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Chimney construction by *Chironomus riparius* larvae in response to hypoxia: microbial implications for freshwater sediments

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Abstract. Many shallow aquatic ecosystems with high nutrient loads experience periods of O_2 depletion that evoke behavioral responses by macrobenthic organisms. The sediment-dwelling midge larva *Chironomus riparius* reduces its deposit-feeding activity and allocates more time to burrow ventilation during periods of hypoxia. We investigated another striking behavioral adaptation of this species, i.e., the elongation of U-shaped sediment burrows to chimneys that tower above the sediment surface. *Chironomus riparius* larvae gradually abandoned burrow construction and took up chimney construction when exposed to hypoxic conditions in laboratory microcosms. Microsensors were used to show that the chimneys were oxic sediment compartments that were periodically irrigated by the larvae with oxygenated surface water. O_2 uptake rates per unit interface area were significantly higher for chimneys than for the flat sediment surface. This observation was consistent with the dense colonization of the chimneys by bacteria. Chimneys may facilitate the larval acquisition of both O_2 for respiration and microbial biomass for food. Given the mass abundance of *C. riparius* in many polluted and O_2-deficient habitats, the chimneys also may contribute significantly to the patchiness of the benthic microbial community in terms of structure and function. In particular, the presence of chimneys might favor aerobic bacterial populations and their metabolism.

Key words: Chironomidae, macrofauna, freshwater sediment, oxygen depletion, biogenic structure, sediment bacteria, diffusive oxygen uptake, animal–microbe interaction, sediment microcosm, microsensor.

Periods of O_2 depletion are common in many aquatic ecosystems, including coastal upwelling regions (Levin 2003), fjords (Rosenberg et al. 2001), streams receiving sewage water (Hellmann 1994), and lake hypolimnia during temperature stratification (Hamburger et al. 2000). Common features of ecosystems susceptible to hypoxia or anoxia are high nutrient concentrations, extensive organic sedimentation, and poor exchange of bottom water (Gray et al. 2002, Levin 2003). High nutrient concentrations can be of natural origin (e.g., coastal upwelling regions, Brüchert et al. 2003), or they can result from anthropogenic eutrophication and pollution (e.g., in streams, ponds, and lakes, Hellmann 1994, Hamburger et al. 2000).

Benthic macro- and microorganisms must cope with the consequences of hypoxic events, including shortage of O_2 and accumulation of toxic substances (e.g., sulfide). Macrofauna may have physiological adaptations to these conditions (e.g., hemoglobin, Choi et al. 2000; anaerobiosis, Hamburger et al. 2000), or they may respond to hypoxia with changes in behavior. Ventilation of sediment burrows is intensified in shallow waters (Leuchs 1986, Heinis and Crommentuijn 1992), bioturbation activity is reduced (Levin 2003), and sediment burrows are elongated above the sediment surface (Kon and Hidaka 1983, Jørgensen and Revsbech 1985). However, stress avoidance and behavioral compensation are of little importance to sediment bacteria (except for mobile taxa). Instead, external conditions regulate the metabolism of bacteria

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from full activity to dormancy. On the microscale, sediments can be considered model habitats for studying the bacterial response to hypoxia and anoxia because sharp O_2 gradients determine the metabolic activity of aerobic and anaerobic bacteria within the sediment (Wieringa et al. 2000, Altmann et al. 2003).

Here we present an example of the behavioral response of an abundant macrofaunal organism to hypoxic conditions and the implications of this behavior for bacterial colonization patterns in freshwater sediments. *Chironomus riparius*, a nonbiting midge with aquatic larval stages, is a typical inhabitant of polluted and temporarily O_2-deficient shallow waters (Pinder 1986, Bairlein 1989, Penttinen and Holopainen 1995). The larvae regularly occur in masses, construct U-shaped burrows within soft sediments, and feed on the detritus particles and the attached bacteria that are deposited onto the sediment surface (Pinder 1986). *Chironomus riparius* larvae tolerate O_2 deficiency because their hemolymph contains hemoglobin with a high affinity for O_2 (Choi et al. 2000). The larvae also can switch to anaerobiosis, but they are less efficient than larvae of other species (e.g., *Chironomus anthracinus*, Penttinen and Holopainen 1995, Hamburger et al. 2000). *Chironomus riparius* larvae increase their undulation activity during periods of low O_2 concentrations in the water column (Leuchs 1986). Occasionally, the larvae elongate their burrows to reach above the sediment surface, a behavior described as *chimney-projecting behavior* (Kon and Hidaka 1983, von Danwitz 1983). Biogenic structures that pierce through the diffusive boundary layer are thought to improve O_2 availability for the inhabiting animal (Jørgensen and Revsbech 1983). Projecting biogenic structures also may generate small-scale habitat heterogeneity for the benthic microbial community by promoting advective nutrient transport and trapping organic particles (Eckman and Nowell 1984, Eckman 1985, Eckman and Thistle 1991, Soltwedel and Vopel 2001).

Our study addressed the following questions: 1) Does a threshold O_2 concentration induce the chimney-projecting behavior at the expense of sediment burrow construction or is the response to increasing hypoxia graded? 2) Does the erect shape of chimneys make them a residual oxic sediment compartment under hypoxic conditions? 3) Are chimneys preferential sites for bacterial colonization? 4) How does sedimentary O_2 uptake vary between inhabited and uninhabited sediments and the chimneys? Laboratory microcosm experiments were conducted using homogenized freshwater sediments that were exposed to different O_2 concentrations (factor 1) and to which *C. riparius* larvae (factor 2) were added or not. The number and size of burrows and chimneys were quantified, total bacterial abundance was determined, and O_2 concentrations were measured on a microscale in all treatment combinations.

Methods

Origin of sediment and animals

Sediment was sampled in a freshwater ditch ~20 km west of Köln (North-Rhine Westphalia, Germany). The ditch runs through an intensely farmed area. The water column is characterized by high nutrient concentrations and transient O_2 depletions. Large populations of various *Chironomus* species inhabit the silty and organic-rich sediment of the ditch. A flat shovel (10 × 15 cm) was pushed 10 cm into the sediment and the load of sediment was lifted carefully out of its place. Five loads were placed in a 10-L plastic bucket and covered with 2 L of ditch water. The sediment was sieved through a 1-mm-mesh screen, homogenized, and stored at 4°C in the dark until use. Egg masses of *C. riparius* were provided by the Institute for Inland Water Management and Waste Water Treatment (RIZA) in Lelystad, The Netherlands. Larvae were bred in laboratory tanks (40 × 50 × 15 cm) filled with aerated, aged tap water and a 5-mm thick layer of sediment. A food suspension of ground leaves of stinging nettle (*Urtica* sp.) and fish food (Trouvit, Tetramin®) was added 3 times a week. The culture was kept at 20°C under a light:dark cycle of 16:8 h. *Chironomus riparius* larvae grew to a body length of 5 to 7 mm within 10 to 15 d (3rd-instar larvae) and were then ready for use in the hypoxia experiments. A group of larvae was tested for its sensitivity to a standard toxicant ($K_2Cr_2O_7$, Grootelaar et al. 1996) before the actual experiment. The sensitivity of larvae taken from our culture lay within the frame of acceptability for use in laboratory exposure experiments.

Laboratory microcosms

Sediment was added to the center compartment (10 × 5 × 5 cm) of transparent aquaria

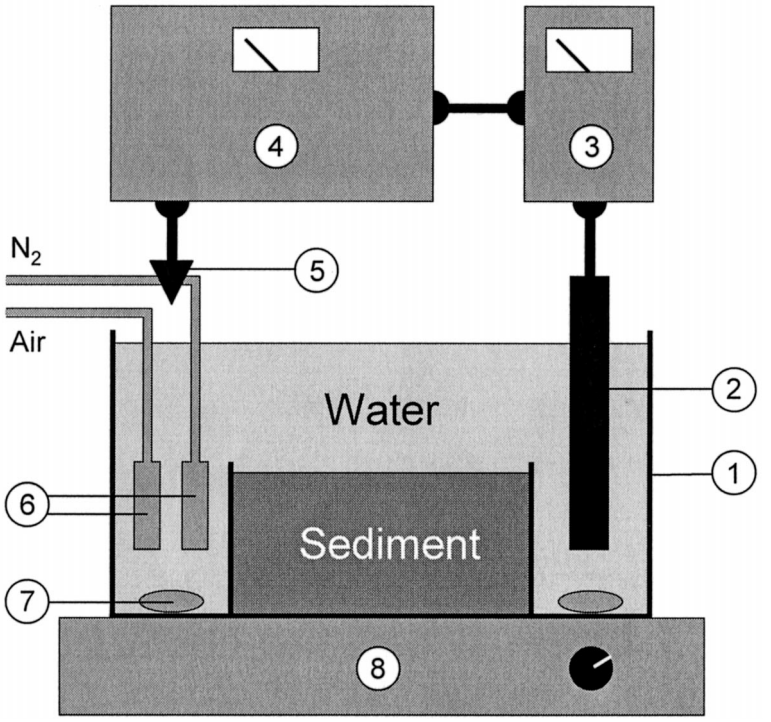

FIG. 1. Microcosms used for hypoxia experiments (side view). Numbered parts are acrylic aquarium (1), O_2 macroelectrode (2), oxymeter (3), O_2 control unit (4), magnetic valve (5), gas dispenser (6), stir bar (7), and magnetic stirrer (8).

(20 × 5 × 10 cm; Fig. 1). The remaining volume of the aquarium was filled with aged tap water. Two stir bars, rotating on either side of the central sediment compartment, homogenized the water. The O_2 content of the water body was regulated using an O_2 control unit (Aquastar V 2.0, iks ComputerSysteme GmbH, Karlsbad, Germany). The O_2 concentration of the water was measured next to the sediment compartment with a calibrated macrosensor. A magnetic valve opened automatically and N_2 gas was blown into the water via a gas dispenser when O_2 concentrations were above the selected value. The valve closed as soon as the selected value was reached. The O_2 concentration (expressed as % air saturation) of the water could be held constant with an accuracy of 5%.

Experimental schedule

Experiments were carried out in 6 consecutive runs. Three runs with chironomid larvae (animal treatments) and 3 runs without larvae (control treatments) were done in random order. Each run began with a 2-wk preincubation period during which 4 aquaria were filled with sediment and allowed to equilibrate at 100% air saturation and 15°C in the dark. After this preincubation period, fifty 3rd-instar larvae (1 larva/cm^2) were added to each aquarium in animal treatment runs. No larvae were added to aquaria in control treatment runs. *Chironomus riparius* typically dug into the sediments in <30 min. The O_2 content of the aquaria was adjusted after 2 d of acclimation. One aquarium was kept at 100% air saturation, and one each was kept at 45, 30, or 15% air saturation. Larvae were fed every 3rd day with 5 mL of food suspension. Aquarium water was replaced the day after food addition to prevent accumulation of detrimental NO_2^- concentrations. Daily checks of the overlying water showed that NO_2^- concentrations remained well below the tolerance level for the larvae (20 μmol/L; Neumann et al. 2001) at all times. All manipulations carried out in animal treatments also were carried out in control

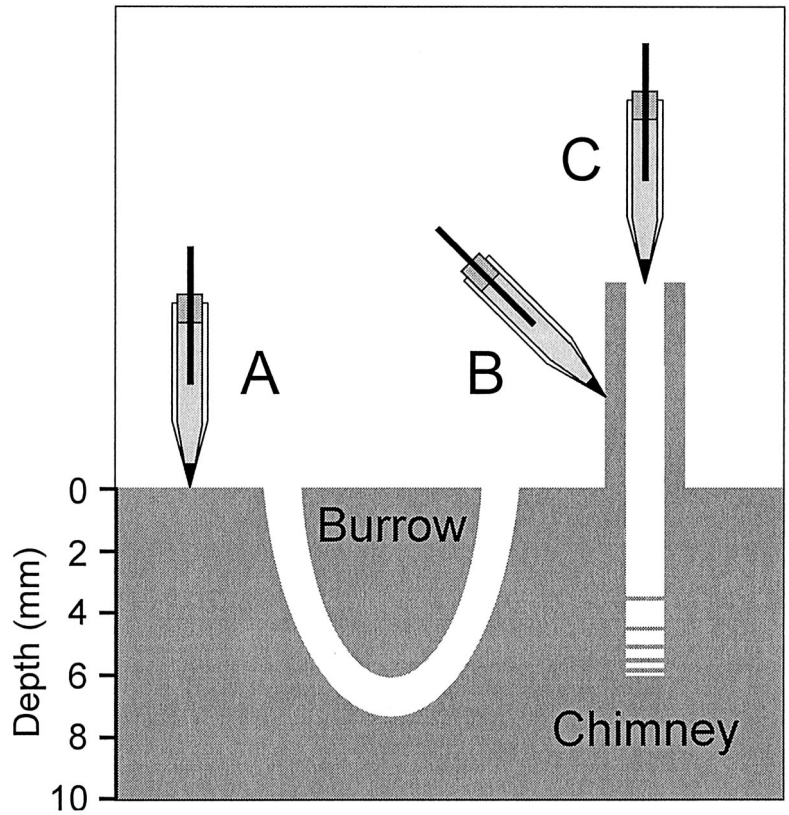

FIG. 2. Representative positions of O_2 microsensor during measurements for O_2 concentration profiles near burrows and chimneys constructed by *Chironomus riparius* larvae in hypoxia experiments. Letters refer to probe positions when measuring vertical profiles across the sediment–water interface (A), profiles within the diffusive boundary layer surrounding a chimney (B), and time-series profiles at the opening of a chimney (C). Representation of the chimney (C) is a simplification: chimney is actually an extension of the U-shaped burrow. Microsensor drawings are not to scale.

treatments. Larval performance, O_2 uptake rates of sediments and chimneys, and total bacterial abundance in sediments and chimneys were studied during the 3-wk experimental incubation period after the addition of larvae.

Larval performance

The 2 types of larval tubes (burrows and chimneys) were quantified in terms of total number and average size (length, diameter, and thickness of walls) on day 14 of the experimental incubations. A cylindrical shape was assumed for the chimneys, and the dimensions were used to calculate the relative gain in sediment–water interface area and oxic sediment volume. A few *C. riparius* larvae left the sediments and were found in the water on either side of the sediment compartment. These larvae were regarded as having escaped from adverse conditions in the sediment, and their number was recorded during daily inspections. Dead larvae and pupae and the exuviae left behind from emerged animals were collected and counted. The cumulative % of escaped, dead, and emerged animals was calculated over the duration of the experiments. The periodic undulation behavior of the larvae inside the burrows was verified qualitatively with observations through the transparent aquarium wall. Larval undulation inside chimneys was documented in the form of fluctuating O_2 concentrations near the chimney opening (Fig. 2C). Microsensors (see below) were used to make sev-

eral 240-min time-series recordings (temporal resolution = 3 s) of O_2 concentrations near chimney openings.

O_2 uptake rates

Microsensors were used to quantify the diffusive O_2 uptake by sediments and chimneys during weeks 2 and 3 of the experimental incubations. O_2 microsensors were constructed, calibrated, and used for concentration profiling as described previously (Revsbech 1989, Stief and de Beer 2002). The sensors had tip diameters of 10 to 20 μm and allowed measurements with a spatial resolution of 50 to 200 μm. Sensor tips were positioned relative to the sediment or chimney surface (Fig. 2) with a dissection microscope and a motorized micromanipulator that drove the sensors stepwise into the sediments or towards the chimney surface. Sensor signals were logged on a computer using the program InSight (R. Thar, University of Copenhagen, Denmark). Diffusive O_2 uptake by the sediments and chimneys was calculated from linear concentration gradients within the boundary layers covering their surfaces using Fick's 1st Law of Diffusion. The diffusion coefficient of O_2 at 15°C was taken as 1.83×10^{-5} cm^2/s (Broecker and Peng 1974). O_2 microprofiles were repeated at as many different positions as possible to average out lateral heterogeneities of the samples. H_2S and total S^{-2} concentrations and pH were measured using microsensors (Revsbech et al. 1983, Jeroschewski et al. 1996) in 100% and 15% air saturation treatments.

Bacterial abundance

Total bacterial abundance on sediments and chimneys was measured at the end of each experiment. Up to three 2.5-cm-diameter sediment cores were taken from each aquarium. Sediment cores were positioned to avoid inclusion of chimneys in the sample. A 2-mm-thick sediment slice (i.e., the feeding layer of *C. riparius* larvae, Stief and de Beer 2002) was removed from the top of each core and fixed in 4% paraformaldehyde for 1 h. Chimneys >3 mm in length were removed as whole units using tweezers and fixed. Fixed samples were washed 3× in phosphate-buffered saline (3 mmol/L NaH_2PO_4, 7 mmol/L Na_2HPO_4 [pH 7.2], 130 mmol/L NaCl) and stored in 3 volumes of a 1:1 mixture of phosphate-buffered saline and 96% ethanol at −20°C. Samples were diluted 100× with phosphate-buffered saline and sonicated with an UW70 probe (Bandelin Electronic GmbH, Berlin, Germany) at a setting of 3 × 60 s, 20% pulse, and 109 μm amplitude. A volume of 75 μL of the sonicated dilutions was immobilized on black polycarbonate membrane filters (pore size = 0.2 μm; Osmonics, Minnetonka, Minnesota). Cells were stained with 4′,6-diamino-2-phenylindole (DAPI) at a concentration of 0.5 μg/mL for 5 to 10 min. Bacterial cells were counted in 20 to 30 randomly chosen microscopic fields on each filter, giving a total of 700 to 1500 cells counted/filter. Cell counts were related to the wet volumes of the sediment slices and the chimneys. In treatments with larvae, the chimney dimensions were used to calculate the relative increase in oxic sediment volume (see above) and the relative increase in the number of bacteria in the oxic sediment volume.

Statistical analysis

One-way analysis of variance (ANOVA) was used to identify significant effects of % air saturation on the number of burrows and chimneys built by the larvae. Each experimental run delivered a single number of burrows and chimneys in each % air saturation treatment and was treated as a replicate. Homogeneity of variances was checked with Levene's test. Scheffé and Games–Howell post hoc tests were run for pairwise comparisons when variances were homogenous or nonhomogenous, respectively. Two-way ANOVA was used to account for the block design of the experiment whenever multiple values were obtained in a single treatment (i.e., depth of burrows, height of chimneys, O_2 uptake rates, and bacterial numbers). In these cases, the experimental run (block) was used as a 2nd fixed factor for the analysis. Significant interactions between block and % air saturation were not detected. The dependence of cumulative percentages of escaping, emerging, and dying larvae on % air saturation was analyzed using a χ^2 test. The dependence of the % gain in bacterial cell abundance in the oxic sediment volume of animal treatments on % air saturation also was analyzed using a χ^2 test. Multiple pairwise comparisons were made, so α (= 0.05) was Bonferroni-corrected ($\alpha/6 = 0.0083$).

Results

Larval performance

Burrows and chimneys were observed inside and on top of the sediments, respectively, within 24 h after introducing the larvae into the aquaria. Surface sculpturing of the sediment by the larvae was fully developed after 2 wk. Some of the burrows were filled with sediment and some of the chimneys had disappeared by the 3rd week of the experiments. Therefore, the 2 types of tubes were routinely quantified on day 14 of all experimental runs (Fig. 3). The number of U-shaped burrows was not affected by % air saturation (ANOVA, $F_{3,8} = 0.710$, $p = 0.573$), whereas the number of chimneys increased significantly as % air saturation decreased (ANOVA, $F_{3,8} = 6.306$, $p = 0.017$; Fig. 3A). The number of chimneys was significantly greater at 15% than at 100% air saturation (Scheffé, $p = 0.020$).

The depth of burrows and height of chimneys varied significantly with % air saturation (ANOVA, burrows: $F_{3,140} = 6.542$, $p < 0.0001$, chimneys: $F_{3,192} = 7.540$, $p < 0.0001$; Fig. 3B). Burrows were significantly less deep under all hypoxic conditions than at 100% air saturation (Games–Howell, 45% air saturation: $p = 0.050$, 30% air saturation: $p = 0.003$, and 15% air saturation: $p = 0.002$). Chimneys built at 15% air saturation were significantly taller than those built at all other air saturations (Games–Howell, 30% air saturation: $p = 0.013$, 45% air saturation: $p = 0.001$, and 100% air saturation: $p = 0.035$). At 100% air saturation, burrows reached through the black, sulfidic sediment layer (located at a depth of 4–7 mm) and extended down to a maximum depth of 25 mm. Under all hypoxic conditions, the deepest point of the burrows generally was in the sulfidic sediment layer, i.e., the burrows never reached >7 mm into the sediment. Chimneys did not persist in their size and shape over the 3 wk of the experiment. Instead, some of them decreased in height or disappeared completely only a few days after their construction. However, new chimneys were constructed until the end of the experimental incubation. Whether the larvae destroyed or ate the chimneys and what the larvae did after the removal of the chimneys could not be determined.

The cumulative proportion of escaped larvae was independent of % air saturation ($\chi^2 = 4.555$, df = 3, $p = 0.207$). At ≥30% air saturation, only a small number of larvae (<10%) escaped from the sediment compartment (Table 1). At 15% air saturation, the cumulative proportion of escaped larvae was 23.6%. Emergence of adult midges was independent of % air saturation ($\chi^2 = 0.317$, df = 3, $p = 0.957$) and was <13.1% in all % air saturation treatments. Mortality was independent of % air saturation ($\chi^2 = 5.827$, df = 3, $p = 0.120$). Mortality was ~25% at 45% and 100% air saturation, ~38% at 30% air saturation, and ~18% at 15% air saturation.

Spurts of larval undulation in burrows occurred every 3 to 4 min and lasted for ~20 s each (PS, unpublished data). Spurts of undulation in chimneys, inferred from O_2 concentration measurements made at the opening of the chimneys, were less frequent (every 7–8 min) but lasted longer (3–6 min). A current of oxygenated water was drawn into the chimney during each ventilation spurt, whereas anoxic conditions prevailed at the chimney opening during resting periods (Fig. 4).

O_2 uptake rates

Vertical O_2 concentration profiles were measured across the sediment–water interface (Fig. 5A, B), and O_2 microdistribution profiles were measured around chimneys (Fig. 5C). These profiles were used to calculate the oxic sediment volume (Table 2) and the diffusive O_2 uptake rates by sediments and chimneys (Fig. 6). O_2 penetration into the sediments decreased with decreasing % air saturation in the water column, but O_2 penetration was not affected by the presence or absence of larvae (Fig. 5A, B). The oxic sediment volumes were 2200, 800, 800, and 300 cm^3/m^2 for the 100%, 45%, 30%, and 15% air saturation treatments, respectively. The presence of chimneys increased the oxic sediment volume substantially (Table 2). The diffusive O_2 uptake rates by the sediments decreased significantly with decreasing O_2 concentration in the water column (ANOVA, control treatment: $F_{3,17} = 19.887$, $p < 0.0001$, animal treatment: $F_{3,75} = 108.213$, $p < 0.0001$; Fig. 6). Diffusive O_2 uptake rates by the chimneys also were significantly influenced by % air saturation (ANOVA, $F_{2,20} = 16.829$, $p < 0.0001$; Fig. 6). Rates were the same at 45% and 30% air saturation, but rates were significantly lower at 15% than at 30% and 45% air saturation (Scheffé, 30% air saturation: $p <$

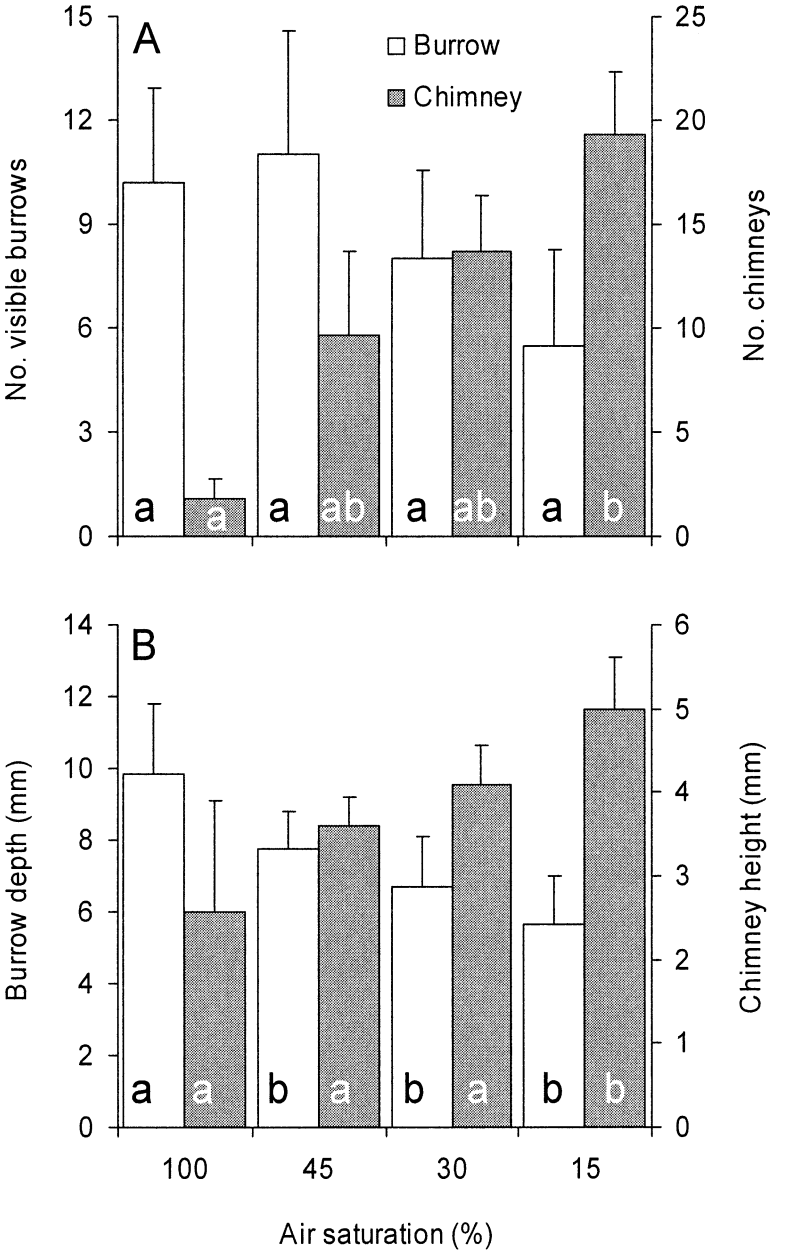

FIG. 3. Mean (+1 SE) number (A) and depths and heights (B) of burrows and chimneys of *Chironomus riparius* larvae. Within each tube type, columns that share the same letter are not significantly different.

0.0001, 45% air saturation: $p = 0.007$). Moreover, within each air saturation treatment, the diffusive O_2 uptake per unit interface area was always significantly higher for chimneys than for the sediment surface (control and animal treatments) (ANOVA, 45% air saturation: $F_{2,22} = 4.412$, $p = 0.024$, 30% air saturation: $F_{2,25} = 32.112$, $p < 0.0001$, and 15% air saturation: $F_{2,38} = 11.112$, $p < 0.0001$).

The representative H_2S measurements in the 100% and 15% air saturation treatments showed that free H_2S was not present in the sediments

TABLE 1. Mean (±1 SE) % of larvae added to microcosms that escaped, emerged, or died in laboratory microcosms under different O_2 (% air saturation) conditions. Treatments sharing the same letter within a column were not significantly different.

	Cumulative %		
% air saturation	Escaped larvae	Emerged larvae	Dead larvae and pupae
100	7.3 ± 2.0[a]	10.7 ± 5.9[a]	27.3 ± 8.9[a]
45	7.5 ± 3.5[a]	11.8 ± 7.9[a]	25.3 ± 8.3[a]
30	9.4 ± 5.0[a]	13.1 ± 7.1[a]	37.7 ± 12.7[a]
15	23.6 ± 12.3[a]	10.5 ± 6.0[a]	18.2 ± 9.0[a]

or in the water column in animal or control treatments (data not shown). Vertical profiles of pH typically showed a decrease from pH 8.3 in the water column to pH 7.5 in the sediments (data not shown).

Bacterial abundance

Bacterial abundance in the sediment decreased significantly with decreasing % air saturation in control treatments (ANOVA, $F_{3,8} = 8.874$, $p = 0.006$; Fig. 7), whereas bacterial abundance in the sediment increased significantly with decreasing % air saturations in the animal treatments (ANOVA, $F_{3,35} = 7.668$, $p < 0.0001$). Percent air saturation did not affect total bacterial counts in chimneys (ANOVA, $F_{2,22} = 2.569$, $p = 0.099$). However, total bacterial counts were significantly higher in chimneys (animal treatments) than in sediments (control treatments) at 30% and 15% air saturation (ANOVA, 30% air saturation: $F_{2,16} = 10.640$, $p = 0.001$, 15%: $F_{2,18} =$

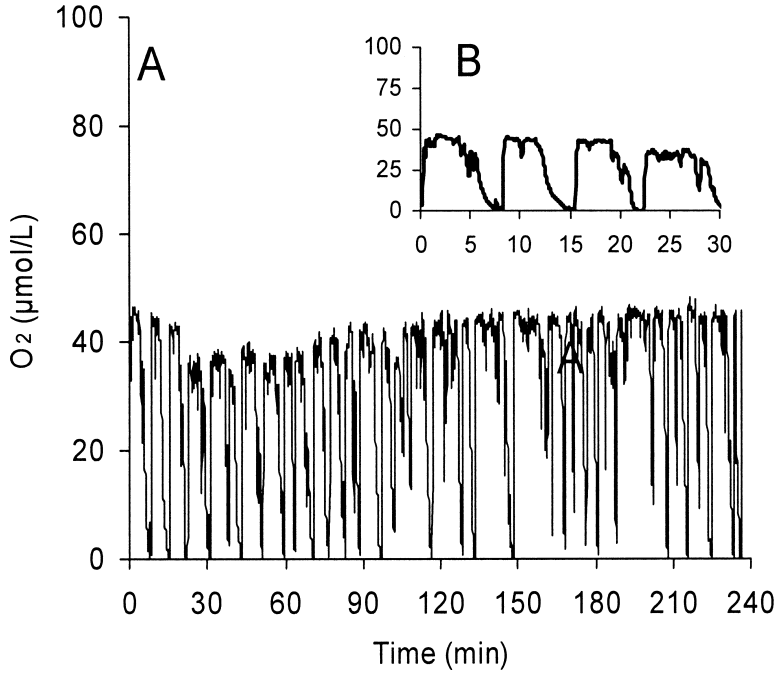

FIG. 4. A.—Time series of O_2 concentrations at the opening of a chimney in a 15% air saturation (~40 µmol O_2/L) treatment. The O_2 microsensor was positioned level with the rim of the 5-mm-high chimney (Fig. 2C). At the start of each larval ventilation spurt, oxygenated water was drawn into the chimney and passed the microsensor tip; between spurts, anoxic conditions prevailed at the opening of the chimney. B.—Expansion of the temporal scale showing the first 30 min from (A).

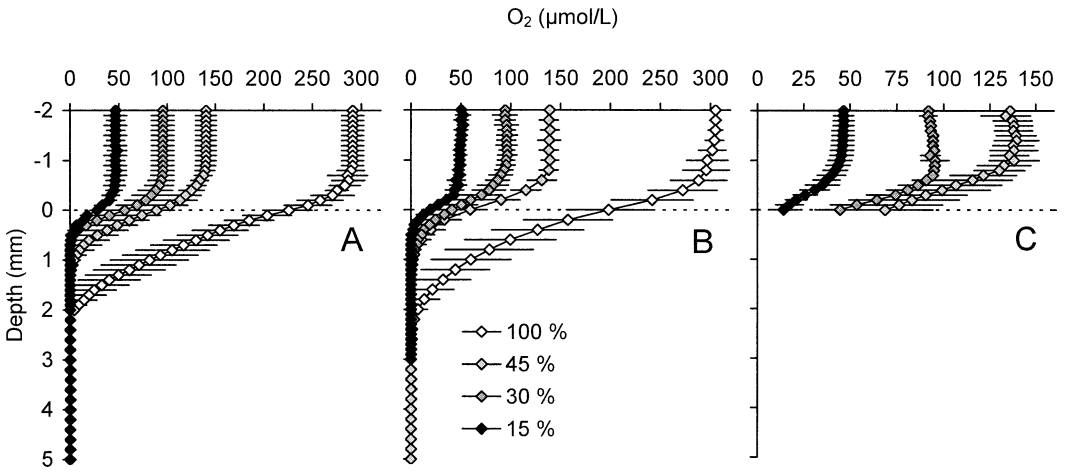

FIG. 5. Mean (±1 SE) O_2 concentrations across the sediment–water interface in control (A) and animal treatments (B) and toward the surfaces of chimneys in hypoxic animal treatments (C). Dotted lines in A and B indicate the sediment–water interface. Dotted line in C indicates the surface of a chimney.

9.120, $p = 0.002$). Moreover, chimneys increased the abundance of bacteria in oxic sediment of the animal treatments (Table 2). The relative gain was dependent on air saturation (χ^2 test, $\chi^2 = 28.39$, df = 3, $p < 0.0001$; Table 2).

Discussion

Importance of chimneys for larvae

Chironomus riparius larvae switched gradually from burrow to chimney construction when exposed to a range of decreasing % air saturations. The burrows inside the sediment became less deep, whereas the chimneys on top of the sediments became more abundant and taller. However, small chimneys were observed even at 100% air saturation. Therefore, we conclude that *C. riparius* chimney-projecting behavior has no threshold % air saturation below which the behavior is induced (Kon and Hidaka 1983).

The microsensor measurements showed that the O_2 concentration at the sediment–water interface (i.e., at the level of burrow openings) was ~50% that of the mixed water column, regardless of the % air saturation in the water column (Fig. 5A, B). Conversely, the O_2 concentration at the rim of actively irrigated chimneys was about the same as that of the mixed water column (Fig. 4). Thus, chimneys pierced through the 400- to 600-µm-thick diffusive boundary layer (DBL) and made higher concentrations of O_2 available for the larvae (Kon and Hidaka 1983, Jørgensen and Revsbech 1985). The apparent O_2 concentration at the sediment–water interface seemed to determine the size of the chimney to be constructed. However, whether the larvae respond

TABLE 2. Mean (±1 SE) % changes in sediment O_2 dynamics and bacterial distribution associated with the presence of chironomid chimneys in laboratory microcosms. Percent air saturation treatments sharing the same letter were not significantly different.

	Chimney-related gain (%)		
% air saturation	Sediment–water interface area	Oxic sediment volume	Bacterial abundance in oxic sediment (relative to sediment bacteria in animal treatment)
100	2.3 ± 0.3	0.5 ± 0.1	0.0 ± 0.0[a]
45	11.8 ± 2.2	7.4 ± 1.4	7.4 ± 1.2[b]
30	8.3 ± 3.0	11.4 ± 1.9	14.6 ± 1.8[b]
15	31.5 ± 7.7	52.4 ± 12.8	57.5 ± 5.0[c]

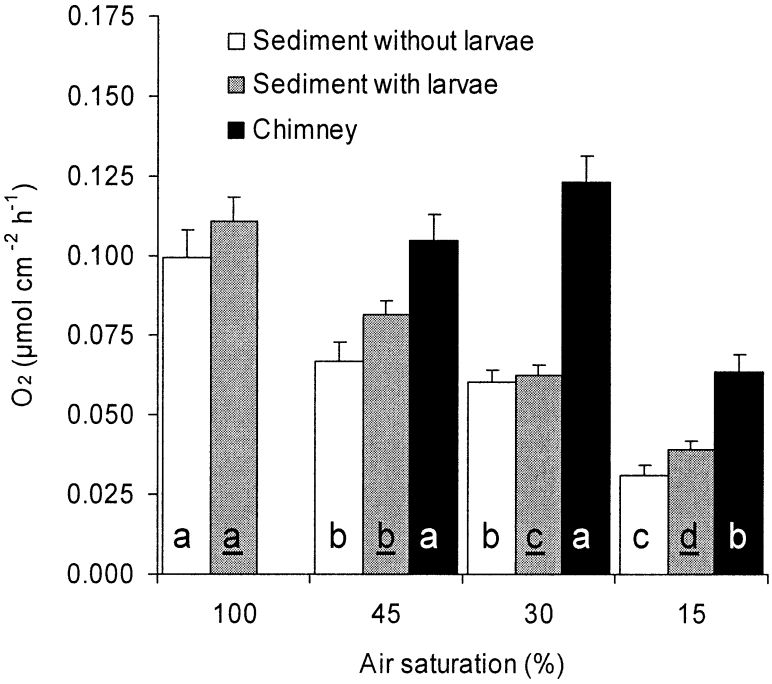

FIG. 6. Mean (±1 SE) O_2 uptake rates of sediments and chimneys in laboratory hypoxia experiments. Rates were calculated from the concentration profiles presented in Fig. 5. Within each sample type, columns that share the same letter are not significantly different.

to the actual O_2 concentration or to a proxy of hypoxic conditions (e.g., CO_2 or H_2S) is unclear. O_2 depletion may favor the production of H_2S close to the sediment surface (Holmer and Storkholm 2001), and H_2S could make the larvae shift their burrows from within the sediment to above the sediment surface, but the microsensor measurements showed that H_2S was not present in our sediments (data not shown). CO_2 also can be ruled out as a proximate factor influencing chimney construction in our study because hypoxic conditions were achieved artificially by blowing O_2 out with N_2 gas. Thus, the low O_2 concentrations should not have been associated with elevated CO_2 concentrations.

One might ask why the chimneys were much higher (up to 8 mm) than the thickness of the DBL (0.4–0.6 mm), and why their height increased with decreasing % air saturation. The smallest chimneys found were tall enough to pierce through the DBL. One reason could be that the thickness of the DBL is inversely correlated with the % air saturation of the water column under natural conditions. Hypoxia near the sediment surface typically develops under stagnant conditions (Gray et al. 2002), and low flow velocities lead to the expansion of the DBL from a few hundred µm to >1 mm (Jørgensen and Revsbech 1985). Thus, chironomid larvae may somehow associate the perceived % air saturation with a certain thickness of DBL to be penetrated. If so, the inherent response by the larvae to natural DBL dimensions was meaningless in our experiments because the DBL always had the same thickness.

Chimneys also may provide larvae with food. Larvae might elongate their burrows above the sediment surface if they were in need of bacterial biomass that could be scraped from the burrow walls (Olafsson and Paterson 2004). Three lines of evidence support this hypothesis. 1) Extrapolation of the O_2 microprofiles indicated that the 500-µm-thick chimney walls were not completely anoxic (Fig. 5C), and the lumens of the chimneys were flushed periodically with oxygenated water because of larval undulation (Fig. 4). Chimneys might allow the attachment and growth of aerobic bacteria rather than the anaerobic bacteria that would grow in less oxygenated burrows. Aerobic bacteria have shorter

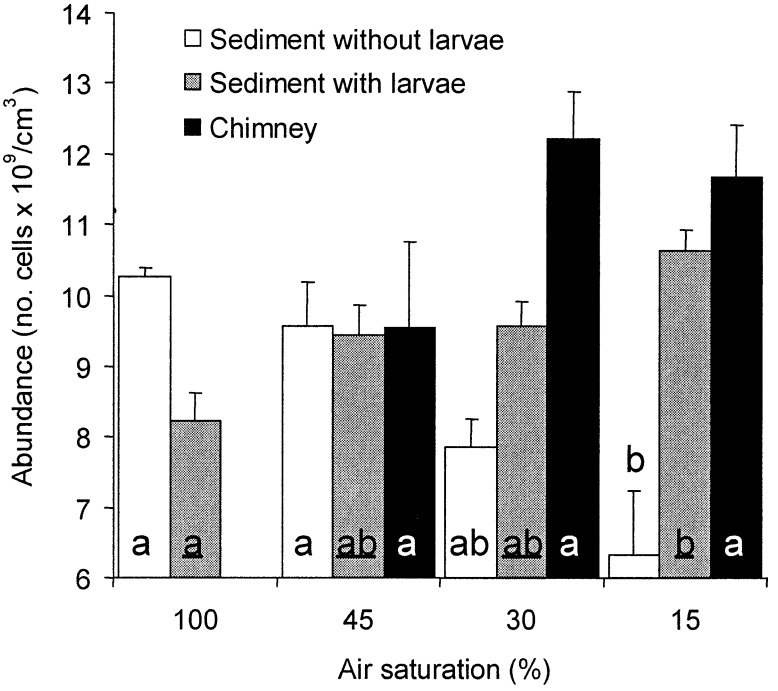

FIG. 7. Mean (±1 SE) bacterial abundance at the sediment surface (top 0–2 mm) and in chimneys in laboratory hypoxia experiments. Within each sample type, columns that share the same letter are not significantly different.

doubling times than anaerobic bacteria. 2) Bacterial abundance per unit sediment volume was generally higher in chimneys than in the feeding layer of the sediment (i.e., upper 0–2 mm; Stief and de Beer 2002, Olafsson and Paterson 2004). 3) Some of the chimneys disappeared a few days after they had been constructed. These data and observations suggest that chimneys might play a role in the diets of chironomid larvae. Construction of a bacterial colonization site with distinctly different conditions (i.e., O_2 supply) than the sediments and harvesting bacterial biomass after incubation could be considered a gardening strategy (Hershey et al. 1988, Plaganyi and Branch 2000). Direct observation of larval feeding and tracing of fluorescent particles (from chimney to larval gut) could provide evidence for this new hypothesis.

If *C. riparius* do use chimneys for dietary purposes, then one must ask why the larvae apply this strategy only under hypoxic conditions. One possible reason could be that low O_2 concentrations increase the energy requirements of the larvae (Bairlein 1989, Penttinen and Holopainen 1995). New metabolic costs arise from the increased undulation activity required to obtain an adequate O_2 supply (Leuchs 1986), and larvae require energy for foraging and burrow construction. Energy might be conserved if larvae were to use the chimney itself as a food resource rather than expend additional energy collecting food further away from the burrows.

Importance of chimneys for the benthic microbial community

Chironomid chimneys were a residual oxic sediment compartment even under severe hypoxia in our experiment. Chimneys made up 50% of the oxic sediment volume in our experimental aquaria at 15% air saturation, they were densely colonized with bacteria, and they had high O_2 uptake rates. Therefore, aerobic microbial pathways (e.g., nitrification or sulfide oxidation) probably were more important in sediments when chimneys were present than when they were absent. Our study did not address possible aerobic microbial pathways, but we did quantify the diffusive O_2 uptake by the sediment surface and the chimneys. This uptake is a composite

of overall microbial O_2 consumption and the chemical re-oxidation of reduced compounds (Canfield et al. 1993). Diffusive O_2 uptake by the flat sediment surface decreased with decreasing % air saturation in the water column regardless of the presence of larvae. This positive correlation probably was caused by the decreasing volume of sediment in which oxidative respiration and chemical oxidation could take place (Fig. 5A, B). However, chimneys had significantly higher diffusive O_2 uptake rates than the sediment surface at all % air saturations. The erect shape, hollow structure, and periodic irrigation of the chimneys made these biogenic structures accessible to dissolved O_2 in the water column. Moreover, the chimneys are likely to be enriched in easily degradable organics from larval saliva excretions used for stabilization of the chimney (Leuchs and Neumann 1990) and from trapped suspended organic particles (Soltwedel and Vopel 2001). The dense bacterial colonization on chimneys might have been both a consequence of O_2 and organic substrate availability and a cause of the significantly higher diffusive O_2 uptake rates of the chimneys than sediment (Eckman 1985). In any case, chimneys contribute to the patchy distribution of bacterial biomass and small-scale O_2 dynamics in the sediment (Eckman 1985, Soltwedel and Vopel 2001).

Larvae changed their feeding behavior when exposed to hypoxic conditions. Deposit-feeding at the sediment surface was visibly reduced at lower % air saturations. In normoxic conditions, *C. riparius* larvae collect and ingest organic particles deposited on the sediment surface (Rasmussen 1984). Larvae reduce the abundance and metabolic activity of particle-attached bacteria within the feeding layer, and they reduce the diffusive O_2 uptake by the sediment (Stief and de Beer 2002, Altmann et al. 2004). At 100% air saturation in our study, bacterial abundance in the sediment was lower when larvae were present than when they were absent (Fig. 7), but the diffusive O_2 uptake rate did not differ between inhabited and uninhabited sediments. The addition of organic food particles to the sediments in both inhabited and uninhabited sediments may have obscured larval effects on the metabolic activity of sediment bacteria.

Bacterial abundances in inhabited sediments in hypoxic conditions were similar to bacterial abundances in uninhabited sediments in normoxic conditions. Larvae did not reduce bacterial abundance in the sediment in hypoxic conditions, probably because they showed reduced grazing at the sediment surface. If reduced grazing in hypoxic conditions were the only reason for similar bacterial abundances in inhabited hypoxic sediment and uninhabited normoxic sediment, then bacterial abundances should have been similar in inhabited and uninhabited hypoxic sediments. However, under hypoxic conditions, bacterial abundance was lower in uninhabited sediment than in inhabited sediment. Therefore, we assume that physical changes in the sediment were induced by the larvae and contributed to the observed pattern of bacterial abundance. Hypoxia causes other sediment-dwelling macrofauna to favor advective over diffusive water exchange with the water column because of compensatory bioirrigation (Forster et al. 1995). This shift also should occur for chironomids that increase their ventilation activity when exposed to low O_2 concentrations (Leuchs 1986). Advective transport of O_2 and nutrients stimulates the metabolic activity of bacteria that are otherwise diffusion limited (Ziebis et al. 1996). Thus, inhabited sediment may have been a favorable site for bacterial colonization because of intense irrigation in the absence of significant grazing effects.

Altered behavior of *C. riparius* larvae under hypoxic conditions causes spatial rearrangement of sediment bacteria and diffusive O_2 uptake by the sediment. Chimney-projecting behavior creates distinct biogenic structures with high bacterial abundance and high diffusive O_2 uptake. In contrast, reduced feeding activity under hypoxic conditions leads to high bacterial abundance, but low diffusive O_2 uptake in the surficial feeding layer. We hypothesize that the particular geometry of chironomid chimneys (i.e., an erect and hollow structure that is periodically irrigated with oxygenated water) favors the growth of metabolically active aerobic bacteria. Thus, chimneys may not only facilitate the larval acquisition of both O_2 for respiration, but also of microbial biomass for food.

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