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Habitat selection during spawning season of the spined loach, *Cobitis* sp. 'yamato' complex, in the Kyushu Island, Japan

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Abstract. Habitat selection of the spined loach yamato complex (Cobitidae) was investigated at the River Saigo, Fukuoka Prefecture, Kyushu Island, Japan, during both the non-spawning (from January to March) and spawning (from April to June) seasons. The study site had one pool, two riffles, and one flood region during the spawning season and is 140 m long, located 4 km stream from the river mouth. The number of yamato complex individuals was checked, and 10 physical environmental parameters were measured to assess the microhabitat in 45 quadrates. The number of individuals was counted each month, and environmental measurements were conducted four times from January to June 2010. Akaike's Information Criterion (AICc) and Generalized Linear Model (GLM) were utilized for analysis to verify the effect of the important environmental variables on the habitat of the yamato complex. A total of 184 individuals were captured during the non-spawning and spawning seasons. The populations of yamato complex correlated positively with the water depth during the non-spawning season and with short emergent hydrophytes during the spawning season. Individuals of the yamato complex were observed in the riffle part of the river with depth during the non-spawning season and in the temporary water area with vegetation during the spawning season.

Key words: microhabitat, reproductive season, flooded bank, temporary water, Cypriniformes

Introduction

The *Cobitis* sp. 'yamato' complex is known to have originated from by hybridization between *Cobitis biwae* and *Cobitis striata* (Kyushu form), and there are five allopatric races in the yamato complex, characterized by different chromosome numbers (82, 86, 90, 94, and 98) (Sezaki et al. 1994, Saitoh et al. 2000, Saitoh unpublished data). This species complex is found in the western Honshu (Yamaguchi Prefecture) and Kyushu Islands and inhabits the sandy bottoms of middle to lower river reaches (Nakabo 2002). Analysis of mitochondrial and satellite DNAs and the geographic distribution pattern of the yamato complex have been conducted and, in the study area of the River Saigo, the yamato complex was identified as an independent tetraploid population on the basis of morphological and genetic examination (Saitoh et al. 2000, Kitagawa et al. 2009). However, no analysis has been performed on the ecology of this species.

One of the most important aspects in the ecology of fishes is their reproductive behaviour. The adult specimens strongly influence the survival rate, growth and the general welfare of their offspring by simply choosing the appropriate spawning site. In cobitid loaches like in most fishes, the earliest ontogenetic stages are the most vulnerable, and the mortality rate during these stages usually influences the size of the cohort or the year class (Gulland 1965, Rickman et al. 2000, Bohlen 2008). Additionally, the early stages have a very restricted potential of migrations, which makes habitat selection by the spawning adults a very important ecological constrains that governs the future of the population (Bohlen 2000, Bohlen 2003).

The aim of the present study is to describe the habitat selection of the yamato complex which is

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now endangered and is currently considered for conservation by analysis of environmental factors in the non-spawning and spawning seasons.

Material and Methods

Study area

The study was conducted in the River Saigo, Fukuoka Prefecture, northern Kyushu Island, Japan (Fig.1). The study area is 140 m long, located 4 km upstream of the river mouth (33°45′ N, 130°30′ E), and the substratum was composed mainly of sands with some pebbles. The river has two riffles and one pool and is usually stable with a low variation of habitat structure below 50 cm in depth unless heavy rains affect the river. In the study area, the level of the river was divided by cement from the path road in the west and by a track of paddy fields in the east. The edge of the permanent water was covered by vegetation.

Collection of individuals and habitat analysis

Between January and June 2010, the occurrence of the yamato complex was surveyed in the study area. Identification of the species followed predominantly on morphology (Nakabo 2002) and also studied population has examined as tetraploid type, spotted color pattern verified as yamato complex (Kitagawa et al. 2009). Based on the knowledge that the spawning period of the yamato complex is from the April to May (Nakabo 2002), the non-spawning season was defined as January to March and the spawning season was defined as April to June 2010. In the studied year, the juveniles of yamato complex started to occur from June in this area. Furthermore, Kawamura & Minamori (1947) have researched, 52.5 ± 0.73 hours (n = 123), estimated time from fertilized egg to newly-hatched larvae in the yamato complex and the time from newly-hatched larvae to juvenile took 44 days reported on *C. hankugensis* closely related to the yamato complex (Uchida 1939). The practical experiment on embryonic and larval development stages of the yamato complex was examined as about 50 days artificially in the same spawning period (Kim, unpublished data). Considering both references and actual experiment, it would be taken approximately two months from egg fertilization to juvenile stage. Therefore, the spawning season would be estimated to be about between April and June in this study area. We presumed that their movement was limited at each site because the yamato complex is a benthic fish that prefers inhabiting sands. Individuals were captured with a hand net (mesh size: 2 mm) each month. The quadrates were designed by referring to the study by Nakajima et al. (2008), and the individuals were counted in an area of 45 quadrates per 1 m^2 area located spaced 10 m apart for 15 min between 10:00 and 14:00 (Fig. 1).

We measured the following 10 physical environmental parameters four times in the 49 quadrates to assess the microhabitat structure between January and March of the non-spawning season and May and June of the spawning season: water depth, dissolved oxygen, water temperature, electricity conductivity, pH, water velocity, oxygen and oxidation reduction potential, sediments, existence of short emergent hydrophytes,

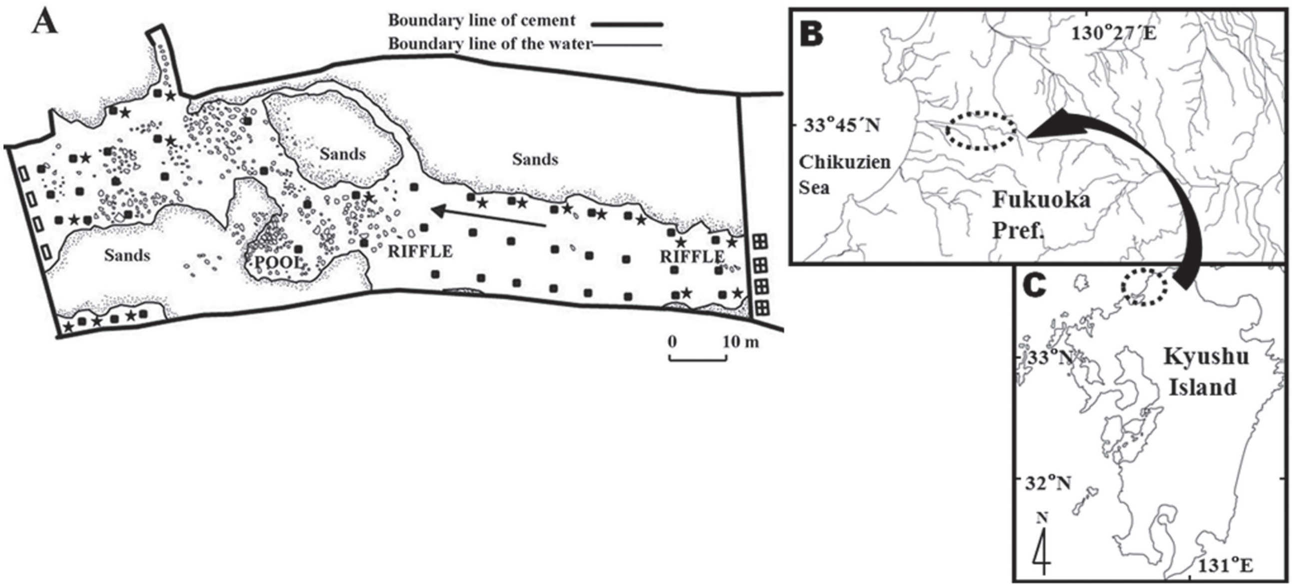

Fig. 1. *The survey site in the River Saigo (A), in Fukuoka Prefecture (B), northern Kyushu Island (C). ←, direction of water flow; ■, quadrates for assessing the microhabitats; ★, regions with emergent hydrophytes related to the microhabitat.*

and long emergent hydrophytes. We sounded the three deepest areas of water three times with an iron rod and calculated the average values. The dissolved oxygen and water temperature were measured once in the center of the quadrate from a depth of 20 cm water depth (YSI 95 Dissolved Oxygen, YSI Inc., USA). Electrical conductivity, pH and oxygen, and oxidation reduction potential were measured once at 10 cm from the surface in the central part of the water (Waterproof pHTestr 30, Waterproof ORPTestr 10, Waterproof ECTestr 11+; Oakton Inc., USA). Water velocity was measured 3 times from the surface of the water to 30 % of its depth, and the values were averaged (model 3631; Yokogawa Electric Co., Tokyo, Japan). The collected sediments from a cube (10 cm wide, 10 cm long, and 2 cm in depth) of each quadrate were sieved and separated into 6 levels (< 0.063 mm, 0.063-0.125 mm, 0.125-0.25 mm, 0.5-1 mm, 1-2 mm, and > 2 mm). The existence of long emergent hydrophytes (Fig. 3B) was set at 0, and that of short emergent hydrophytes (Fig. 3C) were set at 1.

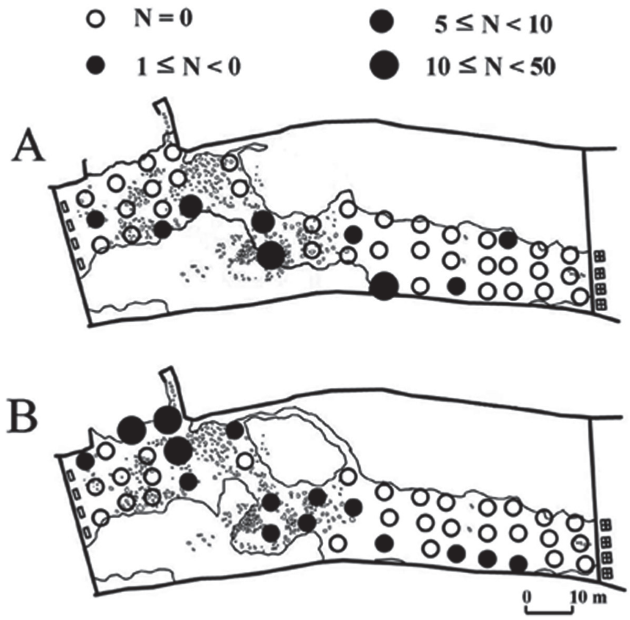

Fig. 2. *Presence (closed circle) or absence (opened circle) of the yamato complex and its abundance at the survey sites of the River Saigo, Fukuoka Prefecture, Kyushu Island, Japan, in the non-spawning season (A) and spawning season (B).*

Statistical analysis

Akaike's Information Criterion (AICc) and Generalized Linear Model (GLM) were used with the 10 environmental factors as explanatory variables and the collected individuals as object variables in each quadrate to identify habitat selection of the yamato complex in the non-spawning and spawning seasons. All measured values were transferred by z-scores to standardize the differences in the measurement units. Analysis was carried out using software R ver. 2. 11. 2 (R Development Core Team, 2010).

Results

A total of 184 individuals were captured and the sex ratio was 1 : 0.64 in favor of females (SL > 40 mm). We observed first occurrence of juveniles on June, confirming that spawning season also started from about April in this area at this year. Among them, 132 individuals were captured during the non-spawning season and 52

Fig. 3. *Major microhabitat of the yamato complex (A) during the spawning season with abundant vegetation including long (B) and short (C) emergent hydrophytes.*

Table 1. *The number of specimens collected during the non-spawning and spawning seasons in the River Saigo from January to June 2010.*

	Range of SL (mm)	Non-spawning season			Spawning season		
		Jan.	Feb.	Mar.	Apr.	May	Jun.
Female	42.1 ~ 114.9	23	37	31	9	7	5
Male	54.7 ~ 94.5	9	12	20	17	6	8

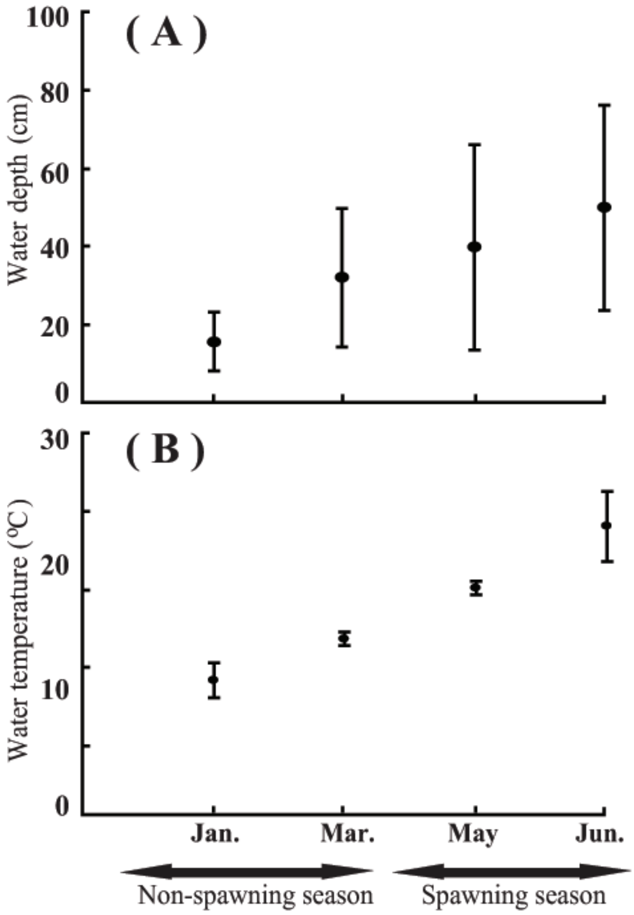

Fig. 4. *Average water depth (A) and water temperature (B) of all the examined sites of the River Saigo, Fukuoka Prefecture, Kyushu Island, Japan, in each month in the non-spawning and spawning seasons. The vertical lines show standard deviation.*

individuals were collected during the spawning season (Table 1). In the non-spawning season, individuals of the yamato complex inhabited the riffle part of the river, and this correlated with depth, whereas during the beginning of the spawning, they were observed in the nearby vegetation on the temporary water, which was not related to water depth (Fig. 2).

In January 2010, water temperature and depth were 10.3 ± 1.36 °C and 15.7 ± 7.5 cm, respectively, in the study area, and reached their peaks of 22.06 ± 2.76 °C and 49.9 ± 26.1 cm, respectively, in June 2010 (Fig. 4). Vegetation patterns changed when the spawning season started, with an increase in the water temperature and inundation of the edge of permanent water by temporary water. The edge of the permanent water was covered with vegetation such as long emergent hydrophytes in January, February, and March 2010. However, the edge of the permanent water inundated with increased temporary water was covered with short emergent hydrophytes expended around the edge of the originally existing vegetation from the non-spawning season through the spawning season in April, May, and June 2010 (Fig. 3).

The results of the analysis are shown in Table 2. On analysis of the environmental factors associated with the yamato complex in the non-spawning season, the AICc value of the final model was found to be 252.42 (Table 2A). The top three models included water depth, water velocity, and the existence of short emergent hydrophytes. These three models showed small ΔAICc values; the model fit to assess the Akaike weight was relatively larger than the other two models. All the models included water depth as an independent variable, and the final model included only water depth (Table 3). In the spawning season, AICc value of the final model was 130.37, and the values of the top 2 models, which included short emergent hydrophytes and water depth, were lower than those of the other models (Table 2B). All the models included short emergent hydrophytes as independent variables, and the final model included only short emergent hydrophytes (Table 3). Water depth was found to positively correlate to the population of the yamato complex during the non-spawning season, and its habitat showed a strongly positive correlation with an area where short emergent aquatic plants were extant during the spawning season.

Discussion

Previous reports on habitat affinities of Cobitidae fishes indicate that *Cobitis elongatoides*, *Misgurnus fossilis*, and *Sabanejewia balcanica* generally prefer a specific type of fine or hard substratum (Meyer & Hinrichs 2000, Pekárik et al. 2008), suggesting that the type of riverbed plays a key role in habitat selection. This study showed that under non-spawning season conditions, habitat selection of the yamato complex is influenced by water depth, suggesting that it shows a positive effect on water depth but that the habitat is not affected by substrate size level. This result is consistent with the finding that a strong positive relationship exists between water depth and the size of the largest fish within a stream (Harvey & Stewart 1991).

On comparing the water temperature and depth between the non-spawning and spawning seasons, we found that the values were significantly higher in spawning season than in the non-spawning season (Fig. 4, Mann-Whitney U test, WT: $z = -7.97$, $p < 0.01$; WD: $z = -2.48$, $p < 0.01$). During winter and early spring in the non-spawning season (January to March 2010), the low water level due to drying of the river would influence

Table 2. *Physical conditions influencing the presence of the yamato complex in the River Saigo during the non-spawning (A) and spawning (B) seasons as determined by model selection. The top 5 Generalized Linear Models selected (GLMs) by Akaike's Information Criterion (AICc) are shown. The plus symbols indicate the parameters to be included in the model.*

Physical conditions (Independent variable)									AICc	ΔAICc	Akaike weight
WD[a]	WV[b]	WT[c]	EC[d]	pH	DO[e]	ORP[f]	MPS[g]	SEH[h]			
(A) Non-spawning season											
+									252.42	0	0.045
+	+								253.5	1.08	0.026
+								+	253.6	1.18	0.025
+			+						254.19	1.77	0.019
+					+				254.19	1.84	0.018
(B) Spawning season											
								+	130.37	0	0.046
+								+	131.07	0.7	0.033
						+		+	131.36	0.99	0.028
			+					+	131.79	1.42	0.023
		+						+	132.02	1.64	0.02

WD[a], water depth; WV[b], water velocity; WT[c], water temperature; EC[d], electrical conductivity, DO[e], dissolved oxygen; ORP[f], oxygen and oxidation reduction potential; MPS[g], median particle size of the sediments; SEH[h], short emergent hydrophytes.

Table 3. *The best model determined from the physical conditions and the individuals of microhabitats, using the Generalized Linear Model identified using Akaike's Information Criterion for the non-spawning (A) and spawning seasons (B).*

Physical conditions (Independent variable)	Estimate	Standard error
(A) Non-spawning season		
Water depth	–1.357	1.126
(B) Spawning season		
Short emergent hydrophytes	0.034	0.13

survival rather than when the water level of the river was high in the summer of the spawning season (May to June 2010) (Fig. 4A). The effect of the low water level might suggest that this species migrates deep in the water for overwintering. Water temperature and depth were significantly higher during the spawning season than during the non-spawning season (Fig. 4). This means that the loaches preferred the relatively deeper parts during the low-water period. Such a preference of deeper areas in streams with very low water level is common in freshwater fishes (Katano et al. 2003, Onikura et al. 2009). The fish fauna recorded at study site showed almost omnivorous fish assemblage including *Cyprinus carpio*, *Carassius auratus langsdorfii*, *Zacco platypus*, and *Oryzias latipes* which might not affect the risk of predation to the yamato complex (Nakajima et al. 2006). However, it is thought as a one possibility that the distribution pattern of stream fish minimizes the risk of predation as they prefer deeper habitats so as to escape from predatory birds or mammals (Power 1987).

As the spawning season of the yamato complex began with increased water quantity starting in April 2010, the amount of temporary water increased by the rainy season. During this season, our analysis result shows that habitat selection of the yamato complex did not correlate with water depth, and that they preferred the vegetation in the temporary water. Habitat selection in *Cobitis taenia* strongly correlates with dense vegetation, but there was also slight correlation with current velocity, water depth, and the substrate level, and it is believed that such vegetation-related habitat specialization is a significant environmental factor during the spawning season (Bohlen 2003). In this study, vegetation was classified into two categories: long and short emergent hydrophytes. Short emergent hydrophytes had a positive effect on habitat preference during the spawning season, and this corresponds to the observation that *C. taenia* does not show a preference toward the plant species, but toward the structure of the vegetation for spawning (Bohlen 2003). Short emergent hydrophytes started to grow from the beginning of the spawning season on the edge of the temporary water area (Fig. 3A), and we believe that because the yamato complex moved to the vegetation area, species collection was difficult in the spawning season. Vegetation supplies a nursery habitat and protects against loss of eggs and from predation and flowing water (Bohlen 2003). Additionally, temporary waters provide an environment rich in microorganisms, and the offspring benefit from the good food supply (Williams & Coad 1979, Saitoh 1990).

The occurrence pattern of the yamato complex observed in this study shows that temporary water regions, which show a high abundance of the yamato complex (Fig. 2B and Fig. 3A), are essential as spawning sites and that the depth of the permanent waters is important during the non-spawning season. Therefore, the movement between temporary and permanent waters is a very significant issue for future conservation of species designated as vulnerable (VU) level on the Japanese red list (Ministry of Environment, Japan 2007).

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