SUBSTRATE SELECTION IN LARVAL *CALOPTERYX SPLENDENS* (HARRIS) (ZYGOPTERA: CALOPTERYGIDAE)

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Under experimental conditions, the relationship between substrate composition, with and without the presence of an emergence support, and larval distribution was investigated. Results revealed that *C. splendens* larvae showed a clear preference for a pebble substrate as opposed to sand or silt, when all 3 substrates were equally available. However, the substrate type decreased in importance as the density of the emergence support increased. Results suggest that the morphological adaptations of *C. splendens* larvae, to cling to a substrate, can be utilised equally in a vegetated habitat and a habitat predominated by pebbles and cobbles. This has implications for the dispersal of *C. splendens* to areas containing, traditionally, less favoured habitat. Range expansion of *C. splendens* on its northern borders, where aquatic habitat characteristics can differ markedly from waterways in lowland southern England, is discussed.

INTRODUCTION

The substrate in an aquatic environment can consist of various types of organic and inorganic materials and provides something sufficiently stable for invertebrates to crawl on, cling to or burrow in (MINSHALL, 1984). The leaves and stems of riparian vegetation and aquatic macrophytes constitute the organic substrate. The nature of the inorganic component is largely determined by the underlying geology. Inorganic substrates are usually sedimentary materials and can range from microscopic silt and clay particles to large boulders.

A preliminary study on invertebrate-substrate relationships was made by PERCIVAL & WHITE (1929), who investigated the macroinvertebrate fauna associated with seven substrate types with differing amounts of plant cover, in streams

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in northeastern England. More recent studies of aquatic invertebrate larval habitat selection have concentrated on burrowing species, including gomphid odonates (KEETCH & MORAN, 1966; HUGGINS & DUBOIS, 1982; SUHLING, 1994, 1996), and research on substrate selection with regard to odonate species that do not burrow is scarce.

Previous work has shown that substrate particle size and composition, and the presence or absence of aquatic vegetation, can directly affect the microdistribution of aquatic insects (CUMMINS & LAUFF, 1969; SUHLING, 1996), although other factors such as prey distribution and predation, as well as competition for space, can result in a variation in abundance from one substrate type to another (BAKER, 1981; BRUSVEN & ROSE, 1981; McPEEK, 1990). In addition, the behaviour of ovipositing females determines, to some extent, the types of resources available to their offspring, and many larval species are aggregated initially as a result of the patchiness of the oviposition substrate. These offspring normally select microhabitats within the general vicinity of the oviposition site (JAENIKE & HOLT, 1991). Nevertheless, movements between microhabitats are commonplace during larval development (CORBET, 1962). MACAN (1966) reported that larval *Lestes sponsa* disperse soon after hatching from the marginal plants in which the eggs were laid and JOHANSSON (1978) showed that *Erythromma najas* larvae move from open mud beneath oviposition sites to reed beds. Other factors implicated in the microdistribution of larvae include seasonality (LAWTON, 1970) and the larval stage (MIYAKAWA (1969) cited in CORBET, 1999).

Recent investigation of the distribution of *C. splendens* in the UK suggests that the species has undergone range expansion (WARD & MILL, 2007). Indeed, in 2004 it was reported in large numbers near Dalbeattie, south-west Scotland by B. & R. Mearns (BATTY, 2007) having previously been unrecorded in Scotland, except for casual sighting. In 2006 *C. splendens* was recorded on the stony Urr Water in Kirkcudbrightshire, again in south-west Scotland (BATTY, 2007). This contradicts the widely held view that *C. splendens* larvae preferentially inhabit silt substrate. Previously considered as a 'southern species' (e.g. BROOKS, 2004), where there are extensive slow flowing rivers in lowland habitats, with well-vegetated banks, *C. splendens* can now be considered as more wide ranging. Indeed, the presence of the species in south-west Scotland, on waterways with contrasting physicochemical features to those further south, suggests it can adapt to conditions which facilitate its movement northward.

Morphologically, *C. splendens* larvae have an elongate abdomen and clasping legs (CORBET, 1999). These adaptations facilitate their habitation of lotic habitats by allowing them to cling to a substrate, such as vegetation stems. Indeed, the larvae have been observed to inhabit the root and stem regions of submerged vegetation, in silt and mud-bottomed streams and rivers (MERRITT *et al.*, 1996; BROOKS, 2004).

This study aims to determine the relationship between substrate compositions, with and without the presence of an emergence support, and the spatial distribution of F-1 and F-2 instar larvae of *C. splendens*.

METHODS

The larvae of *C. splendens* used for substratum selection experiments were collected from the River Medway in Kent, south east England (supplied by Blades Biological Supplies). All larvae were sorted into instars according to head width (C. Schütte, pers. comm.), and were classified as stages F-1 and F-2.

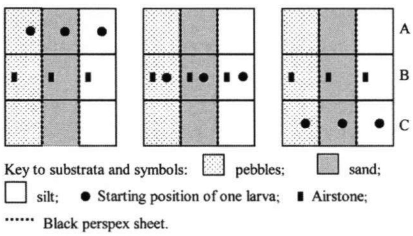

Fig. 1. Aquaria design used for Experiment 1.

Each of three glass aquaria (0.48×0.36 m) were divided by two longitudinal black perspex sheets into three sections of equal dimensions. Within each section there were three equal sized areas, each containing one of three substrate types: coarse gravel and small pebbles, medium sand, and silt/clay (after the Wentworth Classification of substrate particle size). Prior to the experiment the substrata were set out in the aquaria to a depth of 0.02 m and the aquaria filled with water to a depth of 0.12 m. The water was left to stand for 36 hours with airstones to aerate the water. In order to keep the water aerated throughout the experiment, one airstone was positioned above the centre substrate in each block of three, in such a position that the larvae could not cling to it. In all experiments a water temperature of 12°C and a 12:12 hour light:dark photoperiod were maintained.

During all experiments the larvae were fed by placing one *Chironomus* larva on each substrate type, unless otherwise stated. *C. splendens* larvae were fed in this way on alternate days to prevent the settling of uneaten food items influencing substrate selection. Observations of the position of the larvae were recorded every hour from 1000 until 1800 (GMT) for ten days, between February and April 2001.

EXPERIMENT 1. – An experiment was carried out to control for larval preference for position in the aquaria. Each section contained a single substrate type and one larva was placed on each substrate (i.e. in each section) in one of three starting positions (A, B or C). This was carried out for each starting position (Fig. 1).

This preliminary investigation revealed a significant difference in the observed positions of *C. splendens* in the aquaria ($\chi^2 = 16.47$, d.f.= 2, $P < 0.001$). A total of 40 % of the observations (n = 810) were in the middle region (B), whereas 29.3 % and 30.7 % of the observations were in regions A and C, respectively.

EXPERIMENT 2, SUBSTRATE SELECTION. – The following experiments were performed to determine the relative importance of larval choice for substrate type and/or vegetation. Each area within each section contained a diferent substrate. A randomised order of the position of the substrates was used in all experiments (Fig. 2):

(a) In the absence of a vertical support – In the first tank (1a to 1c) one individual was placed in each pebble area, in the second tank (2a to

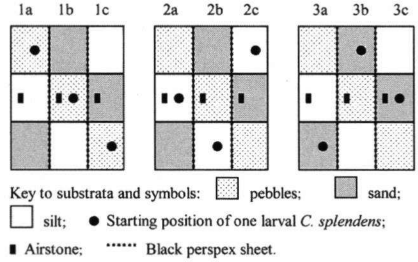

Fig. 2. Aquaria design used for Experiment 2.

2c) in each silt area and in the third tank (3a to 3c) in each sand area (Fig. 2). Two trials were conducted with nine larvae used in each (18 replicates).

(b) In the presence of low density 'vegetation' – One piece of artificial 'vegetation', in the form of a dowel rod (5 mm diameter), was placed in the centre of each substrate (i.e. in each of the nine areas) to simulate sparse vegetation and thereby to investigate if sparse vegetation is more important than a sediment substrate or vice versa. The experimental procedure was the same as in experiment 2(a).

(c) in the presence of high density 'vegetation' – Sixteen dowel rods were spaced evenly in each substrate (i.e. in each of the nine areas) to simulate dense vegetation and hence its importance in relation to sparse vegetation and substrate. The experimental procedure was the same as in experiment 2(a).

However, due to the high mortality rate of the experimental animals, only one trial of experiments b and c was performed. A total of 12 individuals were used in these experiments, which had all been used in the previous substrate-only experiment.

RESULTS

SUBSTRATE SELECTION IN THE ABSENCE OF A VERTICAL SUPPORT

In the absence of a vertical support, the distribution of *Calopteryx splendens* differed significantly between the three substrate types ($\chi^2 = 832.45$, d.f. = 2, $P < 0.0001$) with observations of larvae on the pebble substrate being recorded significantly more often than those on either the silt or the sand substrates (Tab. I).

SUBSTRATE SELECTION WITH A LOW DENSITY OF VERTICAL SUPPORTS

The introduction of a vertical support, in the form of one dowel rod per block, reduced the proportion of observations recorded in the pebble substrate areas in comparison to the absence of a vertical support (Tab. I) but the number of observations in the pebble substrate areas (i.e. those on the substrate itself together with those on the dowel rods in that substrate) were still significantly greater than in either of the other two substrate areas (($\chi^2 = 182.31$, d.f. = 2, $P < 0.0001$) (Tab.

Table I

Influence of substrate and of dowel rods (simulated vegetation) on substrate selection by larval *Calopteryx splendens*. Data are larval occupancy of each substrate area (%) with the number of observations in parentheses

	Sediment area choice			P
	Pebbles	Silt	Sand	
Substratum alone	66.9 (1084)	19.8 (321)	13.3 (215)	< 0.001
1 dowel rod	52.6 (562)	26.0 (278)	21.4 (228)	< 0.001
16 dowel rods	32.1 (343)	32.0 (342)	35.9 (383)	N.S.

N.S.= not significant

I). However, the larvae were recorded significantly more often on the dowel rods (53.7%) than on the substrate (46.3%) ($\chi^2 = 5.99$, d.f. = 1, P = 0.01).

Of those clinging to the dowel rods, 38.2% of the observations were in the silt substrate areas compared with 34.2% and 27.7% in the pebble and sand substrate areas, respectively (Fig. 3); this difference was significant ($\chi^2 = 9.58$, d.f. = 2, P = 0.01). Nevertheless, considering only those larvae that were located on the substrate itself, they were still recorded significantly more often on the pebble substrate ($\chi^2 = 367.67$, d.f. = 2, P < 0.0001) than on either the sand or silt substrates (Fig. 3).

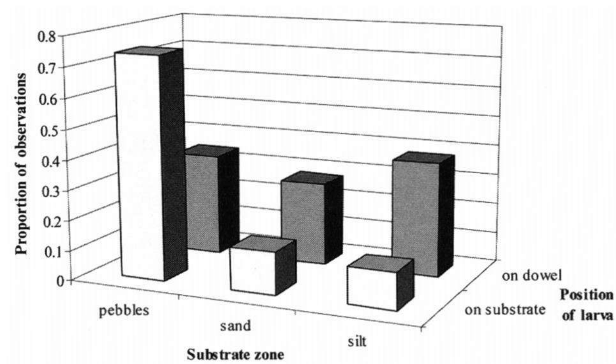

Fig. 3. The proportion of observations (n = 1068) of larvae in each substrate area in the presence of one dowel rod per substrate type.

SUBSTRATE SELECTION WITH A HIGH DENSITY OF VERTIAL SUPPORTS

With the increase in density of vertical supports to 16 dowel rods per block, there was no significant difference between the number of observations in the three substrate areas (i.e. those on the substrate itself together with those on the dowel rods in that substrate) ($\chi^2 = 3.07$, d.f. = 2, P = 0.22) (Tab. I).

The number of observations of larvae on the substrate itself decreased significantly ($\chi^2 = 226.65$, d.f. = 1, P < 0.001), with 73% of the observations recorded on the dowel rods and the number on the latter was significantly greater in the sand substrate areas ($\chi^2 = 17.22$, d.f. = 2, P = 0.0002), than in either the pebble or silt substrate areas (Fig. 4). Again, considering only those larvae

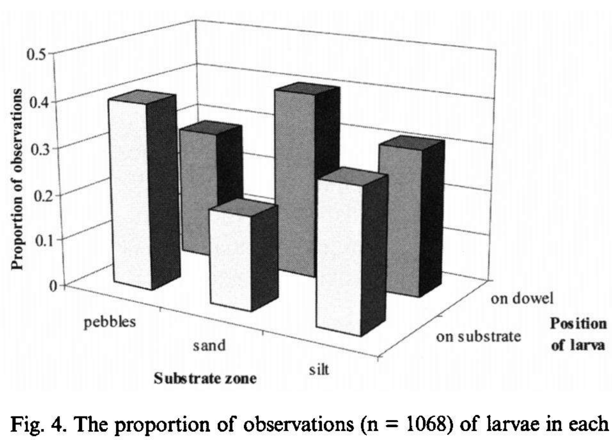

Fig. 4. The proportion of observations (n = 1068) of larvae in each substrate area in the presence of 16 dowel rods per substrate type.

that were located on the substrate itself, the number of observations of larvae on the pebble substrate was still significantly greater than on either sand or silt (χ^2 = 13.56, d.f. = 2, P = 0.001) (Fig. 4).

DISCUSSION

In the current study, *Calopteryx splendens* larvae showed a clear preference for a pebble substrate as opposed to sand or silt, when all three substrates were equally available. A similar preference has been found for *Pteronarcys californica* (Plecoptera), *Arctopsyche grandis* (Trichoptera), *Ephemerella grandis* (Ephemeroptera) and *Brachycentrus* sp. (Trichoptera), under comparable laboratory conditions (BRUSVEN & PRATHER, 1974). Furthermore, under natural conditions, aquatic insects with relatively long larval life cycles of two to three years, such as some dragonflies and stoneflies (*Pteronarcys, Paragnetina*), are commonly associated with boulder substrates, possibly because they are more likely to find shelter during high water flow (DE MARCH, 1976; MINSHALL, 1984). In addition, several authors have stated that habitation of stony substrates may result in reduced predation by fish (SUHLING, 1994; McPEEK, 1990; BRUSVEN & ROSE, 1981). In addition to predation, other important factors in microhabitat selection are competition (SUHLING, 1996) and the physical characteristics of the habitat.

Calopteryx larvae have a high oxygen requirement, which effectively limits members of the genus to lotic habitats (WILDERMUTH, 1994). Furthermore, ERIKSEN (1966) has shown that the presence of silt and fine sand can cause a significant reduction in the oxygen content within the substratum. In addition, silt may significantly affect the habitability of a substratum by altering water movement and food quality (MINSHALL, 1984). Heavy silting generally results in reduced insect species diversity and productivity (MINSHALL, 1984). Several authors have noted that the presence of large amounts of silt and sand coincide with a reduction in the abundance and diversity of stream insects (CHUTTER, 1969; MINSHALL, 1984; NUTTAL, 1972; PENNAK & VAN GERPEN, 1947). This is of significance to *C. splendens* whose larvae are carnivorous.

Several authors have reported a gradual increase in species abundance from bare substrate to the presence of vegetation (PERCIVAL & WHITE,1929; WHITEHEAD, 1935; MINCKLEY, 1963) and it is established that the presence of aquatic plants can lead to increased diversity of fauna in aquatic habitats (MACAN, 1977; MACAN & MAUDSLEY, 1968), thus providing a ready food supply for predatory invertebrates such as *C. splendens*.

The current study showed that the substrate type decreased in importance as the density of dowel rods increased. *C. splendens* are generally considered to inhabit silty areas (BROOKS, 2004), although this observation presumably results from the presence of aquatic vegetation in silt substrates, which larval *C. splendens* use for cover, as an emergence support and, in the adult stage, as a perch. Where the flow rate of a river is more rapid, sediment does not settle and the resultant substrate is predominantly pebbles and boulders. These conditions are not conducive to easy establishment of vegetation communities. In contrast, in slower flowing regions, where silt settles out on the river bed, plant communities are more readily established.

GOODYEAR (2000) compared the environmental requirements of larval *C. splendens* and *C. virgo*. In general the predominant substrate where *C. splendens* was found was silt and detritus, although at three out of ten sites, sand and gravel was the dominant substrate type. GOODYEAR (2000) concluded that, for *C. splendens*, the general pattern is a strong preference, but not a necessity, for muddy bottoms, where ready establishment of macrophyte and riparian plant communities occur.

MINSHALL (1984) suggested that certain species of Ephemeroptera, Trichoptera and Odonata are restricted to vascular plants, partly because they provide a substrate for clinging to. Additionally, since oxygen levels are much lower in silt and mud substrates (see above) a high oxygen intake may be achieved directly from a plant surface since, during daylight, plants produce oxygen. However, for species with a particularly high oxygen requirement, such as *Calopteryx virgo* (MERRITT et al., 1996; GOODYEAR, 2000), which are predominantly found on a pebble substrate, the flow rate of the river is generally more rapid, thus generating the required oxygen level and hence the need for oxygen produced from aquatic vegetation is reduced.

As a species inhabiting lotic habitats, larval *C. splendens* have adaptations for clinging to a substrate and have been described as thigmotactic in habit (CORBET, 1999). Thus, in the absence of a vertical support, such as a vegetation stem, pebbles would be the favoured habitat because they provide a firm substrate for attachment.

Formerly considered a southern species, there is no doubt that the northern range of *C. splendens* has expanded into north-eastern England and, from its base in north-western England, into southern Scotland (CLARKE, 1999; JEFFRIES, 2001; WARD & MILL, 2004). Rivers in the north of England and in Scotland tend to be fast flowing over much of their length and, consequently, the substrate particle size tends to be greater in this sort of lotic habitat. *C. splendens* larvae have the potential to thrive in this habitat since the morphological adaptation to clinging to a substrate can be utilised equally in a vegetated habitat and a habitat predominated by pebbles and cobbles.

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