Persistence of benthic invertebrates in polluted sediments.

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CHAPTER 4

HABITAT SELECTION BY *CHIRONOMUS RIPARIUS* LARVAE: FOOD PREFERENCE OR TOXICANT AVOIDANCE?

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This study examined the habitat selection by *Chironomus riparius* larvae of different sediments. Seven floodplain lake sediments, differing in both food quality and concentrations of sediment-bound toxicants, were offered pair wise to the chironomid larvae and their settlement in the paired sediments was determined after 10 days. The larvae showed a clear preference for sediments with higher food quality, which overruled the avoidance of the sediments with higher toxicant concentrations. Our observations explain the persistence of this opportunistic chironomid species in organically enriched aquatic ecosystems independent of the toxicant levels.

INTRODUCTION

Chironomid larvae are often a quantitatively important component of benthic invertebrate communities. Consequently, their relationship with the sediments they inhabit has been well studied and reviewed by various authors (e.g. PINDER 1986, ARMITAGE et al. 1995). Sediments have been considered as a habitat (ARMITAGE et al. 1995), a cover from predators (MACCHIUSI & BAKER 1992, BAKER & BALL 1995), a source of food (VOS 2001, DE HAAS et al. 2002), and a source of potential toxic compounds (RISTOLA et al. 1996, DE HAAS et al. 2002). Hence, many factors may determine the distribution of chironomids, but sediment-bound toxicants (DIGGINS & STEWART 1998, PEETERS et al. 2000b) and food quantity and quality (PEETERS 2001, VOS 2001), are thought to be the key factors.

The growth rate of *Chironomus riparius* larvae, for example, is often related to the amount of detritus available as a food source (RASMUSSEN 1985). This matches with the field distribution of this opportunistic tube dwelling deposit feeder, which prefers eutrophic and organic enriched waters (ARMITAGE et al. 1995) and has been predominantly found in sediments with high organic matter content (GROENENDIJK et al. 1998). This is confirmed by many laboratory studies, which observed that habitat selection was highly related to the amount of available food (MACCHIUSI & BAKER 1992, BAKER & BALL 1995, SIBLEY et al. 1998, VOS et al. 2002). The food source offered to the chironomids in these studies was, however, always a commercial fish food, which is very high in nutrition, whereas natural detritus is often much lower in nutrition and highly variable (VOS 2001). Moreover, larvae of *C. riparius* can selectively feed on these additional high quality food sources rather than on the natural food sources in the offered substrates (ÅKERBLOM & GOEDKOOP 2003).

Hence, the question remains whether habitat selection under natural conditions is also steered by food availability and how confounding factors, such as sediment-bound toxicants, influences this selection. The latter is especially relevant for *C. riparius* because this species belongs to the particular genera of chironomids (*Procladius* sp., *Chironomus* sp., and *Cricotopus* sp.) that often become increasingly dominant at more contaminated sites (CANFIELD et al. 1996, PEETERS et al. 2000b), including the metal polluted lowland River Dommel (GROENENDIJK et al. 1998). The aim of this study is, therefore, to examine whether habitat selection by *C. riparius* larvae results from the preference for food, avoidance for sediment-bound toxicants, or a combination of both.

Habitat selection by chironomids in their early life stage is of critical importance for their further development, because chironomid larvae are highly sedentary for most of their lifespan. The first instar larvae are mainly pelagic until a suitable habitat has been found (OLIVER 1971). The second to fourth instars often inhabit the upper layer of the sediment, in which they build protective tubes from small particles (ARMITAGE et al. 1995), which are joined with their salivary secretions (EDGAR & MEADOWS 1969). The larvae keep in physical contact with their tube and therefore the foraging area is restricted to the immediate surroundings of the tube entrance (RASMUSSEN 1984). If larvae leave their burrow they will have to find a new site that meets their demands, but meanwhile they are prone to predation (MACCHIUSI & BAKER 1992). Site choice is not restricted to the early instar larvae, although larval mobility decreases with increasing age (BAKER & BALL 1995).

For this study seven floodplain lakes located along the River Waal, a branch from the River Rhine, with different levels of contamination and food were selected (see Figure 1.2). The preference of *C. riparius* for those sediments was assessed using a 10-day choice experiment in which the chironomid larvae could choose between two sediments. It was analysed if the preference of the larvae was correlated with food quality (chlorophyll *a*, fatty acids, bacterial fatty acids, and polyunsaturated fatty acids) and metal concentrations (Cd, Cu, and Zn) in the sediments.

MATERIALS AND METHODS

Sediment sampling, storage and treatment

Sediments for the choice experiments and chemical analyses were collected from seven floodplain lakes located along the River Waal, The Netherlands, in September 2003. About 15 L of sediment was collected using an Ekman-Birdge grab, which was adjusted to sample the upper 5 cm of the sediment. The sediments were transported to the laboratory, where large debris was picked out by hand. Next the sediment was homogenized, and stored at -20°C in 500-ml polyethylene bottles within 6 h after sampling in order to exterminate autochthonous organisms.

Sediment analyses

The OM content was measured as loss-on-ignition by combustion of dried sediment samples (60°C until constant weight) at 550°C for 6 h (LUCZAK et al. 1997) in triplicate. Chlorophyll *a* (chl *a*) and phaeophytin were measured according to LORENZEN (1967) in triplicate using freeze-dried sediment samples.

The acetone solution was centrifuged in closed test tubes to avoid optical disturbance by suspended sediment. Chl *a* and phaeophytin contents were summed, because in sediments chl *a* is already partly degraded into phaeophytin.

Lipids were extracted from 0.5 g of dry sediment with 6 ml methanol containing 2.5% H_2SO_4 for 90 min at 80°C in closed test tubes. Then 500 µl hexane and 1 ml 0.9% NaCl were added to the samples, which were placed for 1 min on a shaker and centrifuged at 12.000 rpm for 1 min 200 µl of the supernatant was transferred to a 200-µl vial. Fatty acid methyl esters (FAMEs) were measured using a Varian CP3800 gas chromatographer (GC) with a Varian Saturn 2000 MS (Varian Inc., Middelburg, The Netherlands) by injecting a 2-µl aliquot in a polar 30-m HP5-MS column (0.25 mm I.D.; 0.25-µm film thickness). GC conditions were as followed: initial temperature 35°C at 4 min, then to 120°C at a rate of 30°C per min and to 240°C at a rate of 8°C per min; injection was made in splitless mode (2 min); carrier gas was helium.

For the analyses of the total sediment Cd, Cu, and Zn concentrations 50 mg of sediment (triplicate) was digested in 2-ml polyethylene tubes with 500 µl 70% HNO_3 Ultrex® (J.T. Baker, Phillipsburg, NJ, USA) at 100°C using a DB-3D Dri-Block® (Techne, Duxford, UK). After digestion, the samples were diluted with 2 ml acidified deionized water (5 ml 70% Ultrex® per L). Metals were analyzed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B). Every 25 samples a blank (no sediment) and a reference (NIST:SRM 2704, National Institute of Standards and Technology, Gaithersburg, MD, USA) was digested for quality control. The measured values of the reference material were in agreement with the certified values (< 10% deviation); recovery of SRM 2704 was 99.6 ± 2.0%.

Choice experiment

The experiments were conducted at 20 ± 1 °C, moderate light (~ 10 µmol/m^2/s) and a 16:7 hour light:dark regime with 30 min of twilight (~ 5 µmol/m^2/s) before and after each light period. The experiments were started with first instar larvae (< 24 h), obtained from a culture maintained in our laboratory. Three days prior to the test 3 newly deposited egg ropes were removed from the culture and transferred into a petri dish with Elendt-M7 medium and placed at 20°C. Sediments were thawed at 4°C four days before the start of the experiment.

Each possible combination of the seven sediments was replicated 3 times, including pairings consisting of the same sediment, which served as no-choice controls. The experimental unit consisted of a polyethylene container (10 × 20 × 10 cm) in which two smaller polyethylene containers (9.5 × 9.5 × 3.5 cm) filled with test sediment were placed. The remaining gaps were filled with quartz sand

(Sibelco® M32, Antwerp, Belgium) 1 L of Elendt-M7 medium (OECD 2001) was added with minimal suspension of the sediments and the test systems were placed under aeration overnight.

At the start of the experiment the aeration was stopped and 30 larvae were placed in between the two sediments, aeration was started again after 3 days, and after 10 days the sediment containers were closed with a lid and retrieved from the test systems. The larvae were sieved from the sediment using a 300-μm sieve, and the number of recovered larvae was counted.

Data analyses

Significant differences in distribution of larvae between pair wise tested sediments were analyzed using a Paired t test ($\alpha = 0.05$).

To group the main variables that explained the pattern of distribution among substrates, a principal component analysis was performed with the different concentrations of metals (Cd, Cu, and Zn) and food quality parameters (chlorophyll *a*, fatty acids, bacterial fatty acids, and poly unsaturated fatty acids). For both metals and food quality one significant factor was extracted, which served as the new variable for respectively metal concentration and food quality. The lowest factor score was set to zero for both variables.

To identify if differences in food or metals determined the distribution of the larvae among the substrates a linear regression was performed using the differences in food variable or metal variable between each pair of sediments and the percentage of larvae found in the sediment with the higher food variable or the lower metal variable. All statistical analyses were conducted using the program SPSS® 10.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Sediment characteristics

The sediment characteristics of the studied floodplain lakes are listed in Table 4.1. Concentrations of metals were lowest in G1, and highest in D4. The concentrations of Cd, Cu, and Zn were highly correlated ($P < 0.01$). Hence, a clear gradient in contaminant concentrations was observed, allowing a ranking of the sediments from relatively clean (G1) to contaminated (D4) using a factor analysis with Cd, Cu, and Zn as the variables (factor metal).

Sediment organic matter content (OM) was lowest in G1 (2.3%) and highest in D4 (12.9%). Chl *a* concentration was lowest in G1 (16.8 mg/kg) and highest in G3 (58.8 mg/kg). The fatty acid (FA) and polyunsaturated fatty acid (PUFA) contents ranged from respectively 218 and 15.7 mg/kg in G1 to 343 and 36.1 mg/kg in O2. Bacterial fatty acids (bacFAs) ranged from 19.3 mg/kg in 3A to

32.6 mg/kg in O2. A classification from low food quality (G1) to high food quality (O2) could be made using a factor analysis with chl *a*, FAs, bacFAs, and PUFAs as the variables (factor food). No correlations were observed between metals and food quantity and quality parameters.

Table 4.1. Sediment characteristics of the sampled floodplain lake sediments: Cd, Cu, Zn, chl *a*, sum of fatty acids (ΣFAs), sum of bacterial fatty acids (ΣbacFAs), and sum of polyunsaturated fatty acids (ΣPUFAs) in mg/kg dw, and percentage organic matter content (OM). Factor metal is the factor score calculated with Cd, Cu, and Zn concentrations; Factor food is the factor score calculated with food quality parameters (Chl *a*, ΣFAs, ΣbacFAs, and ΣPUFAs).

	G1	D2	02	G3	3A	3B	D4
Cd	0.42	0.39	1.04	1.09	1.25	2.02	2.37
Cu	5.0	14.1	26.1	27	39.7	44.4	54.1
Zn	42	70	201	218	351	422	487
factor metal	0.00	0.22	1.00	1.08	1.66	2.24	2.71
OM	2.30	4.6	8.5	11.1	8.7	8.5	12.9
chl *a*	16.8	28.0	42.8	58.8	21.7	16.4	44.2
ΣFAs	218	265	342	287	247	291	283
ΣbacFAs	23.1	26.4	32.6	26.5	19.3	22.1	28.9
ΣPUFAs	15.7	25.5	36.1	25.3	19.7	25.7	21.7
factor food	0.00	1.21	2.95	1.83	0.24	0.97	1.58

Choice experiments

The no-choice control pairs showed an almost equal distribution between sediment pairs (43-57%) (not shown), which indicates that other experimental factors (e.g. aeration, position of light source) did not significantly influence the choice of larvae between the substrates. In contrast, when two different substrates were offered in 12 out of 21 pairings the larvae showed a significant preference for one of the two substrates.

A clear preference for the high food quality substrate O2 was indicated by the significant higher abundance of larvae on this substrate when offered together with each of the other substrates ($P < 0.001$) (Figure 4.1), except when G3 was offered as an alternative. In contrast, a significant lower abundance of the larvae on the low food quality substrate 3A was observed when offered together with G3, O2, 3B, and D4 as a substrate ($P < 0.001$) (Figure 4.1).

In 17 out of the 21 possible sediment combinations, larvae were more frequently found on high food quality substrates. In 13 out of the 21 possible sediment combinations, larvae were more frequently observed on the substrates with lower metal concentrations. This suggests a stronger preference for food, than avoidance of toxicants.

To analyze this preference and avoidance behaviour in more detail, in Figures 4.2 and 4.3 the distribution of the larvae in reaction to the difference in metal concentration and the difference in food quality between each pair of sediments are shown. When differences in metal concentrations between sediments increased, in most cases a larger proportion of the larvae inhabited the sediment with the lower metal concentration, although this relationship was not significant (Figure 4.2). When differences in food quality between sediments increased a larger proportion of the larvae was observed in the sediments that had higher food quality ($R = 0.615$, $P = 0.002$) (Figure 4.3).

In cases where a smaller proportion of the larvae were observed in the substrate with the higher food quality, the metal concentrations in these higher food quality substrates were much higher than in the low food quality substrates (see outlying points Figure 4.3). This confirms that the preference for food was stronger than the avoidance of toxicants.

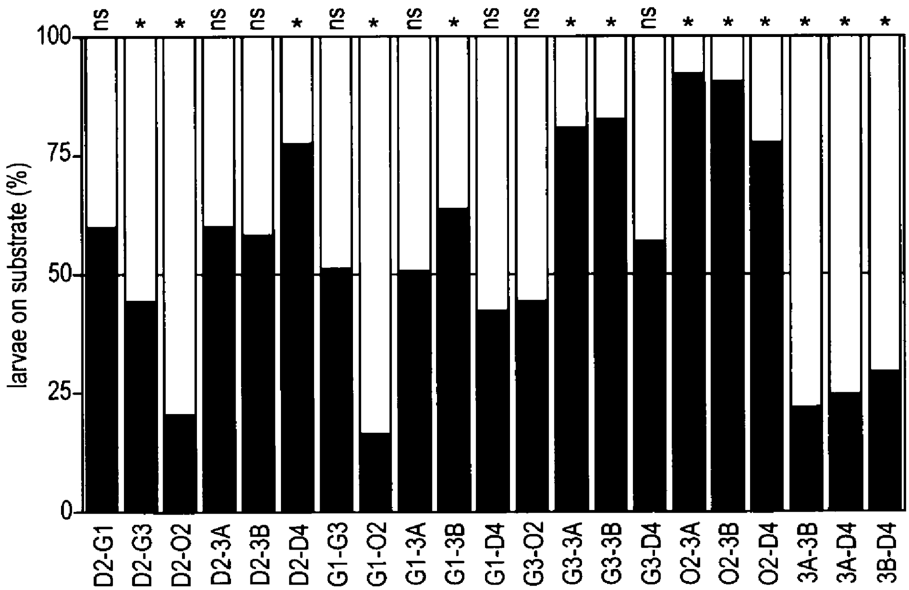

Figure 4.1. Preference of *Chironomus riparius* larvae for each combination of pair wise compared substrates. Black bars represent the percentage of larvae on the first mentioned substrate; white bars represent the percentage of larvae on the second mentioned substrate. The solid line indicates equal distribution among substrates; ns = no significance between distribution among substrates; * significant difference between distribution among substrates with $P < 0.05$ ($n = 3$).

DISCUSSION

Our results clearly indicate that the chironomid larvae could identify differences in both toxicants and food quality between sediments and this discrimination depends highly on differences in the concentrations of high quality food, and to a lesser extent to differences in the concentrations of toxicants. The chironomid larvae showed a high preference for natural sediments with higher food quality. Our findings are supported by experiments under less natural conditions in which larvae were offered a high quality fish food, resulting in a larger proportion of the larvae at the higher food levels (MACCHIUSI & BAKER 1992, BAKER & BALL 1995, SIBLEY et al. 1998, VOS et al. 2002).

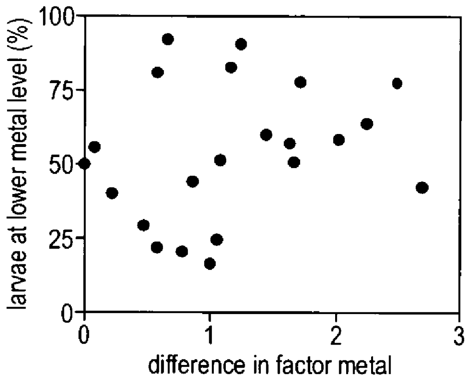

Figure 4.2. Relationship between the difference in metal concentrations (factor metal) between each pair of sediments and the corresponding percentage of *Chironomus riparius* larvae observed at the substrate with the lower metal concentration.

The influence of sediment-bound contaminants on habitat selection of chironomid larvae is less well documented than the influence of food. Nevertheless, WENTSEL et al. (1977) observed that third instar larvae of the midge *Chironomus tentans* started to avoid metal contaminated sediments when metal concentrations were extremely high (> 774 mg Cd/kg and > 8330 mg Zn/kg). HARE & SHOONER (1995) did not observe avoidance of cadmium spiked sediments (303 mg Cd/kg) by field collected *Chironomus* (*salinarius* gr.) larvae. The concentrations of metals at which avoidance is observed in the present set of sediments are much lower, although it must be realised that other, not measured, toxicants may have contributed to the avoidance response. In a previous study, with the same set of sediments, a strong positive correlation between metals, PAHs, and PCBs was observed (DE HAAS et al. 2002). Also the larval instars used at the start of the experiments may influence the outcome: in the present study experiments were started with first instar larvae and it has often been observed that with increasing larval instar the sensitivity of the larvae to toxicants decreases (WILLIAMS et al. 1986, NAYLOR & HOWCROFT 1997).

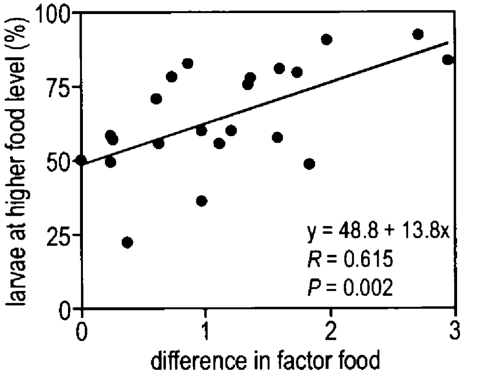

Figure 4.3. Relationship between the differences in food quality (factor food) between each pair of sediments and the corresponding percentage of *Chironomus riparius* larvae observed at the substrate with the higher food quality. Line = linear fit to the data.

Our results clearly demonstrated that the preference for food quality overruled the avoidance of sediment-bound toxicants. This is in concordance with results obtained from whole-sediment bioassays (DE HAAS et al. 2002), in which growth rate and time to emerge of *C. riparius* larvae was positively related to food quality in the sediment, in spite of the amount of toxicants present. Thus, the food quality in sediments is the main driving force in the selection of a suitable habitat, even if this leads to the exposure to toxicants. This explains the persistence of this opportunistic chironomid species in aquatic ecosystems that suffer from both organic enrichment and elevated toxicant levels.

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