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Microhabitat breadth in salmon parr family groups

Autumn microhabitat breadth differs between family groups of Atlantic salmon parr (Salmo salar) in a small chalk stream

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Abstract - The effect of family traits on the microhabitat use by six genetically-distinct groups (three in each year of study) of juvenile Atlantic salmon tagged with passive integrated transponder (PIT) tags was studied via PIT-tag detectors installed on the river bed in a small chalk stream of southern England during Autumn in 2006 and 2007. Canonical correspondence analysis of the molecular and microhabitat data revealed considerable overlap in the microhabitat use of the family groups and notable differences in microhabitat breadth, which was partly influenced by sample size. The data suggest that microhabitat breadth and preferences of wild salmon are influenced by family of origin.

Key words: genotype, adaptive traits, microhabitat use, kinship, family traits.
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Introduction

Atlantic salmon *Salmo salar* (L.) have distinct habitat requirements in the wild (Klemetsen et al. 2003). Juvenile Atlantic salmon become highly territorial during the parr stage and growth rates and size distributions are strongly influenced by habitat availability (Armstrong 2010). Modelling studies have demonstrated that parr establish a territory that provides the best balance between energy gain and energy crucial for metabolism, growth and reproduction (Fausch 1984) with a preference shown towards large cobbled areas (Beland et al. 2004). In-stream macrophytes, ligneous debris and marginal bank-side vegetation are also important refuges from predators especially for juveniles (Armstrong et al. 2003; Riley et al. 2006). However, only a small proportion (2–20%) of stream bed microhabitats has suitable characteristics for salmonid territories (Allen 1969), and the loss of suitable habitat is considered a major reason for the decline in stocks of wild Atlantic salmon (Hendry et al. 2003).

Adaptive traits (genotype) play an important role in habitat use, and interactions between genotype and environment lead to localised adaptations (Kaweki & Ebert 2004). Exploitation of optimal microhabitats, i.e. those to which the individual is best suited, will help optimize fitness gains (Kawecki & Ebert 2004), suggesting that microhabitat choices are genetically pre-disposed. It is therefore possible, though as yet unproven, that fish from different parentage will specialise through different microhabitat usage (McLaughlin 1999), and thus family groups are expected to exhibit similar microhabitat preferences.

The aim of the present study was to test the hypothesis that different family groups will differ in the breadth and character of their microhabitat use, each family group exhibiting a distinct microhabitat profile (i.e. preferences/avoidances with respect to environmental variables).

Materials and Methods
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Experimental Set Up

Juvenile salmon were reared from known parents (eggs of one female fertilised with the milt of one male) caught in the River Frome just prior to spawning, anaesthetised (2-phenoxyethanol) and stripped as per Edwards (1978), to create six distinct full-sibling family groups in each of two years (hence, 12 family groups). Adipose tissue samples from the parents were stored in 100% ethanol at 4°C for genetic analysis. Each batch of fertilised eggs (family groups) was placed into stream-side incubators (as per Government of Canada, 1980), one incubator per family group, situated at Watergates Hatchery (Dorchester, Dorset, U.K.) at the source of the Tadnoll Brook, a tributary of the River Frome.

While the salmon emerged from the gravel as fry, baseline weight and fork length (FL) measurements were recorded. Of the 12 distinct family groups created over two years (six family groups in 2006 and six family groups in 2007), three family groups of juvenile Atlantic salmon of similar length (24.8–27.2 mm FL), weight (0.126–0.177 g) and emergence time were chosen to be used in each year, thus providing fish of comparable size and life histories. Within 24 hours of emergence from each incubator, juveniles were stocked in a 1.5 km stretch of the River Cerne during 3 to 9 April 2006 and from 21 March to 9 April 2007. This stream section has not been subjected to management measures, thus providing fish with an undisturbed stream stretch with natural bank-side and in-stream vegetation. Other fishes and lampreys present in the experimental stretch of river included trout Salmo trutta (L.), European grayling Thymallus thymallus (L.), northern pike Esox lucius (L.), European minnow Phoxinus phoxinus (L.), European bullhead Cottus gobio (L.), stone loach Barbatula barbatula (L.), European eel Anguilla Anguilla (L.), brook lamprey Lampetra planeri (B.) and river lamprey Lampetra fluviatilis (L.). Although the River Cerne is suitable for juvenile salmon, barriers to migration prevent adult salmon ascending the river so there was no resident salmon parr population at the time of the experiments.

In April 2006, six sites on the river were designated as full-sibling sites and fry from each full-sibling group were released into two sites. An additional six sites were designated as mixed-sibling sites. The full- and mixed-sibling sites were alternated along the river to prevent stream altitude from influencing the results (Fig. 1A). Stream sites were 30 m in length, on average 4 m wide and were separated from one other by 100 m, a distance based on models of existing data (Crisp, 1995)
which show that dispersal distance of most newly hatched salmon is < 20 m downstream. In 2007, to further ensure the genetic integrity of stocked areas, all full-sibling sites were situated upstream from mixed-siblings sites (Fig. 1B) and the distance between stocked sites was increased to 250 m. To utilise the river to its full capacity in the second year of the experiment, the length of full-sibling and mixed-sibling sites was increased to 50 m and 150 m respectively. Initial stocking density in 2006 and 2007 was approximately 2.7 and 4.1 fish m$^{-2}$ respectively. (see Fernandes et al. In press for further details)

After stocking, the fish were given time to establish territories and grow before the sites were sampled by electric fishing, at which time all juvenile salmon caught (parr stage) were tagged with passive integrated transponder (PIT) tags as per Riley et al. (2003) to enable repeated individual identification and the recording of small and large scale movements. Tagging took place during 8–22 August 2006 and 26 July 2007 – 08 August 2007. At the time of tagging in 2006, mean fish FL was 9.45 cm ± (SE) 0.65 cm and mean wet weight 10.48 g ± 0.23 g. In 2007, mean FL was 8.61 cm ± 0.06 cm and mean wet weight 8.07 g ± 0.17 g. The tagged fish (n = 243) differed significantly in FL (Students’ t-test: $t_{205} = 9.77, P < 0.001$) and wet weight ($t_{205} = 8.63, P < 0.001$) between years, however habitat use analysis did not overlap between years. A tissue sample (adipose fin clip) was also taken at the time of tagging (and stored in 100% ethanol) for subsequent genetic analyses to enable family group assignment of juveniles to their parents. Fish were released back to their location of capture following recovery from anaesthesia.

To determine the microhabitat use of each family group, two portable PIT multi-point decoder (MPD) units (Riley et al. 2003) were installed at one single-family and one mixed-family stocked area simultaneously. This was called a ‘replicate’. Each MPD unit consisted of 16 flat, plate-shaped antenna discs and PIT tags were detectable when ≤9 cm above each antenna (Riley et al. 2003). Each antenna takes a reading at ≈3.2 s intervals and each reading may be considered as a ‘point sample’ (i.e. the fixed occurrence of an individual fish in time and space). As such, the antenna location constitutes a sampling point (c.f. Riley et al. 2003), i.e. a ‘point sample’ as defined in point abundance sampling for fish microhabitat studies (for a review, see Copp 2010).
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In each replicate configuration, the first antenna of each line was placed as close to the river bank as possible (in this case, the left bank) so that bank cover (if present) could also be included as a variable in the data analysis (Fig. 1C). Each antenna was covered with the substrata from the immediate surroundings. The antennae were not visible above the water. Gap size between each antenna within a line (60 cm in 2006 and 30 cm in 2007) was determined from the width of the river sections where the MPD units were installed. In both years, the gap between each line of antennae was 5 m so that as much as possible of each river section was covered within the constraints of the antenna cable lengths (< 10 m). The MPD antenna configuration covered a total area of 6600 m² in 2006 and 4500 m² in 2007.

The MPD units were moved and installed in the designated sites to generate data for each replicate. In 2006, one entire single-family site and one entire mixed-family site formed the basis of one replicate. In 2007, owing to larger stocked sites, five areas within the single-family sites, and five areas within the mixed-family sites formed the basis of five replicates. Therefore, the MPDs generated data for six replicates carried out over the two-year period. Data collected on the day of installation, and on removal of MPD antennae, were discarded in order to avoid bias due to fish movements in response to disturbance. MPD-generated data was included for four entire days in each of six replicates: i) 5–8 September 2006; ii) 17–20 August 2007; iii) 25–28 August 2007; iv) 4–7 September 2007; v) 12–15 September 2007; vi) 17–20 September 2007.

Detections of individual PIT tagged fish, including re-occurrences, over the 32 MPD disc antennae (i.e. at the same microhabitat) at the two sites over four-day periods were collated into a fish data matrix (192 samples [i.e. antenna records] as rows × 57 tagged fish as columns), which included those antenna (samples) at which no fish detections were recorded. Owing to the large number of detections (of a new fish, or of a fish moving between antennae), the MPD output data were log₁₀ transformed to produce a more even distribution for ease of interpretation.
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Microhabitat Data Analysis

At each MPD disc during installation, available microhabitat within a 10 cm radius from the edge of the disc was measured either quantitatively (depth in cm, water velocity in m s\(^{-1}\)) or as a proportion (%) of the area: weed cover, bank cover, and three substratum types (as per Copp et al. 1994), i.e. gravel (0.2–5.0 cm), sand (< 0.06–0.2 cm), and silt (< 0.06 cm) (Table 1). Substrata > 5.0 cm were seldom found where antenna were placed and therefore were not included in our analysis. Owing to the limited discharge variability of chalk streams, available microhabitat was assumed to remain constant during each four-day sampling period (e.g. Copp et al. 2005). Comparisons between single-family and mixed-family sites were undertaken using one-way analysis of variance (ANOVA) for water depth and velocity and the Mann-Whitney U-test for the other variables.

| TABLE 1 |

In preparation for multivariate analysis, the microhabitat data were collated into a data matrix (192 point samples \(\times\) 7 microhabitat variables) and then converted to semi-quantitative categories, based on the frequency distributions of the variables (as per Copp et al. 1994). Patterns in the microhabitat use of family groups were examined using canonical correspondence analysis (CCA; ter Braak 1986). In CCA, the microhabitat variables were combined into the artificial gradients that best distinguish and separate ‘microhabitat breadth’, which is only one component of ‘niche’ (see Copp 2008). The best synthetic gradients, i.e. those that maximise separation of the tagged fish, were selected from the graphical representation of the corresponding eigen values (Persat & Chessel 1989; Mercier et al. 1992) as described in Copp (1990). From the CCA outputs, ‘triplots’ were generated (\textit{sensu} ter Braak 1986), combining the ordinations for vectors of the microhabitat variables, of the samples coded according to the trial in which they appeared (a = single-family, b = mixed-family. Microhabitat breadth of family groups from the 57 PIT-tagged salmon parr was assessed using ellipses that represent the 90% confidence intervals (Green 1971), whereby the ellipses were plotted on an equal ordination scale (\textit{i.e.} eight units) and the surface areas (in cm\(^2\)) of the ellipses were calculated using...
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MacDraft P.E. v5.5.8 to determine a proxy of mean microhabitat breadth and deviations thereof for each family group.

The distance between the points for each tagged fish (coded by ‘a’ and ‘b’, as above) approximates to their similarity/dissimilarity in terms of appearance in the sampling record, as determined by chi-square distances. The total number of occurrences of tagged fish at each sample (i.e. MPD antenna) was calculated for each family. These figures were converted to log10+1 to reduce skewness, and the matrix cross-tabulated with that of the variables to calculate electivity indices, which were defined as the difference between the frequency of occurrence at sample sites with a specific microhabitat variable and the frequency of that family across all samples (Copp 1992): negative values approaching −0.5 indicate avoidance, whereas positive values approaching +0.5 indicate preference.

Differences in the resulting microhabitat profiles (i.e. 28 electivity indices per family) were tested using the Wilcoxon pair-comparison test. Deviations from expected occurrence of families and microhabitat categories were tested using the Fisher Exact test. The multivariate analyses were undertaken using programmes of the ADE software library (Thioulouse et al. 1997; Chessel & Thioulouse 1998) and the microhabitat profile graphs generated using GraphMu (Thioulouse 1990).

Molecular Analyses

To assign juveniles to their parents, and therefore determine to which family groups they belonged, genomic DNA was extracted (Qiagen tissue DNA extraction kit; catalogue no. 69506) from parental and juvenile fin tissue using the manufacturer's protocol for extracting DNA from animal tissue. DNA yield was quantified on a 1% agarose gel and visualised on a UV transilluminator. Nine microsatellite loci were used in the assignment of parentage, chosen on the basis of their reliability in the use of parentage assignment based on their use in previous salmon genetic studies (Sanchez et al. 1996; McConnell et al. 1995; O'Reilly et al. 1998) and their distinct allelic size range (see Fernandes et al. In press for further details of molecular methods).

The program CERVUS v3 (Marshall 2007) was used to assign each MPD detected parr \( n = 57 \) to their original parental pairs. CERVUS uses an inclusionary approach, which compares the candidate parents’ genotypes with the offspring’s and assesses the relative likelihood (logarithm of
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odds) at each offspring’s genotype having been inherited from all possible parents. The parent with the highest LOD score is usually assigned as the true parent if its likelihood is significantly higher than the next most likely parent. The mean proportion of sampled candidate mothers and fathers was 100% (six mothers and six fathers: three parent pairs in 2006 and three different parent pairs in 2007). The error rate in likelihood calculations was assumed at 1%.

Results

The number of detections (log₁₀ transformed) did not differ significantly (Kruskal-Wallis test, \( P = 0.787 \)) between the days (\( n = 4 \)) of the MPD installation in each replicate site (\( n = 12 \)), suggesting there was no bias due to possible changes in behaviour following MPD installation. Analysis of microhabitat variables recorded at each MPD disc showed that substratum variables and bank cover did not differ significantly (Mann-Whitney U test, \( P = 0.25 \)) between single-family and mixed-family sites, however there was a significant difference (ANOVA) in depth (\( F_1, 801.521 = 29.441, P = 0.001 \)) and velocity (\( F_1, 0.294 = 5.748, P = 0.003 \)), with mixed-family sites being shallower and faster flowing.

Fish from all family groups were detected by the PIT MPD units. Family 4 was the most abundant family group (\( n = 28 \)), followed by Family 3 (\( n = 9 \)), and Family 6 (\( n = 9 \)), Family 2 (\( n = 5 \)), Family 1 (\( n = 4 \)), and Family 5 (\( n = 2 \)). Fish from different family groups were detected in a range of microhabitats (Table 2).

With regard to microhabitat breadth, graphical representation of the CCA eigen values (Fig. 2A–Ca) revealed a break in slope after the third eigen value, indicating that these first three factors (i.e. dimensions) account for the majority of variation in the dataset (Persat & Chessel 1989; Copp 1990; ter Braak & Verdonschot 1995): CA1 for the proportions of gravel, weed cover and bank cover, and to a less extent sand (Fig. 2A-Cb); CA2 for water depth; CA3 for the proportion of sand in the substratum (Fig. 2A-Cc). The CCA triplot revealed considerable overlap in the microhabitat breadth of all family groups (Fig. 2A-Cb and 2A-Cc for CA1 \( \times \) CA2 and CA1 \( \times \) CA3, respectively), where mean microhabitat breadth was 46.29 cm². The family with the largest microhabitat breadth, and
therefore the greatest positive deviation from the mean, was Family 3 (mean / deviation from mean = 90.1096 cm² / 43.8196 cm²). This was followed by Family 6 (70.0379 / 23.7479 cm²) and Family 1 (60.4042 / 14.1172 cm²). The family with the smallest microhabitat breadth, and thus the greatest negative deviation from the mean, was Family 5 (0.0025 / -46.2875 cm²), followed by Family 2 (15.4613 / -30.8287 cm²) and Family 4 (41.7232 / -4.5668 cm²) (Fig. 2A-C).

The microhabitat profiles (Fig. 2A-Cd) were similar for all family combinations except for Family 4, which differed significantly (Wilcoxon’s test, $P \leq 0.05$) from families 1, 3 and 6 (Family 5 was excluded from the analysis due to low sample number; $n = 2$). This difference appears to result from the significant ($P < 0.05$) preference in Family 4 for intermediate water depths and velocities as well as the significant ($P < 0.05$) avoidance of any bank cover – the only such significant deviation from expected for this variable. Family 1 microhabitat use did not deviate significantly (Fisher exact test) from the expected frequencies of occurrence except with regard to depth ($P < 0.05$), revealing a preference for deeper waters. Microhabitat use by families 2, 5 and 6 did not deviate from expected, though Family 2 demonstrated a non-significant preference for moderate proportions of weed cover. Family 3 demonstrated significant deviations from expected frequencies with regard to water depth and velocity, and were frequently found in deeper waters of moderately-elevated water velocities (0.21–0.4 m s⁻¹; see Table 2).

Discussion

Fish from different parentage have been suggested to exploit different microhabitats (McLaughlin 1999), and this was apparent in the significant preferences and avoidances for microhabitat variables (Fig. 2A-Cd) in three family groups of the present study despite considerable overlap in the microhabitat breadth of those families (Fig. 2A-Cb-c). Considerable variation among fish (i.e. wide microhabitat breadth) was evident in most families of the present study.

Preference and avoidance of different habitat types are behaviours that are particularly favoured in the wild, where genetically diverse populations reside. Although the significance of preference and
avoidance from the present study are partly influenced by sample size, and will certainly be influenced by availability of habitat, there is a strong suggestion of a genetic basis for the preference and avoidance of habitat types among the fish in the present study, with different family groups specialising in different microhabitats.

Juvenile Atlantic salmon prefer coarse substrates (> 64 mm) because it provides shelter from high velocities, however in chalk streams where substrate size is generally smaller, macrophytes provide most of the visual barriers between territories and velocity shelters (Hendry & Cragg-Hine, 2000). Juvenile salmon in chalk streams preferred areas with weed cover, which provides shelter from predation risk, particularly at night (Riley et al. 2006, 2009). The highest electivity value observed for weed cover was by Family 2 for proportions of 30–50%, which was the most infrequently encountered category in samples (Table 2). At high densities and limited space, salmonids may use less suitable areas (Riddell et al. 1981), so it is possible that Family 2 in the present study was strongly associated with weeded areas as a result of a trade-off between feeding opportunities and exposure to predation (Milinski & Heller 1978).

In streams, substratum composition is often associated with water velocity (reviewed by Hendry & Cragg-Hine 2003), but this relationship was not apparent in the present study. Water velocity had a minor influence on the ordinations (Fig. 2A-Cb-c), This lack of correlation between water velocity and substratum composition is likely to be due to the relative invariability of the discharge regimes in chalk streams, such as the River Cerne, which results in a relatively uniform bottom substratum (Table 2). Salmon parr typically prefer shallow waters (< 20 cm), with high velocities of 50 to 65 cm s⁻¹ (Hendry & Cragg-Hine 2003). However, in this study only families 1, 3 and 4 were significantly associated with intermediate to high depths, and only families 3 and 4 showed a significant preference for intermediate velocity (i.e. 0.21–0.6 m s⁻¹) and none with regard to substratum type (Fig. 2B:b-c).

Although not significant, virtually all families in the present study avoided silt and were indifferent to sand. Different levels of marked preference and avoidance were observed for the range of gravel coverage. In the laboratory, overhead cover has been shown to decrease stress response rates and increase growth rate (Pickering et al. 1987), however in the wild, bank cover and closed tree canopy have been shown to reduce in-stream macrophyte growth, thereby diminishing macroinvertebrate
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production and diversity (Riley et al. 2009). Despite this, only Family 4 showed a significant
avoidance to bank cover whereas this variable was neither preferred nor avoided by fish originating
from any other family group in the study (Fig. 2B), and this may suggest a genetic basis for
microhabitat specialism.

The number of times individual fish were detected by the MPD was not accounted for. This is
because the current study was to gather data about habitat preference, not territory sharing/overlap or
dominance. However, it is possible that heterogeneous environments enable fish from fast growing,
aggressive family groups to occupy the highest quality habitats. It is also possible that the complexity
of the environment may reduce the fitness (i.e. growth rate) of aggressive dominant individuals in
relation to subordinates (Höjesjö et al. 2004). Therefore, behavioural variation could be maintained in
natural populations, thus allowing subordinate and dominant fish to coexist. Owing to the complexity
of salmonid habitat, if any one habitat component is degraded or inadequate, then the productivity of
salmonids may decline (Hendry et al. 2003). So, if the environment changes faster than the population
is able to adapt, it might quickly become extinct, despite the level of genetic variation present in the
stream (Watters et al. 2003). Therefore, in a genetically diverse population (as simulated in this study),
individuals could be affected to different degrees and face different probabilities of survival. This is
of particular importance for chalk streams where Atlantic salmon are suffering population decline,
and where the habitat structure is often modified during periods of river management.

It is notable that only a few variables can be easily measured in the wild, therefore many habitat
variables may have been unaccounted for in the present study and what is interpreted as habitat choice
may not necessarily focus on the most relevant variable (Bardonnet & Baglinière 2000). Also,
previous methods to classify habitat variables, as well as to assess habitat use and preference, differ
from study to study (e.g Heggenes, 1990; Heggenes et al. 1993; Bardonnet & Baglinière, 2000),
therefore the extrapolation of results from one river system to another may not be simple or
appropriate.

Despite some low sample sizes, the present study has shed light on the microhabitat breadth and
preference/avoidance among distinct family groups of salmon in a chalk stream. Microhabitat choice
is influenced by the family of origin and these findings have important implications for the
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management of chalk streams, in particular those with declining numbers of Atlantic salmon, providing fisheries managers with essential information about mixed-family groups and how to reduce intra-specific competition whilst undertaking salmon stocking and re-introduction initiatives.

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References


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Table 1. Microhabitat variables as converted to semi-quantitative categories (Cat.) from quantitative measurements (depth in cm, water velocity in m s⁻¹) or from proportional (%) measurements (of substratum and cover), based on the frequency distributions of the variables.

Table 2. The microhabitat variables and their semi-quantitative categories for each family of Atlantic salmon (*Salmo salar*) parr in the River Cerne, Southern England, for 5–8 September 2006 and 17 August–20 September 2007. Given are the frequencies of occurrence (f) of each family in all samples (192 antennae), the total number of occurrences of each microhabitat category (‘n’; see also Table 1) in samples (i.e. antennae), and the number of samples in which each family group co-occurred with each category.

Fig. 1. A) Configuration in 2006 of sites stocked with six single-family (dark shaded) sites and six mixed-family (light shaded) groups of juvenile Atlantic salmon *Salmo salar* into on the River Cerne, (Dorset, England). See methods for stocking densities and channel character. B) Configuration in 2007 of three single-family (dark shaded) sites and one large mixed-family (light shaded) site (the size of three single-family sites) of Atlantic salmon. See methods for stocking densities and channel character. C) Schematic configuration of 16 antennae of a multi-point decoder system MPD (not to scale) installed on a chalk stream. Photo courtesy of CEH.

Fig. 2 A–C. Canonical correspondence analysis triplot ordinations (CA1 × CA2) for Atlantic salmon parr families (1–6) and microhabitat profiles (see Table 1) in the River Cerne (Southern England) between 5–8 September 2006 and 17 August – 20 September 2007, with superimposed ordinations for individual fish coded by site in which they appeared (a = single-family, b = mixed-family), the microhabitat vectors (length indicative of that variable’s relative influence on the ordinations) and ellipses representing the 90% confidence intervals (Green, 1971) for that family: A) eigen values for the seven canonical dimensions; B) triplot of CA1 × CA2; C) triplot of CA1 × CA3. D) microhabitat electivity profiles (values approaching +0.5 = preference; values approaching -0.5 = avoidance) for that family (all PIT-tagged fish combined). See Table 1 for microhabitat variables and category
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Significant deviations from expected (Fisher-Exact test) are indicated as * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).
Table 1. (Fernandes, Copp, Riley)

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<th></th>
<th>Cat. 1</th>
<th>Cat. 2</th>
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<th>Cat. 4</th>
<th>Cat. 5</th>
<th>Cat. 6</th>
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<td>48–70</td>
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<td>0.41–0.6</td>
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<td>21–50</td>
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<td>Silt (%)</td>
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<td>60–100</td>
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### Table 2. (Fernandes, Copp, Riley)

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<td>2) 25–47</td>
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<td>2</td>
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<td>3) 48–70</td>
<td>105</td>
<td>6</td>
<td>9</td>
<td>10</td>
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<td>1</td>
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<tr>
<td><strong>Water velocity (m s⁻¹)</strong></td>
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*Silt (%)*

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*Bank cover (%)*

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Figure 1 a, b and c (Fernandes, Copp and Riley)

A

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30m 100m gap

Downstream

B

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50m 250m gap

150m

Downstream

C

Bank

Gap between antenna

Gap between lines

A line of antennae

Bank
Family Group 1

![Graphs showing Family Group 1 data.](image)

Family Group 2

![Graphs showing Family Group 2 data.](image)
Family Group 3

Family Group 4
Family Group 5

Family Group 6