armadillidium* sp.)	1 (0)	14 (0)
	Water boatmen (Corixidae)	5 (0)	10 (0)
	Other terrestrial insects	0 (0)	9 (0)
Mollusks	Snails (Gastropoda)	4974 (55)	10173 (92)
Annelida	Earthworm (Oligochaeta)	3 (0)	1 (0)
	Leeches (Hirudinea)	51 (1)	16 (0)
Plants**		[77]	[61]

*IRI: Index of relative importance, %IRI: Index of relative importance %
**Percentages of fish that had plant matter in stomach (%F)



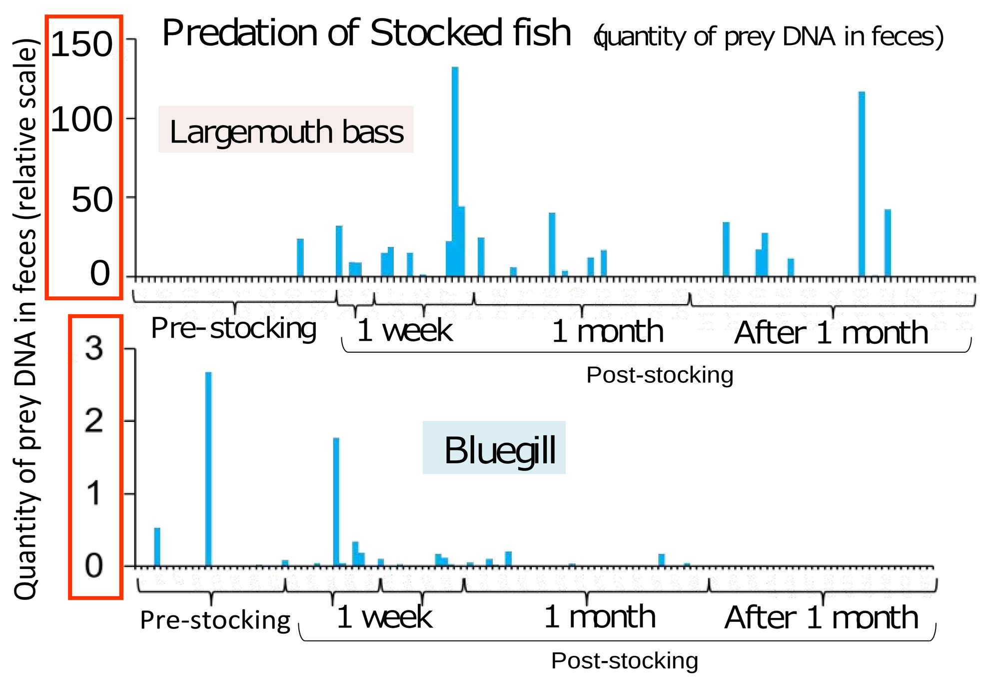


Standard length of largemouth bass (mm)

Feed selectivity index for stocked fish of different sizes or species determined with largemouth bass

Body length (mm) or Species	# of fish in the field	# of fish in the stomach	Selectiveity index (E)
Below 20 mm	148 / 125	2 / 0	-0.63 / -1.00
20-30 mm	226 / 152	15 / 12	0.05 / -0.03
Over 30 mm	26 / 42	7 / 15	0.64 / 0.62
C. grandoculis	124	22	0.39
C. cuvieri	174	1	-0.86

(2010 data / 2011 data)



Feed selectivity index (E) of largemouth bass for prey species (2010 data / 2011 data)

Species	# of fish in the field	# of fish in stomach	E	Freq. of fecal DNA	E
Smelt*	3 / 1	28 / 26	0.98 / 0.95	6 / 2	0.95 / 0.98
Goby*	78 / 42	54 / 54	0.71 / 0.73	28 / 14	0.77 / 0.88
Carp*	770 / 353	33 / 27	-0.46 / -0.44	9 / 0	-0.60 / -1.00

* Smelt: *Plecoglossus altivelis*; Goby: *Rhinogobius* sp.; Carp: *Carassius* spp. (stocked fish)

Identification of fecal DNA of largemouth bass and bluegill using clone libraries (2010 data)

Clones	6/17	6/21	6/23	7/2	7/27	8/23, 9/10
Fishes						
Plecoglossus altivelis			3			
P. altivelis or *Hypomesus nipponensis*			2	(1)	1	
Carassius spp.*1	3	1		1	2	
Carassius cuvieri			2			
Opsariichthys uncirostris		4				
Tribolodon hakonensis	1					
Cyprinid fishes				5	2	
Gobiid A	2	(4)	6			12
Gobiid B		5				1
Other gobiid fishes	(1)		2		(1)	
Unidentified fishes*2	2	1				
Frogs						
Hyla japonica	6 (1)		1	1		4
Unidentified frogs*2	1	(1)	4	3		5
Crustaceans						
Palaemon paucidens	6	1		1		
Macrobrachium spp.		4				
P. paucidens or *Macrobrachium* spp.				1	2	
Atyiid shrimps		(2)	(24)	2 (4)		
Amphipod shrimp (*Gammarus* sp.)	(1)		(2)			
Daphnia spp.	(16)		(1)			
Insects						
Flies (Diptera)	(1)					
Flies or beetles (Diptera or Coleoptera)						(15)
Mayflies (Ephemeroptera)	(3)					
Chironomids (Chironomidae)	(4)			(3)		
Woodlice (*Armadillidium* sp.)				(8)		
Mollusks						
Snails (Gastropoda)	(3)	(1)	(10)	1 (12)	2 (3)	1 (6)
Snail (*Physa acuta*)						(2)

Numbers indicate the numbers of clones recovered (numbers without parentheses: largemouth bass; numbers in parentheses: bluegill).
*1 *Carassius grandoculis* or *Carassius langsdorfi*
*2 "Unidentified species" includes species listed in this table.

Identification of fecal DNA of largemouth bass and bluegill using clone libraries (2011 data)

Clones	6/2	6/12, 15**	6/27, 29	7/8, 11	8/11
Fishes					
Plecoglossus altivelis			2		
Cyprinid fishes				2	1
Tridentiger brevispinis			(1)		
Rhinogobius spp.	5		1(1)		8
Frogs					
Hyla japonica	(2)				
Rana catesbeiana	3 (5)		3		
Unidentified frogs*	3		1		
Crustacea					
Palaemon paucidens	18		5 (9)	26	
Macrobrachium spp.			11		(1)
Atyid shrimps		(8)	(2)	(12)	(7)
Procambarus clarkii					15
Amphipod shrimps	(1)	(12)	(6)		
Daphnia spp.		(2)			
Insects					
Flies (Diptera)					(2)
Beetles (Coleoptera)			(3)		(2)
Aphids (Aphididae)				(1)	
Chironomids	(9)		1		
Chironomids or Coleoptera					(1)
Mollusks (snails)					
Physa acuta	(7)	(4)	4(4)	(2)	(2)
Radix spp.	(6)	(1)		(3)	(2)
Laevapex spp.		(1)			
Semisulcospira spp.	(2)		1		
Pomacea canaliculata					(1)
Unidentified snails			3	2(10)	(4)

Numbers indicate the numbers of clones recovered (numbers without parentheses: largemouth bass; numbers in parentheses: bluegill).
*Unidentified frogs excluding the listed frog species.
**Samples of bluegill only.

Summary

Largemouth bass feed mainly on fishes and shrimps, while bluegill feed on numerous prey organisms including plant matter.

Results of fecal DNA clone libraries revealed some new species that were not identified by visual and microscopic examinations of the stomach contents.

Largemouth bass have a strong preference for larger fishes, and thus smaller fishes are less vulnerable to its predation. Bluegill do not have any size-preference for prey organisms.

Largemouth bass have a strong preference for *Palaemon* and *Macrobrachium* shrimps to Atyid shrimps. Bluegill showed an opposite preference.

Metagenomic analysis of fecal DNA may be particularly useful for larval to juvenile fishes as well as crustacean and molluskan species, for which visual or microscopic examinations of the prey species in the stomach is very difficult or even impossible.

In making clone libraries, the number of PCR cycles and the PCR condition must be optimized in order to minimize the occurrence of template-switching and the formation of heteroduplex, that may lead to artifactual sequences such as chimeras.