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Size-related habitat use by bitterling (*Rhodeus sericeus*) in a regulated lowland river

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Abstract – Habitat use by four size-classes of bitterling (*Rhodeus sericeus*) in a regulated lowland river was studied at two spatial scales. Mesohabitat scale was represented by the bank type (boulder, beach and steep eroded bank). Microhabitat associations with substrate types, vegetation, woody debris and position in the stream were examined at each mesohabitat scale. Three size cohorts of young-of-the-year (0+) fish and a group of adult bitterling were detected. Univariate (χ^2-tests) and multivariate (Outlying Mean Index analysis) methods were applied to determine size-related habitat associations. All the size-classes of bitterling avoided the steep eroded bank and most 0+ bitterling were distributed along the boulder bank. The smallest fish had the most pronounced habitat requirements and occurred mostly in the depositional areas, whereas the larger 0+ fish occurred in deeper water. The size-related change in the habitat use coincided with a change in diet and morphological state.

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Key words: microhabitat analysis; habitat shift; young-of-the-year fish; regulated river; juvenile fish; Outlying Mean Index

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Un resumen en español se incluye detrás del texto principal de este artículo.

Introduction

Ecological studies are carried out at different scales and the chosen scale has a major effect on detection and interpretation of the pattern (Addicot et al. 1987; Wiens et al. 1987). Hierarchical approaches, where fine-scale patterns are analyzed separately within large-scale units, explain variation in habitat use better than the microhabitat studies which do not take into account interactions between habitat effects at different scales (Poizat & Pont 1996). Three habitat levels are usually considered for fish in a flood plain. Macrohabitat considers the landscape scale, e.g. the main river channel and different types of lentic water bodies within the floodplain. Mesohabitat scale usually refers to distances of tens of meters, e.g. different bank types or riffles/pools. Microhabitats are finer subdivisions of space and associations with various structures increasing the heterogeneity of environment are studied at this scale (Caselle & Warner 1996; Poizat & Pont 1996; Vadas & Orth 1997).

Many European floodplain rivers have been regulated and channelized during the previous century which led to the depauperation of their heterogeneity (Schiemer & Waidbacher 1992). These rivers often lack off-channel habitat (e.g. backwaters, floodplain oxbow lakes) and near-shore areas are the primary nursery zones for juvenile fish (Winkler et al. 1997), because they provide low water velocity, increased food supply and refuge from predation (Schlosser 1987; Schiemer & Zalewski 1992; Copp & Jurajda 1993; Hill & Grossman 1993).

Requirements of the specific habitat decrease with the age of fish (Schiemer et al. 1991). Therefore, young-of-the-year (henceforth 0+) fish requirements are considered to play an essential role in the life history of particular species. Consequently, investigations of habitat use by 0+ fish assemblages in several European floodplain rivers have been undertaken (e.g. Schiemer et al. 1991; Copp et al. 1994; Poizat & Pont 1996; Gozlan et al. 1998). Size-related shift in habitat use has been observed in many freshwater fishes (Baltz &

Moyle 1984; Copp 1990a; Sempeski & Gaudin 1995; Toham & Teugels 1997). Thus, fish body size, in addition to the different habitat availability, can have an effect on the detection of habitat requirement that was found to vary among studies.

When the size-specific habitat use of 0+ fish is studied, samples are collected over several sampling occasions to incorporate all developmental stages. Moreover, habitat availability changes during season and therefore many size-related differences in the habitat use can be explained by differences in the habitat availability, rather than an active shift in habitat preference (Grossman & Ratajczak 1998; Welsh & Perry 1998).

Here, we use the bitterling, *Rhodeus sericeus* (Pallas), a small cyprinid fish to investigate the size-specific habitat use in a regulated lowland river. The studied population in the lower River Morava supports a large production of 0+ bitterling (Jurajda 1995). Bitterling spawn over a long reproductive period (from the beginning of May to late August in central Europe (Holčík 1999)) and a wide size range of 0+ individuals is encountered in late summer. Thus, two main assumptions to study size-specific habitat use are satisfied. In the present study, we describe and compare habitat use by four size-classes of bitterling in three different mesohabitats represented by bank types.

Study area

The study was carried out in the lower reach of the regulated and channelized River Morava (Danube basin, Czech Republic). Even if the discharge regimen remained natural (no dams were constructed on the main channel), dikes prevent any flooding of the old floodplain area, and so no fish migration into any off-channel refuges (e.g. backwaters) is possible. The 45 km study stretch was situated between the town of Hodonín (48°49′ 39″ N, 17°07′ 44″ E) and the Morava's confluence with the River Dyje (48°36′ 59″ N, 16°56′ 21″ E). The discharge varied from 8.6 to 40.1 $m^3 \cdot s^{-1}$ during the study (August 1995). The width of river channel was 50–60 m and the maximum depth reached 1–1.5 m.

Three bank types formed the shoreline: (1) artificial rip-rap (boulder banks) created from stones and boulders, (2) steep eroded clay outer banks, and (3) shallow sand–gravel beaches on the inner bank. Small (0.5–2 m^2) shallow marginal lagoons with standing water and deposits of silt were present along boulder and beach banks. The river bed was formed by gravel and sand with muddy deposits in lentic sections above weirs and near banks. Pebbles, stones and boulders (see Materials and methods for categorization) were present at all three bank types.

Riparian cover was present at some parts of the river, such as trees and bushes (*Salix* spp.). Overhanging emergent bank-side vegetation (grasses *Phalaroides arundinacea* (L.) and *Carex* spp.) sometimes reached the water level. Submerged vegetation (*Polygonum amphibium* L.) was rare whilst woody debris and submerged roots were more frequent. The most abundant juvenile fish in near shore areas of the stretch of river under investigation in 1995 were bitterling, roach (*Rutilus rutilus* (L.)), chub (*Leuciscus cephalus* (L.)) and gudgeon (*Gobio gobio* (L.)).

Materials and methods

Fish sampling

Sampling was undertaken from 22 to 29 August 1995 using a petrol DC electroshocker (220 V, 1.5–2.5 A, 20–50 Hz) modified to catch small fish effectively (Copp 1989). Point abundance sampling (Persat & Copp 1989) was used at 26 localities, each with 20 points sampled. The sampling was a part of a long-term monitoring program. Originally, the sampled localities were stratified into the bank type but owing to a course of the monitoring program, changes in river morphology induced changes in bank type at fixed localities. Consequently, some localities comprised two bank types. Localities were approximately 120 m long and points were chosen randomly within the localities with distance from the bank ranging from 0.1 to 9.0 m (median 0.6). The distance between sampling points was 4–7 m.

The anode (diameter 13 cm) on 2 m long pole was quickly immersed into the water and immediately activated for 3–5 s. After this, another operator collected all shocked fish using a small dip net with a 1-mm mesh. A detailed description of the sampling method was presented by Copp (1993). Juvenile fish were preserved in 4% formaldehyde and later identified and measured for standard length (SL) to the nearest 0.01 mm.

Environmental data collection

Bank type (beach, boulder bank, eroded bank) was estimated visually according to the slope and the character of the shoreline. Bank type is considered as mesohabitat scale throughout the article. At each sampling point, microhabitat variables were recorded immediately after the depletion of fish from the sampled area. Measurements were undertaken according to Copp (1992) with several modifications (Jurajda 1995, 1999).

Distance from the bank (di) and depth of water column (de) were measured by a graduated dipnet pole and converted to intervals for the analysis (di1: $\leq$40 cm; di2: 41–100 cm; di3: $\geq$100 cm and de1: $\leq$10 cm; de2: 11–25 cm; de3: 26–40 cm; de4: $\geq$41 cm). Current velocity (cu) was estimated by the movement of the dipnet mesh suspended in the water (for details see Copp 1992) with four categories discriminated (cu1: $<0.1\,\mathrm{m \cdot s^{-1}}$; cu2: 0.1–$0.5\,\mathrm{m \cdot s^{-1}}$; cu3: 0.5–$1.0\,\mathrm{m \cdot s^{-1}}$; cu4: $>1.0\,\mathrm{m \cdot s^{-1}}$).

Substrate type was divided into six categories according to the grain size: mud (<0.06 mm), sand (0.06–5 mm), gravel (5–20 mm), pebbles (2–5 cm), stones (5–40 cm), and boulders (>40 cm) (Bain et al. 1985; Copp 1991). Other microhabitat variables recorded were: clay silt (as a layer when the original substrate was distinguishable, otherwise recorded as mud), fine woody debris (small branches and roots), rough woody debris (large branches, stumps), riparian cover, emergent vegetation and submerged vegetation. Microhabitat variables and substrate types were recorded as present or absent.

Data analysis

From the length–frequency distribution of 1250 bitterling, we detected four size classes of bitterling (Fig. 1); 0A: $\leq$20.00 mm ($n = 129$), 0B: 20.01–30.00 mm ($n = 541$), 0C: 30.01–43.00 mm ($n = 515$) and D: >43.00 mm ($n = 15$). From scale analysis, fish <43 mm were identified as 0+ juveniles and larger fish as 1+ and older. Even though bitterling is a continuous spawner, the peaks of spawning activity (regarding meteorological and hydrological conditions) are observed. Therefore, three size-classes of 0+ bitterling can be considered as age cohorts (Copp 1990b).

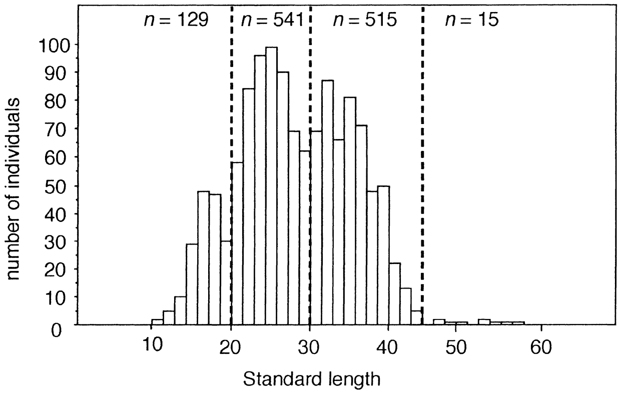

Fig. 1. Length–frequency distribution of 1250 individuals of bitterling. Division of cohorts is indicated by a dashed vertical line. 0A, 0B, 0C: 0+: juveniles; D: – adults. Number of fish in each cohort is indicated.

Quantitative data of bitterling abundance were $\log_2(x+1)$ transformed before analyses to reduce the effect of large shoals and avoid pseudo-replication. A set of χ^2 tests was used to analyse fish distribution at the mesohabitat scale for each cohort of bitterling.

In the microhabitat scale, we employed a hierarchical approach and analysed the microhabitat associations within each mesohabitat separately. We used Outlying Mean Index analysis (OMI) to describe microhabitat associations. This ordination technique has been recently proved successful in studies on niche separation and niche breadth (Dolédec et al. 2000) as well as on species distribution (Fievet et al. 2001). It measures the distance between the mean habitat condition used by species and the sampling area. Therefore, the position of the species in hyperspace depends on its deviation from a hypothetical species distributed without affinity to either of the habitat conditions.

First, we employed a normalized PCA analysis on covariance matrix of absent/present coded microhabitat variables to reduce the hyperspace. Total inertia for the representative axis was computed and used to select PCA axes for further analysis. The first five axes of PCA explained 68.3 and 63.0% of total variability in boulder and beach mesohabitat, respectively, and these were used in later analyses. Second, species table was matched to environmental (i.e. microhabitat) table of the row profiles resulting from the PCA. Four rows in the species table were represented by the different age cohort of bitterling (0A, 0B, 0C and D). The last row was represented by unoccupied sampling points. This allowed us to investigate also sampling points with no bitterling caught. Such samples are often eliminated from habitat use analysis (e.g. Copp 1992, 1993; Copp et al. 1994; Gozlan et al. 1998) but they could represent some important information and should not be omitted.

Then, the OMI analysis was performed on the matched tables. Permutation test (1000 permutations) was used to test the global effect of the average marginality of all species, i.e. a significant influence of microhabitat variables for cohort distribution. Cohort inertia, OMI and Tolerance index (Tol) were calculated for each cohort. The OMI index measures cohort marginality, i.e. the deviation of the average habitat conditions used by a particular cohort to the habitat condition of the total sampled area. Tolerance index shows the dispersion of sites that contain a particular cohort, i.e. the breadth of the habitat requirement. The statistical significance of the observed marginality (i.e. whether microhabitat variables significantly constrained the distribution of particular cohort) was tested by a permutation test (1000 permutations). For a detailed description of the OMI method see Dolédec et al. (2000). The OMI analysis was performed using ADE-4 software (Thioulouse et al. 1997)

In addition to the multivariate analysis, we used univariate χ^2 tests to investigate the direct association of each microhabitat variable with habitat used by the particular cohort. In this analysis, each microhabitat variable was tested with each combination of cohort and mesohabitat. The two highest current velocity categories (cu2 and cu3) were pooled owing to the low number of observation in these categories. In order to obtain an experimentwise error rate of α when all possible combinations were tested, a Bonferroni correction was used to decrease the probability of type I error (Sokal & Rohlf 1995).

Results

Habitat availability

The most frequent bank type sampled along the right bank of the River Morava in 1995 was boulder bank (54.6%, $n = 284$), whereas the sandy–gravel beaches were sampled in 35.6% ($n = 185$) and steep eroded bank in 9.8% ($n = 51$) of the investigated points.

A range of 1–4 (median = 2) types of substrates for one sampling point was recorded. Sand and gravel were the dominant substrate types on beaches whereas boulders and sand were most common on boulder banks. Mud was almost the exclusive substrate on eroded bank (Table 1). Silt was present at all sampled points along the eroded banks. Woody debris and vegetation were observed only occasionally with the exception of emergent vegetation along eroded banks (Table 2). The distribution of sampled points along gradients of di and de is shown in Table 2. Areas with the weakest current velocity ($<0.1\ \mathrm{m \cdot s^{-1}}$) were the most often sampled along all bank types (Table 2).

Mesohabitat scale

In the mesohabitat scale, steep eroded bank was avoided by all bitterling. The only individuals caught were two specimens of 0B cohort. The most distinct distribution with regard to bank type was found in the smallest fish. 0A cohort was significantly positively associated with boulder bank and avoided beaches and eroded bank. Also, the 0B fish tended to occur in boulder bank but were not underrepresented on beaches. The 0C and D fish did not showed any tendency to be associated either to the boulder or beach bank (Table 3).

OMI analysis

As steep eroded bank was avoided by all bitterling cohorts, microhabitat analyses were performed only on boulder and beach banks. The first result of OMI analysis showed a significant influence of microhabitat variables for cohort distribution (permutation test, $P < 0.001$). The first two axes, used for a graphical representation, expressed 77 and 17% of explained variability on boulder bank and 75 and 20% of explained variability on beach bank. Cohort separation by habitat use was higher on beach banks (total inertia = 0.425) than

Table 1. Availability of substrate types and the microhabitat characteristics on sampled points in the River Morava in late August 1995 in the respective mesohabitat.

	Boulder ($n = 284$)		Beach ($n = 185$)		Eroded ($n = 51$)	
	n	(%)	*n*	(%)	*n*	(%)
Substrate types						
Mud	31	(16.8)	73	(25.7)	46	(90.2)
Sand	133	(71.9)	109	(38.4)	1	(2.0)
Gravel	101	(54.6)	31	(10.9)	0	(0.0)
Pebbles	72	(38.9)	25	(8.8)	0	(0.0)
Cobbles	13	(7.0)	107	(37.7)	0	(0.0)
Boulders >40 cm	10	(5.4)	196	(69.0)	19	(37.3)
Microhabitat characteristics						
Silt	33	(17.8)	96	(33.8)	51	(100.0)
Fine woody debris	17	(9.2)	22	(7.7)	1	(2.0)
Rough woody debris	9	(4.9)	7	(2.5)	0	(0.0)
Riparian vegetation	1	(0.5)	19	(6.7)	0	(0.0)
Emergent vegetation	11	(5.9)	6	(2.1)	23	(45.1)
Submergent vegetation	0	(0.0)	3	(1.1)	0	(0.0)

Number of points sampled in the respective mesohabitat are indicated in parentheses. Number of points with the respective variable and its percentage (in parentheses) are shown. More than one substrate type was often present at one sampling point and hence the sum of substrate types does not correspond to the total number of the points investigated.

Table 2. Distribution of sampled points along the gradient of distance from the bank, depth of water column and current velocity on sampled points in the River Morava in late August 1995 in the respective mesohabitat.

	Beach ($n = 284$)		Boulder ($n = 185$)		Eroded ($n = 51$)	
	n	(%)	n	(%)	n	(%)
Distance from the bank (cm)						
<40	17	(9.2)	91	(32.0)	20	(39.2)
40–100	69	(37.3)	169	(59.5)	31	(60.8)
>100	99	(53.5)	24	(8.5)	0	(0.0)
Depth of water column (cm)						
<10	62	(33.5)	36	(12.7)	2	(3.9)
10–25	97	(52.4)	168	(59.2)	23	(45.1)
26–40	21	(11.4)	66	(23.2)	19	(37.3)
>40	5	(2.7)	14	(4.9)	7	(13.7)
Current velocity ($\mathrm{m \cdot s^{-1}}$)						
<0.1	98	(53.0)	209	(73.6)	48	(94.1)
0.1–0.5	44	(23.8)	50	(17.6)	3	(5.9)
>0.5	43	(23.2)	25	(8.8)	0	(0.0)

Number of points sampled in the respective mesohabitat are indicated in parentheses. Number of points within the respective category and its percentage are shown.

Table 3. The distribution of four bitterling cohorts along the banks of the River Morava in the late summer in 1995.

Bank type	Boulder			Beach			Steep eroded		
Fish cohort	χ^2	*P*	P/A	χ^2	*P*	P/A	χ^2	*P*	P/A
0A	9.14	0.010	P	5.29	0.071	A	7.67	0.022	A
0B	5.18	0.075	P	0.40	0.818		17.38	<0.001	A
0C	2.12	0.347	P	0.01	0.996		13.05	0.002	A
D	0.04	0.982		0.67	0.716		1.23	0.542	

χ^2 value and its statistical significance are presented. P/A column indicates whether a particular cohort was overrepresented (P) or underrepresented (A) on the respective bank.

on boulder bank (total inertia = 0.238). The relative contribution of each microhabitat variable on the first two factorial axes is given in Table 4 and the graphical projections of variables on the first and second axis of the OMI analysis are given in Figs 2 and 3. Substrate type, current velocity and water depth categories were the most important descriptors of gradients on the first two factorial axes (Figs 2 and 3).

0A cohort had strongest habitat requirements with high OMI indices and low Tols. In other words, their average use of the habitat was the most distinct from the mean habitat conditions of the sampling area. This was especially apparent on beaches, where 0A fish were underrepresented (Tables 3 and 5). On both boulder and beach banks, non-occupied sampling points were positioned near the centroid and had low OMI and high Tols which indicated that no distinct habitat patch was avoided by all bitterling cohorts pooled, i.e. a hypothetical 'cohort' set to non-occupied sampling points did not show any special habitat requirement (Fig. 4).

A continuous change in habitat use is visualized in Fig. 4. On the boulder bank, the smallest fish

Table 4. The relative contribution of each microhabitat variable to the first two factorial axes (Fac 1, Fac 2) of the OMI analysis.

Bank type		Boulder		Beach	
Habitat variable	Code	Fac 1	Fac 2	Fac 1	Fac 2
Riparian vegetation	rip	0.402	0.354	0.319	0.668
<40 cm from the bank	di1	0.548	0.152	0.761	0.112
40–100 cm from the bank	di2	0.913	0.017	0.964	0.012
>100 cm from the bank	di3	0.564	0.284	0.955	0.032
Water depth <10 cm	de1	0.532	0.323	0.805	0.157
Water depth 10–25 cm	de2	0.843	0.145	0.500	0.490
Water depth 26–40 cm	de3	0.916	0.047	0.063	0.730
Water depth >40 cm	de4	0.378	0.030	0.319	0.668
Current velocity <0.1 $\mathrm{m \cdot s^{-1}}$	cu0	0.846	0.067	0.748	0.232
Current velocity 0.1–0.5 $\mathrm{m \cdot s^{-1}}$	cu1	0.609	0.330	0.830	0.000
Current velocity 0.5–1.0 $\mathrm{m \cdot s^{-1}}$	cu2	0.412	0.492	0.322	0.638
Current velocity >1.0 $\mathrm{m \cdot s^{-1}}$	cu3	0.218	0.766	0.166	0.769
Mud substrate	Mud	0.986	0.001	0.523	0.409
Sand substrate	Sand	0.957	0.070	0.849	0.143
Gravel substrate	2	0.429	0.094	0.929	0.060
Pebble substrate	5	0.063	0.381	0.914	0.080
Stones 5–40 cm in diameter	10	0.988	0.005	0.577	0.366
Boulder >40 cm in diameter	40	0.902	0.061	0.131	0.806
Silt	Silt	0.014	0.975	0.422	0.062
Fine woody debris	fw	0.227	0.628	0.624	0.139
Rough woody debris	rw	0.553	0.062	0.269	0.533
Emergent vegetation	em	0.142	0.480	0.015	0.662
Submergent vegetation	su	0.754	0.000	0.000	0.000

Codes of the microhabitat variables used in Figs 2 and 3 are indicated.

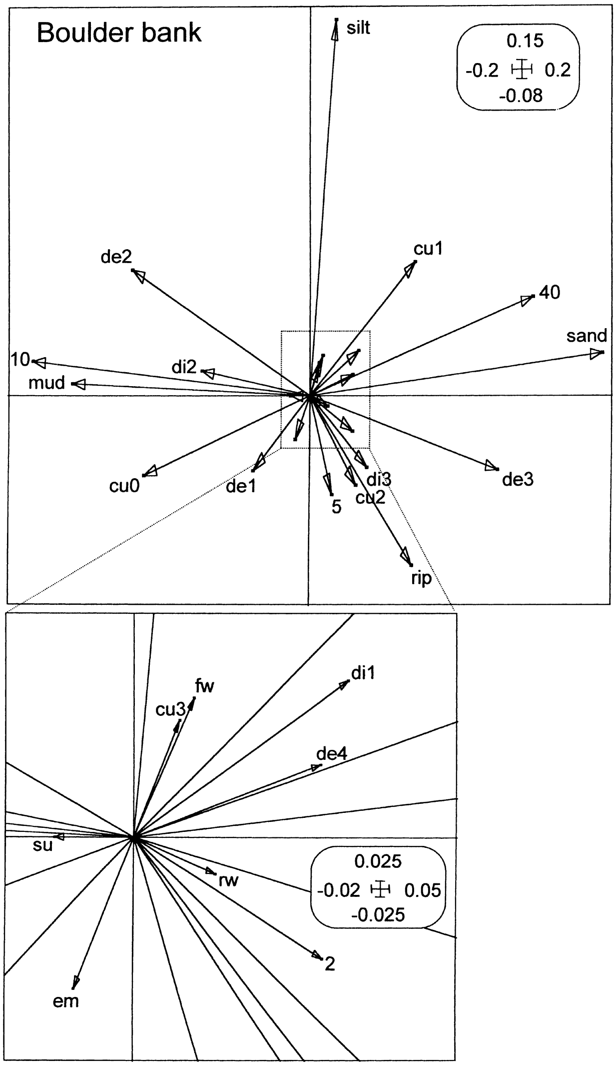

Fig. 2. Graphical representation of canonical weights of the first (horizontal) and second (vertical) axis of OMI analysis on the boulder bank. For codes of the microhabitat variables see Table 4.

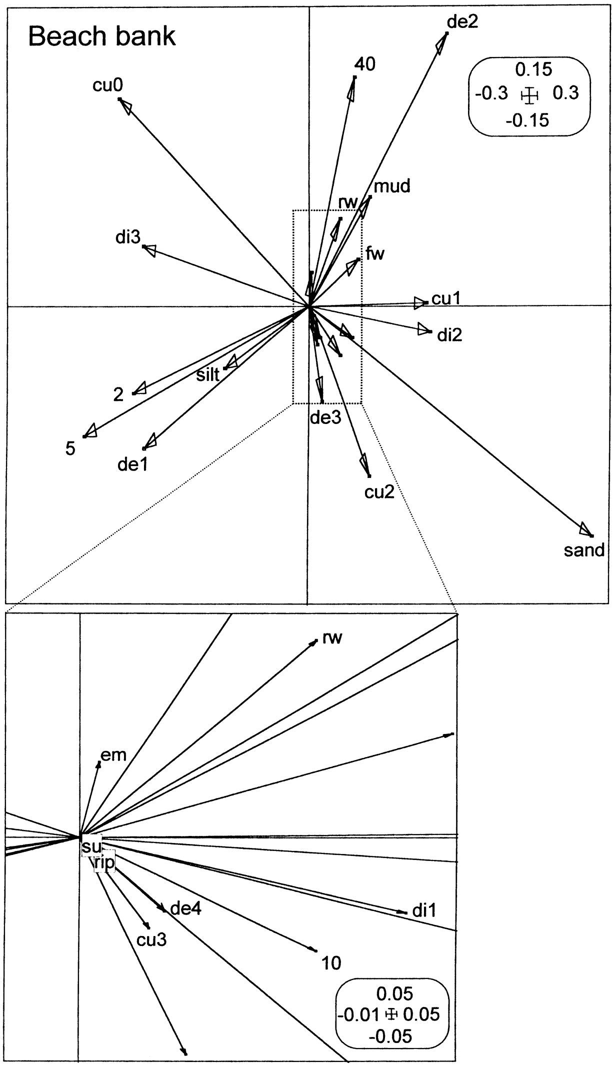

Fig. 3. Graphical representation of canonical weights of the first (horizontal) and second (vertical) axis of OMI analysis on the beach bank. For codes of the microhabitat variables see Table 4.

(0A) were associated with mud and stone substrates, the weakest current velocity and distance from the bank between 0.4 and 1.0 m. 0B fish occupied similar habitat but their tolerance increased. The 0C cohort tended to be present at points with deeper water and riparian vegetation. Adult fish (D) occurred at points with sand substrate, deep water and higher current velocity (Figs 2 and 4). On the beach bank, the change in habitat use was from the points with the weakest current velocity, the smallest depth and gravel to pebble substrate in 0A fish to deeper water with

Table 5. Habitat use parameters of four bitterling cohorts on the boulder and beach banks.

	Boulder bank					Beach bank				
Fish cohort	*n*	Inertia	OMI	*P*	Tol	*n*	Inertia	OMI	*P*	Tol
0A	62.5	2.54	0.756	< 0.001	1.54	15.7	3.04	2.250	<0.001	0.41
0B	119.2	2.80	0.250	< 0.001	2.27	58.0	2.74	0.950	<0.001	1.31
0C	107.7	3.31	0.196	0.01	2.75	61.7	3.24	0.380	0.05	2.45
D	6.3	3.67	1.993	0.01	1.54	6.2	2.85	0.797	>0.05	1.72
Non-occupied	187.0	2.98	0.017	0.01	2.70	143.0	3.10	0.016	0.05	2.78

Number of fish in the particular cohort after $\log_2$ transformation (*n*), cohort inertia (inertia; quantification of the microhabitat position on cohort separation), Outlying Mean Index (OMI; deviation of average cohort position from the mean habitat condition), statistical significance of the OMI analysis for the particular cohort (*P*, permutation test, 1000 permutations) and Tolerance index (Tol; measure of the extent of the habitat use) are presented.

Table 6. Microhabitat associations of four bitterling cohorts with particular environmental variables as revealed by χ^2 test.

	Beach bank (age cohort)								Boulder bank (age cohort)							
Habitat variable	0A		0B		0C		D		0A		0B		0C		D	
<40 cm from the bank	1.6		5.9		0.4		0.6		0.1		0.1		2.7		0.2	
40–100 cm from the bank	6.4		11.5	A	0.0		0.5		11.7	P	12.0	P	0.4		1.0	
>100 cm from the bank	10.2	P	21.9	P	0.0		0.6		19.4	A	15.0	A	5.9		0.6	
Water depth <10	0.6		9.6		8.6		3.1		0.2		0.2		0.5		1.7	
Water depth 10–25	9.9	A	5.1		22.3	P	2.6		13.7	P	9.4	P	7.2	A	5.2	
Water depth 26–40	2		0.2		5.8		0.8		16.2	A	5.3		22.4	P	1.9	
Water depth >40 cm	nc		1.6		1.7		nc		3.2		4.1		2.1		26.3	P
Current velocity <0.1 m · s^{-1}	29.9	P	75.7	P	16.1	P	0.0		29.1	P	18.5	P	0.1		0.7	
Current velocity 0.1–0.5 m · s^{-1}	2.9		6.4		2.2		12.9	P	14.3	A	12.5	A	0.9		1.5	
Current velocity >0.5–1.0 m · s^{-1}	15.8	A	41.4	A	23.7	A	6.2		9.4	A	3.4		0.6		0.0	
Mud substrate	3.2		2.8		8.8	P	0.0		29.8	P	20.8	P	9.2		2.2	
Sand substrate	33.3	A	89.8	A	4.1		0.1		31.0	A	35.1	A	9.2	P	10.2	P
Gravel substrate	9.6	P	20.3	P	5.9		1.2		4.8		0.0		4.5		nc	
Pebble substrate	19.8	P	31.5	P	12.1	A	1.3		6.0		5.1		9.7		nc	
Stones 5–40 cm in diameter	1.2		4.4		1.0		nc		39.7	P	16.7	P	9.9	A	3.8	
Boulder >40 cm in diameter	nc		3.3		84.0	P	nc		37.8	A	12.6	A	0.0		2.8	
Silt	30.0	P	0.0		1.8		0.0		2.0		8.7	A	27.4	A	3.2	
Fine woody debris	3.4		5.9		5.2		nc		0.4		1.3		0.2		nc	
Rough woody debris	nc		3.0		18.2	P	nc		1.6		1.3		2.1		nc	
Riparian vegetation	nc		nc		nc		nc		4.5		3.9		22.0	A	nc	
Emergent vegetation	nc		0.0		1.0		nc		4.8		0.1		4.8		nc	
Submergent vegetation	nc		nc		nc		nc		nc		0.4		1.2		nc	

χ^2 value is presented. Positive (P) or negative (A) association is indicated in the statistically significant ($P < 0.05$) cases. Some combination had insufficient number of observation to perform an analysis (nc).

large boulders, woody debris and mud substrate (Figs 3 and 4).

Univariate analysis

Overall, the univariate test of microhabitat associations gave concordant results to the multivariate analysis. The 0A and 0B fish were caught at sampling points with the weakest water velocity. These were associated with mud and stone substrate on boulder bank and gravel to pebble substrate on beaches. The adult fish used the patches with higher water velocity (0.1–0.5 m · s^{-1}) on beaches. Detailed results are summarized in Table 6.

Discussion

We found that habitat use by 0+ bitterling was size-related. In the mesohabitat scale, all bitterling avoided the steep eroded bank and 0+ fish were the most likely to be found on boulder banks. However, only the smallest fish (<20 mm SL) tended to be underrepresented on beaches (Table 3). The smallest cohort had also the most pronounced habitat requirements in the microhabitat scale (Table 5). The highest overlap in cohort distribution was observed between 0A and 0B cohorts, with co-occurrence on 81% of sampling points. Conversely, low or no overlap in distribution was found between 0A, and 0C and D cohort, respectively (Table 7). On boulder and beach banks, no distinct habitat patch (with specific microhabitat characterization) was avoided by all bitterling cohorts (Table 5, Fig. 4).

Table 7. Co-occurrence of bitterling cohorts at sampled points in the River Morava in late August 1995.

	n	0B	0C	D
(*a*) *Cohort*				
0A	37	30 (81.1)	6 (16.2)	0 (0.0)
0B	82		34 (41.5)	3 (3.0)
0C	84			8 (80.0)
D	10			
(*b*) *Cohort combination*				
	0A	0B	0C	D
0A + 0C		6 (16.2)		
0C + D		3 (30.0)		
0A + D		0		
0C + D	0			

Number of points where the respective cohort occurred (*n*) and the number of points where two cohorts co-occurred (percentage from the total of possible co-occurrence given in parenthesis) (a). Number of observation for co-occurrence of three cohorts for all possible combinations and its percentage from the total possible co-occurrence given in parenthesis (b).

There was a gradual change in the habitat use by bitterling on boulder and beach banks. Among cohorts, the overlap in habitat use was higher on the boulder bank. On boulder bank, small fish (0A and 0B) tended to occupy depositional patches characterized by minimal current velocity and

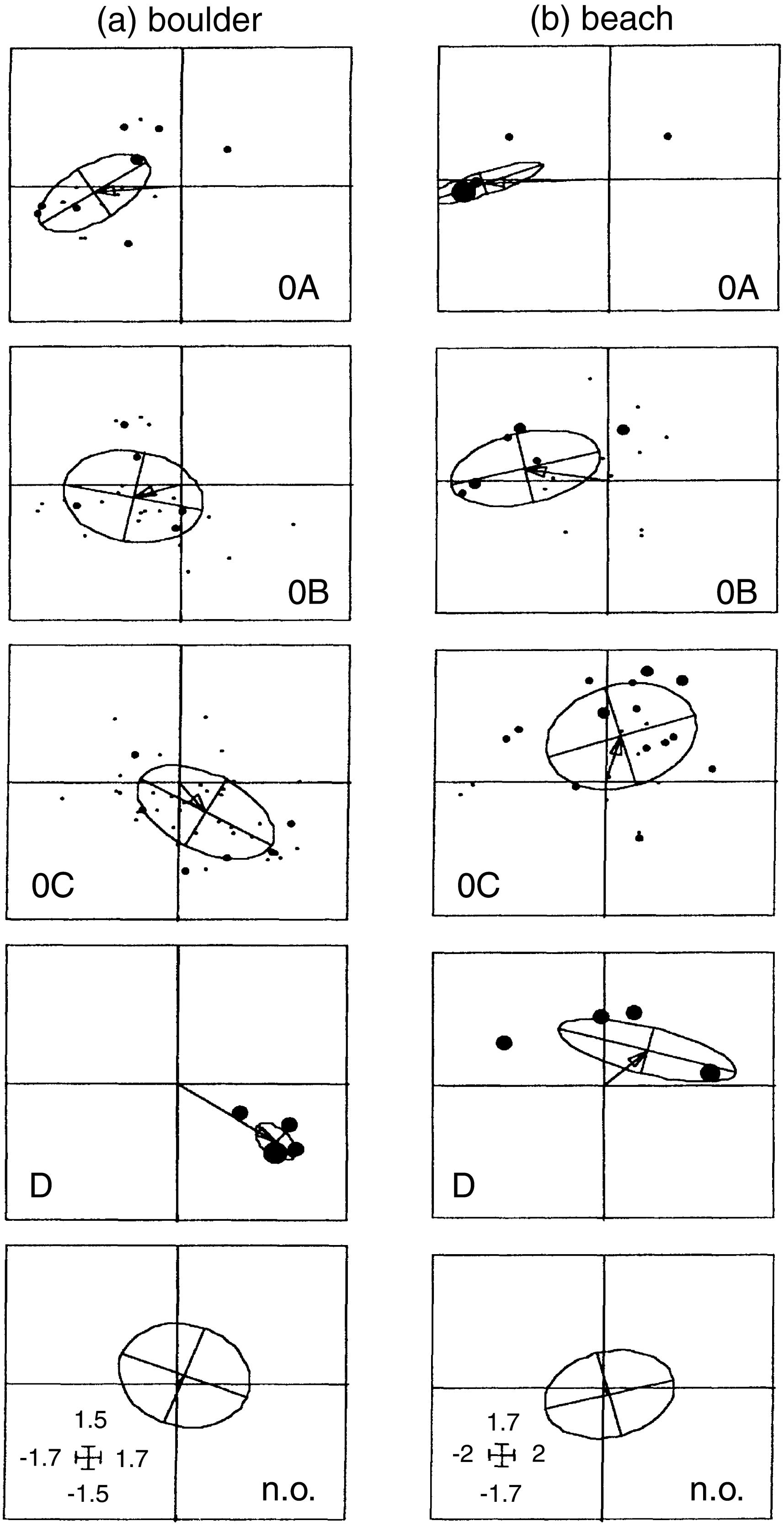

Fig. 4. Microhabitat distribution of each age cohort (0A, 0B, 0C and D) and non-occupied sampling points (n.o.) with regard to the first and second axis of OMI analysis on the boulder (a) and beach bank (b). Points represents fish frequencies and the ellipses show the average niche size for each age cohort and for non-occupied sampling points.

the substrate composed of mud and stones. There, they occurred 0.4–1.0 m from the bank where water was 10–25 cm deep. They also avoided patches with large boulders. The 0C fish had different requirements and occupied deeper water (26–40 cm) above sand substrate. Likewise on beach bank, 0A and 0B cohorts occurred in patches with minimal water velocity, though they were associated with gravel and pebble substrate there. These patches were distributed further than 1 m from the shoreline. The 0C fish also preferred minimal flow, but they were found in different patches nearer to the bank and characterized by boulders and mud substrate. The adult fish were distributed in the patches with higher water velocity.

Habitat use of bitterling

Jurajda (1999) found that the dominant species of 0+ fish in regulated and channelized rivers (as is the case of bitterling in the River Morava) did not show any habitat preferences and/or preferences change from year to year. Two possible explanations may account for this. First, habitat heterogeneity in regulated and channelized rivers is too low to permit any 0+ fish segregation by habitat requirements. Second, the length–frequency distribution of fish is broad and/or interannual variation in fish size occurs and consequently, habitat requirements of particular fish are masked. The later explanation is the most applicable for bitterling as a species with a long reproductive period and fractional spawning.

Habitat use by bitterling and its seasonal changes has been previously investigated in riverine habitat (Przybylski & Zieba 2000) and habitat use by 0+ bitterling has been addressed within studies on several 0+ fish assemblages, e.g. Slovak/Hungarian stretch of the River Danube (Copp et al. 1994), the River Garonne (Gozlan et al. 1998) and the River Morava (Jurajda 1999). However, none of these studies have separated bitterling into the size-classes and compared size-related differences in habitat use.

In riverine habitat, bitterling occurred in near-shore areas with the minimal water velocity and dense submerged vegetation. Substrate type did not affect their habitat use beside its covariation with other habitat variables (Przybylski & Zieba 2000) which is in conformity with our results for 0+ bitterling.

In a complex floodplain area, 0+ bitterling (mean body size 24.9–27.3 mm SL) occurred in abandoned and partially abandoned channels, whereas their occurrence in the river channel was scarce (Copp et al. 1994; Gozlan et al. 1998). Jurajda (1999) found that 0+ bitterling (range 7.1–41.1 mm SL) were most likely to be found on boulder bank in the River Morava during two seasons (1992–1993). However, they tended to occur on beach rather than boulder bank in 1991. Regarding to the present study, this could be explained by differences in the sampling period (October in 1991, i.e. 2 months later than other years) when fish were larger (16.5–39.6 mm SL). Copp & Jurajda (1999) reported a diel pattern of the bank use with a more extensive use of all types of river banks (boulders, beaches) during the night and an eventuality of off-shore migration during the morning. However, these results are based on a single diel cycle and more information is required to substantiate their suggestions. In contrast, other studies have found no evidence of diel migrations and a high degree of mesohabitat loyalty of bitterling (Peňáz et al. 1978; Przybylski 1996).

Although microhabitat use patterns may vary between populations with regards to biotic (e.g. local food supply; Fausch 1984) and/or abiotic (e.g. temperature; Baltz et al. 1982) factors, the corresponding microhabitat associations of bitterling are confirmed by studies from the lotic channel (Jurajda 1999) and the floodplain (Copp et al. 1994; Gozlan et al. 1998). However, the microhabitat analysis of Copp et al. (1994) and Gozlan et al. 1998) found significant associations with numerous habitat variables. It may be a result of unscaled analyses performed in their studies (Poizat & Pont 1996).

Bitterling is classified as a limnophilic species (Schiemer & Waidbacher 1992; Holčík 1999) usually occurring in lentic macrohabitat within the floodplain. However, Copp et al. (1994) and Gozlan et al. (1999) suggested that juvenile bitterling are semi-rheophilic, even when they were found avoiding water current. We have shown previously that bitterling can inhabit the regulated and channelized stretch of large lowland river (Jurajda 1995) and can develop morphological and ecological adaptations in its life history traits (Reichard 1998; Reichard et al. 2001). Its presence is limited by the occurrence of unionid mussels in which the early development of the bitterling is completed. Despite the ability of the bitterling to spend their entire life cycle in lotic habitat, we have documented a preference for low flow patches, especially in the smallest fish. We also documented that the freshly released bitterling larvae (8.5–10.0 mm SL) have drifted downstream in the river current at night when they lost the visual orientation (Reichard et al. 2001; Reichard unpublished data). The presence of depositional areas may be necessary for the survival of newly released bitterling.

Shifts in habitat use

Shifts in habitat use have been reported for some European cyprinids, however, other cyprinids were not found to undergo any habitat shift (Schiemer & Spindler 1989; Copp 1990a, 1992; Rincón et al. 1992; Garner 1996). The first habitat shift occurs when active habitat selection is permitted owing to the development of swimming ability (Copp 1990a). Other shifts are associated with a change in diet (Schiemer & Zalewski 1992; Garner 1996) and predation risk (Schlosser 1987). Distinct patterns of size-related habitat shifts were observed among years which was affected by different habitat availability (Grossman & Ratajczak 1998). Seasonal shifts in habitat use, exhibited in many stream fishes, are often reported as passive responses to changes in the habitat availability (Baltz & Moyle 1984; Grossman & Freeman 1987; Rincón et al. 1992).

Copp & Kováč (1996) found that a shift in habitat use by roach corresponded with stabilization in factors describing swimming ability and visual acuity. Przybylski & Zieba (2000) noted that habitat use by adult and 0+ bitterling considerably differed. In the present study, the most distinct change in the habitat use of bitterling was between fish sized <30 mm (0A and 0B cohorts) and larger (0C and D cohorts) (Table 7). This change in habitat use closely coincides with a shift in diet (Przybylski 1996) and morphological state (Reichard & Jurajda 1999). It confirms that the main components of the ecological niche (habitat use, diet) and morphology are intimately associated.

Resumen

1. La utilización de habitat por cuatro clases de tamaño de *Rhodeus sericeus* fue estudiado a dos escalas espaciales en un río regulado. La escala de mesohabitat estuvo representada por diversos tipos de orilla (piedras, playas y bancos erosionados verticales). A cada escala de mesohabitat examinamos asociaciones de microhabitat con varios tipos de sustrato tales como vegetación, 'woody debris' y posición del río. Detectamos tres tamaños de juveniles del año (0+) y un grupo de adultos y para detectar asociaciones de tamaños relacionadas con el habitat, utilizamos métodos univariantes y multivariantes.

2. Todas las clases de tamaños rechazaron los bancos erosionados verticales y la mayor parte de los individuos 0+ se distribuyeron sobre bancos de piedras. Los peces mas pequeños parecieron tener requerimientos de habitat mas pronunciados y ocurrieron fundamentalmente en areas de depósitos mientras que los individuos 0+ de mayor tamaño ocurrieron en aguas mas profundas. Cambios relacionados con el tamaño en la utilizacion del habitat coincidieron con cambios en la dieta y en el estado morfológico.

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