

THE ROLE OF ACTIVE INDIVIDUAL MOVEMENT IN HABITAT SELECTION IN THE ENDANGERED FRESHWATER MUSSEL *UNIO CRASSUS* PHILIPSSON 1788

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Abstract *Unio crassus*, an endangered freshwater mussel species, inhabits streams with diversified physical structure, subject to frequent changes in channel morphology. It is usually regarded as a sedentary animal, although individual movement has been observed. The hypothesis that adults can use movement in order to actively select a microhabitat was tested in two experiments simulating dislodgement of mussels by a stream. In the first experiment, begun in 2004, individually tagged *Unio crassus* individuals were distributed in a regular pattern throughout a 17 m long riffle-pool reach of Cedron River (S Poland). The mussels changed their positions after displacement. The distances travelled differed significantly between parts of the channel: they covered longer distances in the pool (ca 2 m) than in the riffle (ca 0.6 m), with minimal movements on steep banks and dead water areas and large movements in shallows and deep areas. Individuals from shallow places were found to have moved to the steep bank of the pool, built of fine sediments, and to the area of dead water in the lee of boulders. The movements were not related to the water depth gradient: individuals moved to the steep bank even from the deepest parts of the pool, a finding experimentally confirmed with radio-tracked individuals. The second experiment was begun in 2008 in a smaller reach 5 m long. The mussels were put in the deepest part of the reach. Most of them climbed to a steep bank built of fine sediment with minimal flow, and some of them hid beneath the gravel armouring the fast-flowing part of the channel. It can be concluded that adult *Unio crassus* individuals can move to any place in a channel after being disturbed by natural or artificially created fluvial processes. Steep banks of the pool, built of fine sediments, represented the best place of refuge.

Keywords *Unio crassus*, mussel behaviour, river channel structure, fine sediments, spate, radiotelemetry

INTRODUCTION

The thick-shelled River Mussel *Unio crassus* (Unionidae) occurs in small or medium-size rivers with gravel, sandy to muddy bottoms and clean water. It may be one of the most abundant unionids inhabiting this type of habitat (Zajac, 2004), but due to a significant and continuing decline in its range (Wells & Chatfield, 1992, Zajac, 2004, Zettler & Jueg, 2007) it is now protected under both EU law (Annex II and IV of the EU Habitats and Species Directive) and the laws of many European states.

Artificial alteration of natural fluvial systems is seen as one of the main reasons for the decline of the species, with a demonstrated eutrophication impact (Patzner & Muller, 2001, Zettler & Jueg, 2007). Indeed, river channels in all developed countries have undergone substantial changes of physical structure and function, due to intensive regulation work undertaken in the last 200 years (Ward, 1998). Natural changes in the physical environment such as those occurring in floods also can have a significant impact on freshwater mussel populations, through channel reforma-

tion and large-scale movement of the substrate (Hastie *et al.*, 2001). Whether natural or artificially created, the influence of such physical changes on the population dynamics of declining *Unio crassus*, a species typical of dynamic lotic environments, is poorly understood.

Individuals dislodged by a stream should be expected to have very low survival as they are transported downstream in adverse conditions, buried in sediment, or even crushed by the coarse bedload mobilized during a flood. The geomorphology of coarse bedload movement during floods is reasonably well studied; even small streams can move substrate having particle sizes much larger than the body size of any freshwater mussel (Petit *et al.*, 2005). Despite exposure to such adverse conditions, *Unio crassus* has a life span of over 10 years (Bauer & Wächtler, 2001). Because mussels are distributed non-randomly within the channel (aggregation in beds) and have a long life span, they should be expected to exhibit adaptations to changes in channel conditions.

Of course, with their limited locomotor ability, mussels cannot react to changes in the environment quickly; probably their maximum locomotive ability is engaged during escape in

reaction to declining water level. Without such an overwhelming threat, mussels of the family Unionidae do not move conspicuously. After the larval period spent encysted in fish gills or fins, the mussels spend life as sedentary individuals aggregated in mussel beds. Any movements reported in running waters are rather random (Balfour & Smock, 1995) or cover short distances (Aldridge, 2000; Schwab & Pusch, 2007). However, considering that individuals of *Unio crassus* are frequently threatened with unpredictable environmental events, they might be expected to be able to correct their location within a channel after being dislodged, in order to be always at the optimal sites, that is, sites that are safe but which also fulfill other requirements related, for example, to reproduction (Vicentini, 2005). If such an ability exists, an understanding of it would be very important for effective monitoring and protection of this species (Pfeiffer & Nagel, 2010) both in naturally dynamic rivers and those undergoing rapid human-caused change.

The aim of this study was to verify whether adult *Unio crassus* individuals are able to move actively to a more suitable location, by experimental dislodgement of a sample of mussels and observations of their subsequent movements.

MATERIALS AND METHODS

Study site The Cedron is a small river in the Carpathian foothills (Central Europe, southern Poland), 24.8 km in length and having a catchment area of 93.4 km². In the studied channel reach (close to Leńcze village, N49°53' E19°43') it meanders slightly, flowing through a wide meadowy valley. There is no bare rock within the studied section of the channel. Most of the channel area is covered with gravel, the dominant fraction of stones exceeding 32 mm (Fig. 1a). The banks of the pool sections have areas of fine sediment deposition, with the sand fraction (ca 2/3) and silt fraction (ca 1/3) dominant (Fig. 1b). The area lies within a Natura 2000 site ("Cedron", PLH120060) dedicated to *Unio crassus*. Under the Polish monitoring scheme (Zajac, 2010) the studied population is accorded FV status, in habitat U1, with density reaching 5–50 ind./m² and very good recruitment (Zajac K., unpubl. data).

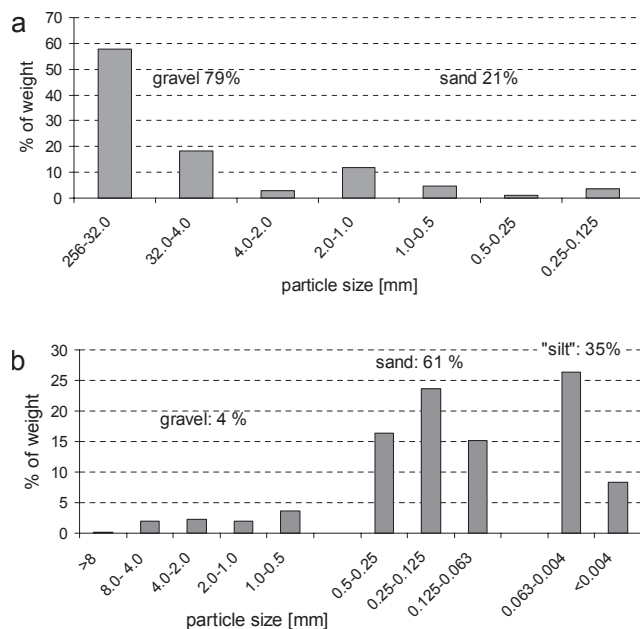


Figure 1 Structure of sediments in channel of Cedron river: a – coarse sediment covering most of the channel, sample weight 1769 g; b – fine sediment deposited on the slopes of the channel bed in pool area, sample weight 450 g.

Experimental plot The river reach studied, beginning in 2004, was 17 m long and comprised a slow deep section (pool) and a fast-flowing shallow section (riffle; Fig. 2a–c). The right bank of the stream was steep and built of fine sediment, exposed and eroded in the riffle area (with some pure clay parts) and silted in the area of marginal dead water in the pool. The middle part of the studied reach was covered with fine gravel. In the middle of the studied reach was a group of boulders. The lower part of the left bank of the river channel was an area of dead water in the lee of boulders, adjacent to a large emerged bar of gravel and sand. In the pool part the area near the left bank was covered by shallows graduating to a dry gravel bar.

The studied reach was mapped in detail along a regular grid of measurement points (1 m intervals along the channel, 0.2 m intervals crosswise). Within that grid, for the purpose of experimental displacement of mussels, 0.5 m × 1 m squares were marked with wooden sticks (20 × 1 × 2 cm) pushed 15 cm into the substrate, as shown in Fig. 2a. At each measurement point the substrate type (sand, gravel, boulders, fine sediments – sand, silt, clay) was noted (Fig. 2a), and the channel and flow measurements were taken (Table 1,

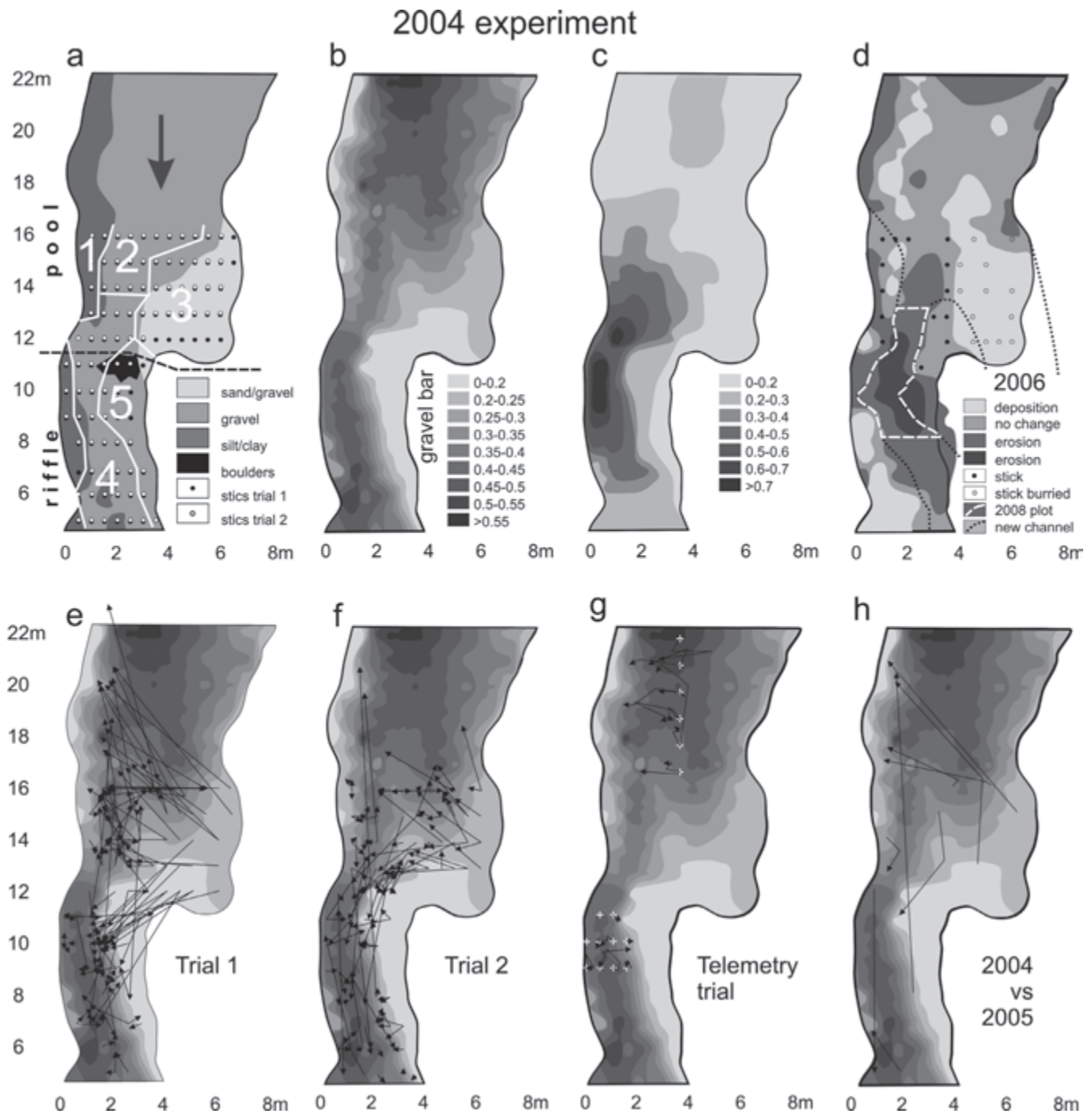


Figure 2 2004 experiment: characteristics of the studied channel reach of Cedron river (2004–05, large study plot) and presentation of tagged mussels' movements, with water depth shown: a – river substrate and grid points (with distinguished pool and riffle parts and areas differing in conditions: 1 – steep bank, 2 – deep pool, 3 – shallows in pool, 4 – riffle, 5 – lee of boulders; large arrow indicates flow direction); b – depth map (D [m]); c – flow velocity map (V [ms⁻¹]); d – the same channel reach after changes in 2006, the white dashed line indicating the size and location of the study plot in the 2008 experiment, the black dotted line indicating the final shape of the channel in 2008; e – movements of tagged mussels during first trial; f – movements of tagged mussels during second trial; g – movements of mussels in radiotelemetry trial; h – movements of tagged mussels between their final position in 2004 trials and autumn of 2005.

Table 1 Channel and flow characteristic of study plots. All measurements in metres (flow: ms^{-1}).

Experiment:		2004		2008
		riffle	pool	riffle/pool
Length		6	11	5
Width	Mean	3.3	6.6	1.0
	SD	3.0	8.9	0.68
Depth	Mean	0.20	0.20	0.17
	SD	0.40	0.14	0.09
	Max	0.47	0.57	0.40
Flow	Mean	0.22	0.13	0.29
	SD	0.17	0.15	0.24
	Max	0.65	0.59	0.93

Fig. 2b, c). Water transparency was usually high throughout the whole stream, enabling detection of the adult mussels during surveys. There were no major floods during the experiments. The data on the morphology of the studied channel were collected before each of the experiments, imported to the GIS and used for construction of the channel model, from which data were derived for subsequent analysis.

In 2006 the study area was transformed by the river due to a large spring flood; the main stream moved towards the left bank, eroding the group of boulders. This change was mapped (Fig. 2d) by measuring the area in 2006 and comparing the depth map from 2004 with the new one, using the GIS. In 2007, again as a result of a large flood, in the riffle area an additional channel was formed (dotted black line in Fig. 2d), going through the gravel bar near the left bank of the river. The old channel was then rebuilt, forming a new pool (white dashed line in Fig. 2d). At the outset of the experiment in 2008, the new pool consisted of two symmetric parts: a gravel part with fast-flowing water on the left side of the channel, and a pool with fine sediment and still water on the right side (Fig. 3a–c). The site was measured and mapped by the method applied to the previous site: it was measured precisely at 146 measurement points (0.5 m intervals along the channel, interpolated to 0.25 in the GIS in order to smooth the interpolating isoclines, and 0.1 m intervals crosswise; measurements summarized in Table 1).

In each of the study plots, just after the morphological measurements of the channel were made, water flow speed was measured with

a water velocity meter (accuracy 0.03 ms^{-1}) at the same points of the grid and at the same water level; velocity was measured just above the channel bottom in order to record the actual conditions encountered by the mussels. Then the data were imported to the GIS and flow maps for the respective study plots were created (Figs 2c, 3c), from which data were derived for statistical analysis.

2004 experiment The 2004 experiment included two trials (Table 2). In each of them, two individually tagged adults of *Unio crassus* were placed at the points of the grid covering the study plot, marked with wooden sticks (Fig. 2a). The mussels had been collected from a mussel bed on the right bank of the stream; the bed was on steeply inclined fine-sediment substrate adjacent to the experimental site (right bank of large pool above the study site). Individuals ca 5 years old were chosen (mean shell length 50.9 mm; SD = 2.75) and marked with tags bearing unique letter/number codes glued to their shells (some of them were marked with oil-penned numbers on the dried shell).

The schedule of trials and subsequent surveys within trials is shown in Table 2. The first trial used 196 individuals distributed in pairs at each grid point, and the second trial used 178 individuals (fewer mussels were distributed then because the water level had dropped in the meantime, leaving some of the points out of the water). Then in each of the trials, two detailed surveys of the study plot were made to locate all the tagged individuals visible on the bottom surface, after which their positions were plotted on a map (Fig. 2e, f). Because one individual was found outside the boundaries of the GIS spatial model, it was not used in further analysis of movements (trial 1, survey 2, thus $n=81$). In 2005 and 2006, additional surveys were made in order to locate mussels tagged during both 2004 trials (Fig. 2h).

Radiotelemetry trial To track individual movements precisely, on 14 October 2004, 2 mussels fitted with radio transmitters (LTM, 1.4 g, Titley Electronics Pty Ltd.) were placed at each of 6 points in the middle part of the pool (Fig. 2g). They were distributed on fine gravel at mean depth $D = 0.36 \text{ m}$ (SD = 0.06), with uniform flow velocity (mean $V = 0.06 \text{ ms}^{-1}$, SD = 0.010). At the

2008 experiment

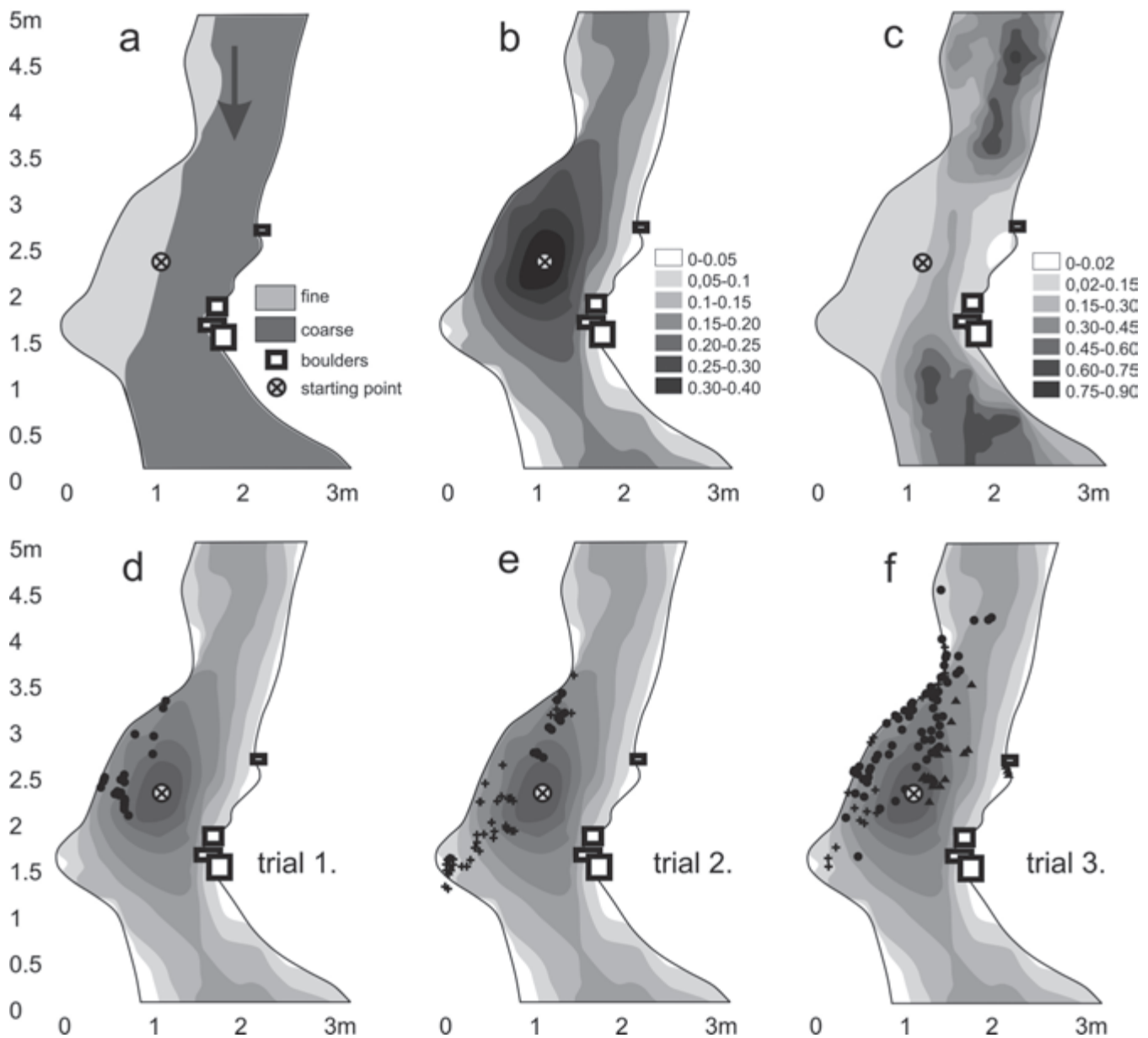


Figure 3 2008 experiment: characteristics of the studied channel reach of Cedron river (2008–09, small study plot), and presentation of tagged mussels' positions, with water depth shown: a – river substrate with sediment type and initial placement of mussels shown (large arrow indicates flow direction); b – depth map (D [m]); c – flow velocity map (V [ms^{-1}]); d – positions of mussels found after the first trial; e – positions of tagged mussels (dots) and wild mussels (crosses) after the second trial; f – positions of tagged mussels (dots), wild mussels (crosses) and mussels found hidden under bottom armoring (triangles) after the third trial.

same time, 10 transmitter-fitted mussels were distributed in the uniform part of the riffle at 10 points in 3 rows across the stream ($D = 0.31$ m, $SD = 0.07$; $V = 0.37$ ms^{-1} , $SD = 0.20$).

The distribution of radio-tracked individuals over the river bottom was determined in 4 sub-

sequent surveys (Table 2). The radio transmitters were tracked with a wand antenna. With proper adjustment of the signal level, the radio transmitter can be located very precisely by touching the transmitter antenna with the wand antenna. There was one transmitter failure in the pool

Table 2 Experimental setup, respective dates of surveys and detectability of experimental *Unio crassus* individuals during subsequent surveys. Parenthesized numbers of individuals in second surveys of each trial represent individuals not found during first surveys but found in second surveys, and the respective percentages refer to the mussels not detected during the first survey but found in the second survey, versus all mussels not detected during the first survey. In the 2008 experiment there are no percentages shown, as the starting number of mussels in each of the trials was 100.

Experiment:		2004 experiment			2008 experiment	
Trial No.	Survey No.	date	Detectability		date	Detectability
			n	%		n=%
1	start	17 Jun. 2004	196	100	7 Jul. 2008	100
	1	8 Jul. 2004	76	39	8 Sep. 2008	24
	2	7 Sep. 2004	82 (37)	42 (31)	—	—
2	start	7 Sep. 2004	178	100	8 Sep. 2008	100
	1	10 Sep. 2004	117	66	15 Jul. 2009	47
	2	6 Oct. 2004	51 (15)	29 (25)	—	—
3	start	—	—	—	15 Jul. 2009	100
	1	—	—	—	3 Sept. 2009	93
Telemetry	start	14 Oct. 2004	12	100	—	—
	1	22 Oct. 2004	10	83	—	—
	2	27 Oct. 2004	10	83	—	—
	3	5 Nov. 2004	10	83	—	—
	4	10 Nov. 2004	10	83	—	—
1–2	1	13 Oct. 2005	9	5	—	—
	1	25 Sept. 2006	2	1	—	—

area, and in each of the next surveys one individual was not located (probably also transmitter failure), reducing the sample sizes.

2008 experiment All mussels regardless of age were collected and removed from the sample plot (Fig. 3a–c) and from the adjacent mussel bed (ca 3 m upstream and downstream). The older ones (mean shell length 53.4 mm, SD = 6.48) were tagged with a unique letter/number code and used in the experiment.

Then in each of 3 trials (Table 2), 100 individuals were placed at one site in the deepest part of the reach (local coordinates X = 2.25, Y = 1.2; Fig. 3a–c) on the boundary between fine and coarse sediment (Fig. 3a) and between still and fast-flowing water (Fig. 3c).

The first trial was done during summer 2008, the second trial was much longer, lasting from late autumn through winter and spring 2009, and the third trial was done during summer 2009 (Table 2). The surveys differed in terms of the search procedure. During the survey of the first trial only individuals visible on the bottom surface were counted, without digging in

the sediment. During the survey of the second trial, the soft sediment was checked carefully with the fingers in order to find individuals not visible on the surface. The coarse sediment was left unchecked, because disturbing the bottom armouring could lead to a change in channel morphology (sediments are not sorted under the armouring and are very prone to erosion). During the final survey of the third trial the fine sediment was checked with the fingers down to the more solid layer of sediment (10–15 cm), and all the cavities under the bank and tree roots were carefully penetrated manually. The bottom armouring was removed from the whole area and the sediment under the armouring was checked carefully, penetrated with the fingers to ca 10 cm. The individuals found each time were identified and their positions were mapped (Fig. 3d–f).

DATA ANALYSIS

In each of the experiments the grid point coordinates and data related to each of them

Table 3 Distances covered by *Unio crassus* individuals during the 2004 experiment in relation to channel character, tested with the Kruskal-Wallis test (H).

Trial	Survey	Part of reach	Mean distance [m]	SD	N	H	P
1	1	riffle	0.48	0.548	36	29.5	<0.0001
		pool	2.11	1.594	40		
	2	riffle	0.96	0.845	30	21.4	<0.0001
		pool	3.29	2.390	52		
2	1	riffle	0.45	0.841	49	12.3	0.0005
		pool	1.07	1.261	68		
	2	riffle	0.53	0.699	29	7.81	0.005
		pool	1.52	1.585	21		

were stored in a database. Then a model of the river channel was generated with a GIS system (Arc-Info, ArcGIS); the model included a flow velocity layer. Next, data on the positions of all the tagged individuals located were imported into the model. The model was constructed to derive and analyze data on the final positions of the individuals (analyzed by X and Y coordinates), distances covered by individuals, water depth (D) and flow velocity (V) at the mussels' initial and subsequent locations. For simplicity, in the analysis of mussel movements (except for distance; Table 3) in the 2004 experiment, only data from the second, final survey were used, as any response of the mussels to local conditions should be more evident after longer periods.

The data from the 2004 experiment were analyzed specifically in relation to the complex morphology of the studied stream reach. First the reach was divided into two parts on the basis of general morphology: riffle and pool. The second analysis was related to more specific morphological features of the channel. The channel area was divided into 5 non-overlapping areas (Fig. 2a): (1) steep right bank of the reach, built mainly of fine sediment (pool) or clay (in the riffle); (2) deep area with flat bottom in the upper part of the reach; (3) very shallow flat area near the left bank of the pool; (4) area of fast-flowing water with flat bottom in the lower part of the reach; and (5) area of dead water in the lee of boulders in the middle part of the reach. The mussel movement distances were analyzed in relation to features of the departure area.

Differences in the layout of the 2008 experiment complicated some of the analyses. During the 2008 experiment, part of the studied channel section was not parallel to the Y axis but was

more diagonal. Such a channel orientation could affect comparisons of cross-channel movements of mussels if they were done in the original coordinate system, so they were compared at X coordinates standardized along the Y axis. In the standardization procedure it was assumed that in each cross-section, $X = 0$ was located at the channel bank regardless of the original coordinates, simulating a channel course parallel to the Y axis. For example, the midpoint of the place where mussels were placed at the start of the experiment was located at original coordinate $X_0 = 1.2$ m, whereas in the standardized coordinates at $X_{0s} = 0.7$ m. In order to analyze their movements in the same morphological setting, the analysis was restricted to the middle, uniform part of the pool (from $Y = 1$ to $Y = 3.5$ m; Fig. 3), which reduced the sample size of the third trial (5 individuals were found upstream, behind the pool; Fig. 3f, $n=93-5=88$).

During the 2008 experiment the study plot was very small, thus the mussels were potentially able to reach any place within the studied pool. In order to exclude the possibility that their movements were caused by escape behaviour (escaping from shallows, which might be suspected after the 2004 experiment), they were placed in the deepest part of the study site, but this procedure made their initial sites very uniform in terms of environmental conditions. To avoid this oversimplification of the experimental setup, during the analysis of habitat features the characteristics of the mussels' positions detected during the surveys were compared with those of all sites that the mussels potentially could occupy. In the case of water depth, the features of the final positions were also compared to the initial conditions, using comparison to a

user-specified constant (conditions in the initial place). In the case of depth, even a random distribution of mussels within the study plot would result in significant differences depending only on the site of reference: a comparison to potential sites (shallow, on average) could result in a preference for deeper areas, whereas a comparison only to the initial place (the deepest one) would show a preference for shallowness.

The final survey in the 2008 experiment, with the highest detectability of mussels, including those hidden in interstitial parts of the bottom, allowed us to compare mussel frequencies in different kinds of sediments. Assuming that the mussels could reach any part of the pool (areas above and below the pool excluded) and assuming their random redistribution, the expected

frequencies of individuals found on different substrates should be proportional to the area covered by the different substrate types. The mussel frequencies recorded from different types of bottom were thus compared with the frequencies expected from a random distribution of individuals within the pool. The results of previous trials and of the 2004 experiment did not offer such a possibility, as the gravel bottom was not removed, so their frequencies could be biased.

The distribution of distance data differed very significantly from a normal distribution, with low values dominating, so all the distance-related data were analyzed with non-parametric tests: the Kruskal-Wallis test and the Wilcoxon matched pairs test.

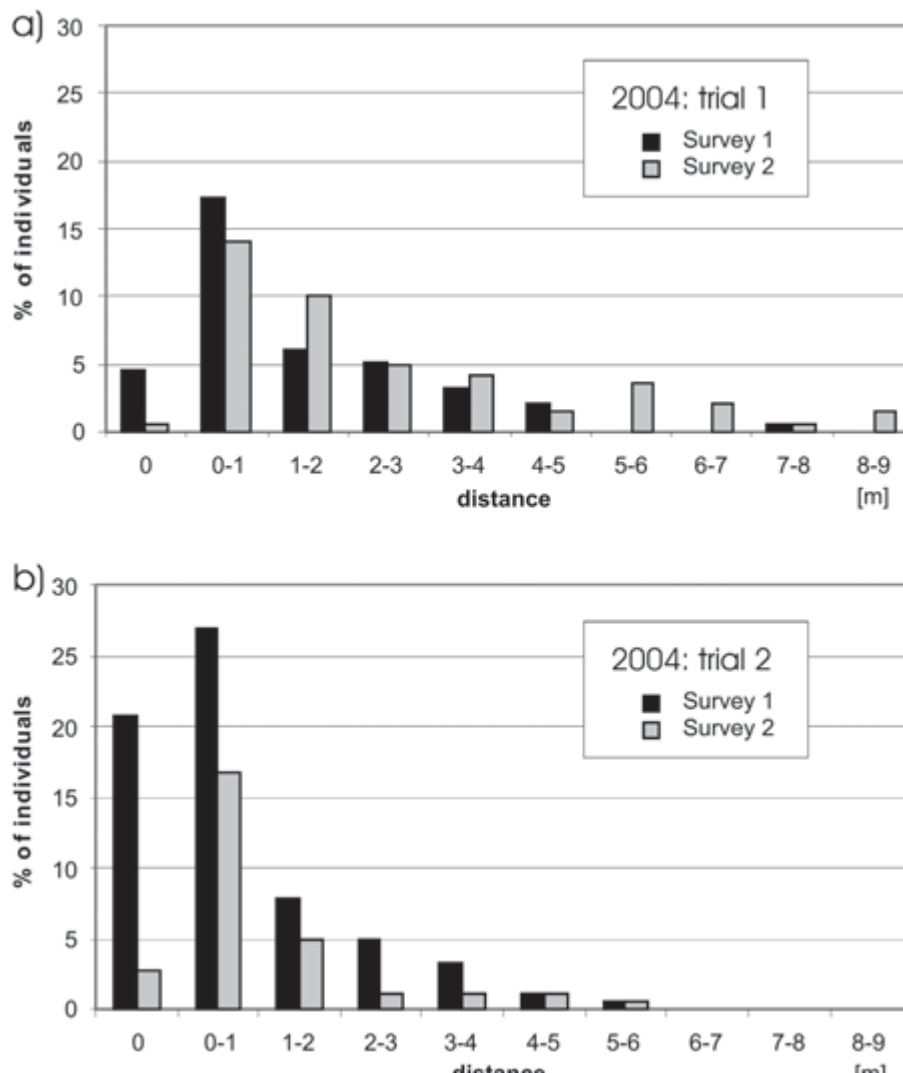


Figure 4 Distribution of distances covered by tagged mussels in successive surveys of both trials of the 2004 experiment.

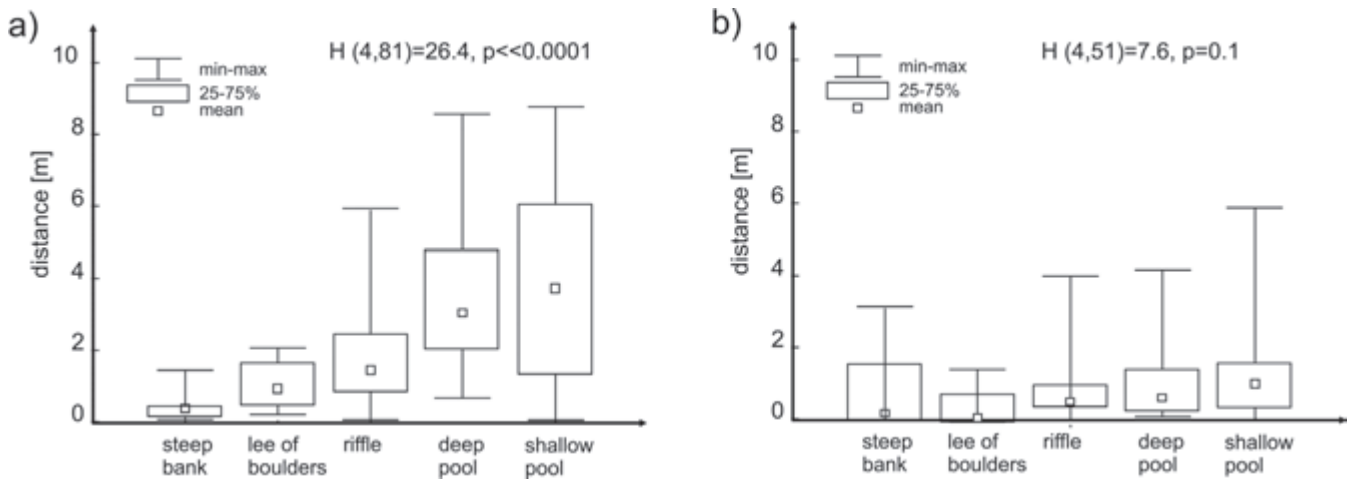


Figure 5 Distances covered by individuals during the 2004 experiment, versus channel area type for the mussel departure site in the first (a) and second (b) trials.

RESULTS

Detectability During the 2004 experiment, in the first trial the detectability of tagged individuals was about 40%; in the second trial it was more variable (Table 2). It is worth noting that a significant proportion ($\frac{1}{4}$ – $\frac{1}{3}$) of the individuals not found during the first survey was found during the second survey, making combined detectability for both surveys much higher than in individual surveys. During the survey in 2005, only 10 tagged individuals were found (9 from the second, 1 from first trial 2004), and in 2006 only two individuals were found, with obliterated codes.

During the 2008 experiment, done in the small study area, detectability during the surveys depended largely on the method applied (see methods; Table 2). Importantly, the area of fine sediment was colonized by a large number of new wild individuals (58 during the second trial, between 2008 and 2009, and 20 during the third trial; Fig. 3e, f).

Distance During the 2004 experiment the distances covered by the tagged mussels differed greatly between individuals, with many individuals not moving very far (<1 m) and a few individuals moving over 5 m (Fig. 4). Movement distance depended on time: much longer distances were covered during the first trial. The first (summer) trial lasted over two months (Table 2) and the individuals moving over 5 m were recorded almost entirely during the

second survey (Fig. 4a). In the second (autumn) trial the distances were much shorter (Fig. 4b), but the trial was much shorter (one month; Table 2) and done later in the year, in colder water.

Versus the last positions recorded in 2004, the mussels found during the next year's survey in 2005, covered distances ranging from 0.12 to 13.35 m (mean 5.45 m, SD = 4.842; Fig. 2h). The longest distance, recorded in 2005, was covered by the individual coded A18, which moved from the dead water area in the lee of boulders to the fine sediment bank of the pool, against the current (13.35 m; Fig. 2h).

During both trials, individuals distributed in fast-flowing parts of the channel (riffle) moved short distances (0.5–1 m; Table 3), whereas those distributed in slow deep parts (pools) covered distances 2–4 times farther.

The distances covered differed significantly in relation to departure area during the first trial; during the second trial there were no significant differences found (Fig. 5a, b). The longest distances were covered by individuals distributed in shallows and the deepest parts of the pool, whereas individuals placed on the steep fine-sediment bank of the pool were almost stationary.

The 2008 experiment was done in much smaller plots and the mean distance covered was short (1.1 m; SD = 0.39); the maximum distance recorded was 5.15 m from the initial place (3rd survey), and the mussels moved mainly towards the right, fine-sediment bank (Fig. 3d–f).

Table 4 Comparison of habitat features (means \pm SD) for sites finally occupied by mussels in relation to habitat features: (1) at the beginning of the experiment (2004 experiment, trial 1: N of found tagged individuals = 81, trial 2: N_{ind.} = 51, data analyzed with Wilcoxon matched pairs test) and (2) at sites potentially available for mussels (2008 experiment, trial 1: N_{ind.} = 24, trial 2: N_{ind.} = 47, trial 3: N_{ind.} = 88, data analyzed with Kruskal-Wallis test).

Channel features	Trial	2004 experiment			2008 experiment		
		Start	Final	Z	Potent.	Final	H
X axis	1	2.7 \pm 1.67	1.46 \pm 0.63	5.98***	0.63 \pm 0.106	0.23 \pm 0.145	20.2***
	2	2.3 \pm 1.63	1.94 \pm 1.17	2.57**		0.34 \pm 0.183	18.0***
	3	—	—	—		0.49 \pm 0.393	7.06***
Y axis	1	12.1 \pm 2.89	12.9 \pm 4.26	2.62**	2.25 \pm 0.540	2.43 \pm 0.330	0.93 ns
	2	10.7 \pm 3.59	10.6 \pm 3.72	0.04 ns		2.36 \pm 0.526	1.20 ns
	3	—	—	—		2.91 \pm 0.520	16.1***
Depth	1	0.16 \pm 0.121	0.25 \pm 0.082	5.68***	0.16 \pm 0.092	0.24 \pm 0.054	17.7***
	2	0.18 \pm 0.122	0.23 \pm 0.105	2.91**		0.25 \pm 0.071	31.8***
	3	—	—	—		0.23 \pm 0.090	30.2***
Flow	1	0.19 \pm 0.162	0.23 \pm 0.139	2.40*	0.29 \pm 0.237	0.00 \pm 0.005	17.6***
	2	0.18 \pm 0.150	0.23 \pm 0.151	2.67**		0.01 \pm 0.031	43.3***
	3	—	—	—		0.12 \pm 0.128	24.5***

ns – non-significant, * $-0.01 < p < 0.05$, ** $-0.001 < p < 0.01$, *** $-p < 0.001$.

Movement patterns Detailed analysis of the data on all individuals found in both trials of the 2004 experiment showed significant patterns of change in position between their initial and final locations. They moved perpendicular to the flow (towards lower values along the X axis, i.e., the right bank of the channel; Table 4). The magnitude of change between the first and last positions during the two trials depended very significantly on the initial crosswise location of the mussels: the more they were located toward the left bank (shallows), the larger their change of position (Spearman rank correlation: first trial: $r_s = -0.94$, $n = 81$, $p < 0.0001$; second trial: $r_s = -0.69$, $n = 51$, $p < 0.0001$).

The differences between the initial Y coordinate position and the position in the final survey in 2004 were much smaller. Although many mussels moved long distances against the current (Fig. 2e–h), the mean position in the first trial shifted only slightly along the Y axis (Table 4), whereas in the second trial there were no significant differences. Other measurements (Table 4) indicated that the mussels changed position towards deeper water and higher flow velocity than at the initial locations.

As a rule, the radio-tracked individuals distributed initially in the deepest part of the pool moved towards the steep right bank built of fine

sediment. In the last check they were found at the base of this bank (Figs 2g, 6; $n = 10$ (12 individuals in 6 places, minus 2 not found)). There were no significant differences in water depth during this movement (Wilcoxon matched pairs test: $Z = 0.52$, $n = 10$, $p = 0.55$). The telemetry survey in the pool area showed that the differences between flow velocity at the last survey (V_4) and at the initial distribution points (V_0) were at the margin of statistical significance (Wilcoxon matched pairs test: $V_0 = 0.061 \text{ ms}^{-1}$ (SD = 0.012) vs $V_4 = 0.048 \text{ ms}^{-1}$ (SD = 0.007): $Z = 1.94$, $n = 9$, $p < 0.052$). The same analysis for individuals distributed in the riffle part did not reveal any significant differences between the initial and final distributions ($n = 10$).

During the survey in 2005 (Fig. 2h), almost all of the tagged mussels ($n = 10$) were found on the steep fine-sediment right bank of the studied channel reach: 6 in the pool and 2 in the riffle. Another 2 were found in the area of dead water in the lee of boulders (1 individual tagged during the first trial had an obliterated code, so there are no data on its initial position). They generally moved across the river (along the X axis) towards the right bank from their previous positions in the second trial (Wilcoxon matched pairs test: initial X position in 2004 (X_0) vs X position found in 2005 (X_{2005}): $Z = 2.54$, $n = 9$, $p = 0.011$). In the

autumn of 2006 (Table 2) only 2 tagged individuals were found, with obliterated codes, in the steep fine-sediment bank of the pool.

The 2008 experiment confirmed that tagged mussels were found more frequently in places with lower values along the X (cross-channel) axis, that is, closer to the right bank than expected from a random distribution among the potential sites (Table 4). After the first and second trials all the found individuals were within the still-water pool (Fig. 3d, e), and only after the third trial were some individuals found in the riffle part of the channel, hidden beneath the bottom armouring or in the lee of boulders (Fig. 3f).

The mussels did not move very far along the Y axis. After the first and second trials their mean distance did not differ significantly from the mean distance to all potential sites (Table 4). Only the results of the final survey of the third trial showed mussels moving significant distances upstream along the Y axis as compared to the mean distance to potential sites (Table 4, Fig. 3f).

During all trials, the mussels were generally found at depths greater than the average of the whole area (Table 4). This would imply that the mussels preferred deeper places within the study plot. However, when depth at the mussels' final positions (0.23–0.25 m; Table 4) was compared with water depth at their initial position (user-specified constant: $D_0 = 0.35$ m) then the result was the opposite: depth at the mussels' final positions was less than the water depth of their initial position; almost all of them climbed upward from the deepest part of the pool. The difference was highly significant in the surveys after the first trial (t-test for single mean: $t = 10.6$, $n = 24$, $p < 0.0001$), second trial (t-test: $t = 10.1$, $n = 47$, $p < 0.0001$) and third trial (t-test: $t = 12.6$, $n = 88$, $p < 0.0001$).

Water velocity at the places when the mussels were found during all three trials also differed from average conditions (Table 4). Most of the mussels were found in the pool, with still water (Fig. 3d–f).

Sediment structure In the 2008 experiment, 69 of the 93 individuals found in the final survey of the third trial were distributed on fine sediment, 21 were found in gravel substrate, and 3 were in between (excluded from the following analyses). When this distribution was compared with the frequencies expected from a random distribution

(i.e., proportional to the area of a given substrate) it gave significant results both for the frequencies expected only within the pool (expected frequency 35 for sand and 55 for gravel; Fisher exact test – $p < 0.0001$) and for the frequencies expected for the whole study plot (expected frequency 24 for sand and 66 for gravel; Fisher exact test – $p < 0.0001$). All except one of the untagged wild individuals that appeared on the study plot in the second trial were also found in fine sediment, and during the third trial all the wild individuals were found in fine sediment.

DISCUSSION

Detectability One of the most important methodological aspects of this type of study is mussel detectability. In the 2004 experiment a considerable proportion of *Unio crassus* individuals not found during earlier surveys were later found during the next survey (30–40%) or some in the next year, indicating that they had been buried or hidden in cavities and thus overlooked. This scenario was confirmed by the final check of the 2008 experiment, where ca $\frac{1}{4}$ of the individuals were visible on the bottom surface (first survey), which means that $\frac{3}{4}$ were hidden. Applying the usual methods to search for mussels can greatly

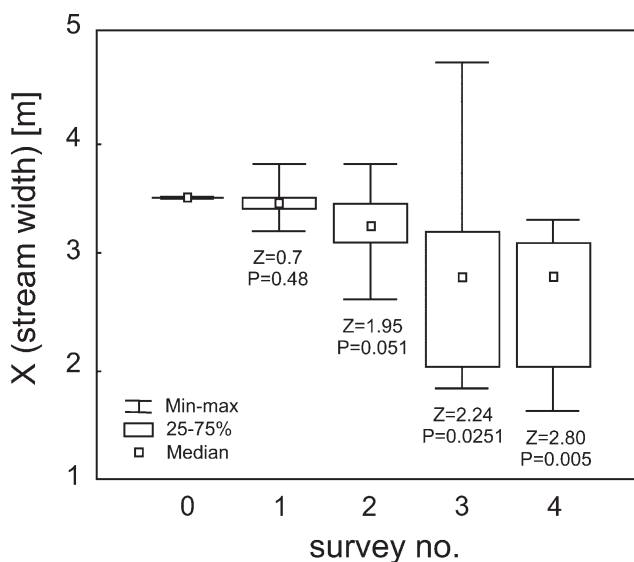


Figure 6 Changes of coordinate along the X (cross-wise) axis resulting from mussel movements in consecutive surveys (nos. 1–4) of radio-tracked individuals (2004), tested with the Wilcoxon matched pairs test in relation to their initial positions (0).

increase these proportions, but the ones hardest to detect are those buried completely under the armouring gravel – in this case $\frac{1}{4}$ of the mussels (in other unionids inhabiting lotic habitats: 47.5% in Balfour & Smock, 1995; about $\frac{3}{4}$ of individuals buried entirely in Schwalb & Pusch, 2007).

The large numbers of individuals differing in identity between the first and second surveys do not negate the generally observed patterns; rather, they imply that both the hidden and visible portions of the experimental mussels finally behaved in the same way. Moreover, the fact that some studied mussels became visible after some time proves their vertical and/or horizontal movement. A total count, including those hidden in the bottom material, cannot be done during the course of this type of experiment, as it requires removal of bottom armouring. That would drastically alter the habitat structure of the experimental plot, affecting not only the mussels' movements but also the stability of the habitat: the sediment under the armouring is unsorted and thus very prone to rapid erosion. Moreover, the imbricated structure of bottom armouring gravel can be rebuilt only during spates. The only way to deal with those problems is to use radiotelemetry or transponders to detect the position of hidden individuals without disturbing experimental plot conditions.

Distances and movement patterns The distances traveled by the displaced *Unio crassus* individuals differed greatly between the riffle and pool in both experiments. The long-distance movements recorded in the pool reflected the long distances between the departure and destination points. The basic mechanism seems to be escape from shallows, which were the starting point for most of the individuals covering long distances (Fig. 2e, f). Many mussels escaping from the shallows were likely to enter the deepest part of the pool, which was also a place to avoid (as shown in the telemetry experiment), finally lengthening the distance to cover. From Fig. 2e it is clear that mussels escaping from shallows headed in two directions: upstream or downstream, probably randomly adopting a given direction. Those moving upstream were going through sediments that were finer, less sorted, and less armoured. Their precise routes remain unknown: they could go directly through the deep area towards the other bank, or else around the deepest area,



Figure 7 The shallow area with stranded mussels, subject to desiccation during summer periods of low water in the Jasiołka river (2008). In the magnified part of the picture, a doomed mussel (indicated by white arrow) made circular, random paths, trying to find a way out.

following some isohypse. Both possibilities can be seen in Fig. 2e, f. Those that turned from the shallows towards the riffle, probably following an isohypse at least initially (in Fig. 2f, see their routes towards the riffle in the shorter, second trial of the 2004 experiment) and did not move far through the riffle (Fig. 2e, f), and most of them harboured at once in dead water in the lee of boulders.

Avoidance of shallow areas, which are subject to desiccation during summer periods of low water, is a common behaviour for mussels (Björk, 1962). In such parts of a stream, a slight lowering of the water level can drastically reduce the water surface area. The water-covered area can shrink at a rate much faster than the speed at which the mussels can move to escape, leaving them stranded and doomed in such flat, shallow bottom areas (Fig. 7). Such a process would be facilitated by the high sediment deposition

rate (notice the buried grid-marking sticks in Fig. 2d).

Unio crassus individuals experimentally distributed in the riffle area during the 2004 experiment did not travel far; this is especially evident in the telemetry data (Fig. 2 g). The mussel concentration observed near the boulder debris was composed of individuals from the shallow parts of the pool (Fig. 2e, f); all of this can be interpreted as avoidance of the riffle. Also in the 2008 experiment, redistributing mussels did not move far into the riffle from their initial place (Fig. 3f), but did move far into the fine sediment pool. There may be a number of factors disadvantageous for *Unio crassus* individuals in a riffle: higher flow velocity and the serious risk of being dislodged (in Fig. 2d, notice the removal by natural factors of almost all grid-marking sticks from the riffle and from parts of the pool with faster water); larger gravel particle size impeding movement; and finally the more imbricated structure of a gravel floor composed of overlapping flat stones of Carpathian flysch. It is also characteristic that almost all the mussels found to have burrowed under the gravel armour of the bottom were found in an area of fast-flowing water (2008 experiment). Such behaviour may suggest that the riffle structure promoted burrowing.

It seems that most of the experimentally displaced mussels changed location according to the cross-sectional variability of conditions (right bank with steep slope built of fine sediment; left bank with graduating gravel bar). There was not much difference in the position of mussels along the Y axis of the study plot (upstream or downstream), but there were very significant changes in position perpendicular to flow direction. Analysis of the individual mussels' routes also suggests that they are inclined to climb to higher areas: individuals distributed experimentally in the deepest parts of the pool (2008 experiment) were later found high on the slope of the right bank. It is surprising that in both experiments, regardless of the wide range of accessible depths, *Unio crassus* individuals were later detected within the same very narrow water depth range: 23–25 cm deep (Table 4). Mussel movements were of course prompted by the need to escape shallows, as discussed above, but the telemetry experiment, and more evidently the 2008 experiment, demonstrate that the deepest areas were avoided. This accords with

observations by Vicentini (2005), who in the same species suggested a reproductive function for climbing: individuals creep to the water line on the bank in order to spurt glochidia-containing jets of water widely, increasing the probability of infecting a host fish.

Another habitat feature apparently important to *Unio crassus* is water velocity. The preference for lower water velocity was especially evident in the 2008 experiment. All experiments also demonstrated a preference for fine sediment. Here it is impossible to decide which of them is more decisive, as in natural conditions fine sediment is inextricably related to low water velocity.

The places in pool areas with fine sediment and still water are less threatened by channel reformation or large-scale movements in the substrate (Fig. 2d). As mentioned above, the grid-marking sticks were saved only in the pool, in areas of slow water flow. The 2005 and 2006 surveys showed that the survivors in the area were found at the fine-sediment slope of the pool. Within the study area the riffle from 2004–2005 reformed substantially during the spring of 2006 and 2007 – a new small pool was formed within the previous riffle. The steep fine-sediment bank of the large pool studied in 2004 (Fig. 2a–d) did not change at all from 2004 to 2010; it looks exactly the same even after catastrophic floods of 2010, which again completely destroyed the small pool used in the 2008 experiment and the adjacent riffle area, forming a completely new channel.

The movements of the studied *Unio crassus* individuals were not merely the result of experimental stimulation. Individuals that should have reached their finally optimal locations in the course of the 2004 experiments were found in different places in the autumn of 2005, the mean distance from their previous locations being about 5 m. All these movements were made long after the experimental disturbance and thus should be seen as spontaneous. The movements were usually directed towards the steep fine-sediment bank, like those during the experiments. From this it follows that in natural conditions these mussels move throughout the channel in a continuous search for better sites, or that they react in this way to natural stimuli. This supposition is confirmed by the numerous presence of wild untagged individuals in the fine-sediment bank (emptied when the experiment was being prepared), recorded during the second and third

trials in the 2008 experiment. Some of them may have been overlooked during preparation of the experiment, being hidden under the armouring, but if so they should have appeared during the first trial, which lasted over two months – in the earlier 2004 experiment that was enough time for some mussels to reappear. The wild individuals appeared during the second very long trial and in very high numbers; it is quite unlikely that all of them were overlooked during plot preparation and during the final survey of the first trial. It is more likely that at least a significant part of them came from outside. It should also be stressed that the pool was newly formed in 2007, meaning that there were no mussels there before, so they had to immigrate in any case. The wild individuals found within the new pool were too old to have dropped off of fish, so the only plausible explanation is spontaneous immigration.

General implications The adaptiveness of the movements of mussels living in running waters has been questioned. Balfour and Smock (1995) recorded significant movement distances for *Elliptio complanata* in Buzzards Branch River (mean 2.9 m over the course of a year, with single mussels covering over 10 m) but reported these movements as erratic, with mussel paths often crossing back upon themselves. In a situation like that presented in Fig. 7, that is a typical movement pattern for mussels in uniform and unfavourable habitat conditions, where they cannot find clear environmental gradients to be guided by. Studying other species of *Unionidae* in a lowland river, Schwab & Pusch (2007) recorded significant net displacement towards the river bank, although the distances were minimal in their work (17 cm). They concluded that mussel movement behaviours are an adaptation to river flow and food conditions.

The results reported here imply that the marginal dead water at the steep fine-sediment bank and the dead water in the lee of boulder debris meet the microhabitat requirements of *Unio crassus*. Following upon similar studies in fish (Railsback *et al.*, 1999), some basic movement rules can be defined for *Unio crassus*: (1) departure: shallow flat area, deep pool, (2) destination: dead water high on steep fine-sediment bank, or dead water in shelters near boulders. Even these simple rules can lead *Unio crassus* individuals to aggregate in certain places. The Cedron river is

one of the best habitats in Poland for this species, but individuals staying in riffle areas during 2006–2010 would certainly have died. Those that stayed in or entered the fine sediment bank of the pool survived longer than others, and young *Unio crassus* were also present there, indicating the success of both recruitment and survival in that place. This conclusion finds support in river surveys: *Unio crassus* occupies sites of the same character as recorded in this study in natural rivers, both in the Carpathians (KZ, unpublished data) and in Western Europe (Engel & Wächtler, 1989). Usually they are found more or less aggregated in areas of dead water near river banks, less frequently in aggregations of fine sediments in the lee of boulders or other kinds of debris. Large and dense mussel shoals sometimes form on firm clay layers exposed by lateral erosion of a river.

It has already been suggested that environmental factors may be responsible for the patchy distribution of some mussel species (Balfour & Smock, 1995). Many factors responsible for mussel grouping in lotic environments can act in concert, reinforcing each other. The presence of mussel beds can be explained without reference to active movements, by differential mortality of juveniles settling evenly on the river bottom but perishing in unsuitable habitats (Strayer, 1999), and that idea was supported in a model by Morales *et al.* (2006). On the other hand, grouping in safe shelters automatically brings additional advantages for adults, promoting active aggregating. In *Unio crassus*, for example, active grouping may decrease the probability of fertilization failure: stream margins are occupied by young fish, a necessary host for glochidium development; areas of dead water minimize the probability of being dislodged; and the steep slopes of a pool minimize the risk of stranding.

In natural conditions the steep bank of pool is a discontinuous element in the river continuum. Such a habitat has a certain probability of occurring. It can be created with a certain probability and later destroyed with a certain probability, by lateral erosion and sediment transport in the channel. Such a distribution of individuals in unstable habitat patches implies that *Unio crassus* populations in natural rivers may function as metapopulation systems (Hanski & Simberloff, 1997). In such a system their persistence depends on the probability of colonizing a new patch

before the occupied one is destroyed. This probability can explain the enormous number of progeny produced by single individuals, as well as the employment of fish as a vector for parasitic glochidia. A similar mechanism has been suggested for swan mussels *Anodonta cygnea* inhabiting oxbows in natural lowland rivers (Zajac, 2002). The accessibility and persistence of patches suitable for *Unio crassus* may be significantly limited by changes in the hydrological regime of a river, especially those due to the effects of river regulation on the dynamics of bank erosion (Petts & Gurnell, 2005). Channel alterations – removal of woody vegetation, channel widening, installation of fascine mattresses, lining with concrete – can quickly eliminate mussel microhabitats such as the ones the mussels in this study apparently chose as optimal. Cross-river construction can present barriers to mussel movements, as they do to fish migration.

In some parts of the range of *Unio crassus*, channel dredging or gravel excavation from the river bed can create a serious problem. Dredging is regarded as very dangerous for lowland species of mussels having less dispersal ability (less than 15 cm after 55 days; Aldridge, 2000). The natural preference of *Unio crassus* for marginal parts of a channel, demonstrated in this study, supports the suggestion (Aldridge, 2000) that dredging should be restricted to the centre of the channel. However, any removal of sediment from the channel also alters sediment transport in other parts of it, even marginal ones. Then the mussels' locomotor ability to correct their positions within the channel becomes very important for their survival.

Information on the rules of mussel movement and their detectability can help in the practice of mussel conservation (Schwalb & Pusch, 2007), especially for projects to relocate threatened mussels. Cope & Waller (1995) reviewed 33 relocation projects and noted that success was generally rather low (50%). Knowledge of mussel movements and microhabitat preferences can be put to use in choosing a suitable relocation site (Hamilton, Box & Dorazio, 1997), as well as in verifying the success of relocation. Those who design and implement monitoring procedures should consider detectability differences related to bottom substrate and the consequences of mussel movements, as they can influence the results (Pfeiffer & Nagel, 2010).

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