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Behavioural, developmental and morphological responses of *Chironomus* gr. *thummi* larvae (Diptera, Nematocera) to aquatic pollution

Almut Gerhardt[1] & Luc Janssens de Bisthoven[2]
[1]*Lund University, Dept of Chemical Ecology and Ecotoxicology, Ecology Building, Sölvegatan 37, S-223 62 Lund, Sweden*
[2]*Catholic University of Leuven, Ecology and Aquaculture, Naamsestraat 59, B-3000 Leuven, Belgium*

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Abstract

Populations of *Chironomus* gr. *thummi* larvae from two differently polluted lowland streams (Dommel, high cadmium and zinc; Ijse, medium copper and organic xenobiotics) were screened for behavioural and morphological responses to pollution. Behaviours such as locomotion (swimming and looping), respiration movements (ventilation) and inactivity were quantified with impedance conversion technique. Chironomids from the Dommel were more active than larvae from Ijse. In Ijse, deformed larvae showed less emergence, less locomotion and more ventilation than non-deformed larvae. In Dommel, deformed and normal larvae were equally fit (behaviour, emergence).

1. Introduction

Benthic macroinvertebrates have been attractive targets for biological monitoring because they are a diverse group that react strongly and, often predictably to human influences on aquatic ecosystems (Cairns & Pratt, 1993). Chironomid larvae (Diptera, Nematocera) have been used as indicators of environmental stress (e.g. community responses (Saether, 1979; Armitage & Blackburn, 1985) and morphological deformities (e.g. Hare & Carter, 1976; Van Urk *et al.*, 1992) as they often exhibit a high species diversity and high larval densities (Rosenberg, 1992).

A relationship between morphological deformities in benthic Chironomidae and micropollutants in aquatic sediments is suggested by many field studies (Hare & Carter, 1976; Wiederholm, 1984; Warwick *et al.*, 1987; Warwick, 1990; Dermott, 1991; Van Urk *et al.*, 1992; Janssens de Bisthoven *et al.*, 1992; Janssens de Bisthoven *et al.*, 1995).

Behavioural responses to chemical stress are among the first and most sensitive parameters (Warner, 1967), which integrate both biochemical alterations at the sub-organism level and ecologically relevant changes at the whole-organism level (Beitinger, 1990; Scherer, 1992).

The impedance conversion technique has been shown to be an appropriate means of detecting and quantifying different types of behaviour, e.g. ventilation, locomotion (swimming, looping) and feeding in different aquatic invertebrate and vertebrate species (Gerhardt *et al.*, 1994). Significant behavioural changes due to acute and chronic metal pollution have been found. Examples include increased ventilation and decreased activity of *Gammarus pulex* (Crustacea) due to Pb and Cu at sublethal levels (Gerhardt, 1995), decreased activity and increased ventilation of *Glyptotendipes pallens* (Chironomidae) due to Cd (Heinis *et al.*, 1990) and decreased activity of *Leptophlebia marginata* (Ephemeroptera) due to Cd (Gerhardt, unpubl.).

2. Objectives

The objectives of the present study were:

(1) to characterise behavioural patters of *Chironomus* gr. *thummi* from different polluted sites with the help of impedance conversion;
(2) to compare deformed and non-deformed chironomids with respect to their behaviour and emergence in different polluted waters.

3. Material and methods

3.1. *Sampling sites*

Fourth instar *Chironomus* gr. *thummi* larvae, sediment and detritus were sampled in July 1994 with a 500 μm mesh-size handnet by sweeping through the superficial layer of the river sediment along the banks of two lowland streams in Belgium, the Ijse (NEI) and the Dommel (DO). Site characteristics are given in Table 1.

3.2. *Deformity screening*

From each population, fourth instar larvae (30 per site for the emergence experiment, 118 for NEI and 14 for DO for the behaviour experiment) were preserved in 70% ethanol for subsequent screening of different types of morphological deformities according to Janssens de Bisthoven *et al.* (1995). Chironomid larvae were also selected (magnification 12–50×) *in vivo* for normal and deformed menta. Menta were considered deformed when bearing a gap (so-called Köhn gap, Köhn & Frank, 1980), lacking a lateral tooth or when the medial tooth was split or fused with the first inner lateral tooth (Fig. 1). The *in vivo* selection of normal and deformed larvae was done prior to the emergence and behaviour experiments (*cfr. infra*).

3.3. *Behaviour experiments*

The behavioural pattern of normal and mentum-deformed larvae was measured at room temperature and under daylight within 24 h after field sampling. Each measurement consisted of two larvae (one deformed and one non-deformed) placed individually in their test chambers filled with 2 ml water and a few detritus particles from their stream. Five to eight replicates of this experiment were done for each sampling site. After an acclimation time of 10 minutes to the test chamber, the behavioural pattern of the larvae was recorded continuously for a period of 440 sec. with a sampling frequency of 50 Hz. Fifty percent of the larvae in the deformed group had a Köhn gap and 50% had either a split or fused medial tooth or a lower number of teeth.

The principle of the impedance conversion method is that movements of the organisms in the electrode chambers change the resistance of the alternating current (impedance) which is generated between a pair of electrodes placed opposite each other along the chamber walls. Changes in impedance are measured by a second non-current producing pair of electrodes (four-electrode principle) (Gerhardt *et al.*, 1994). The analog signals are then transformed into digital signals and different types of behaviour can be distinguished and quantified by their signal pattern using amplitude and frequency as descriptors with help of Super Scope software (GW Instruments) on a Macintosh LC III computer.

3.4. *Emergence experiments and metal concentrations*

Fourth instar *Chironomus* gr. *thummi* larvae sampled in both sites were assigned to two groups (mentum-deformed and mentum-non-deformed) and kept in experimental tanks at a density equivalent to natural densities (ca. 700 individuals m^{-2}). The emergence of the non-deformed larvae (replicates = 2, $n = 15$) and the deformed larvae (replicates = 4, $n = 15$) was followed daily (at 10:00 am) in the tanks, which contained water (1 litre) and sediment (130 g DW/tank) from the respective sampling sites. No additional food was given. The Ijse sediment contained 3.6 + SD 0.8% organic matter (loss on ignition, 3 replicates) and the Dommel sediment, 6.2 + SD 0.5% AFDW. The experiments were performed at 16 °C with a 16h:8h light:dark regime including 30 min twilight.

Concentrations of Cd, Cu, Pb and Zn were determined in 4th instar larvae (3 to 5 pooled larvae per analysis) according to Timmermans & Walker (1989) and Janssens de Bisthoven *et al.* (1992).

3.5. *Statistical analysis*

Behavioural data were generated by simultaneous pairwise measurements of two animals each (e.g. deformed and non-deformed) and analysed pairwise with Student's *t*-tests to detect significant differences in behaviour, such as time spent on locomotion, time spent on ventilation, number of ventilation phases and dominant ventilation frequency.

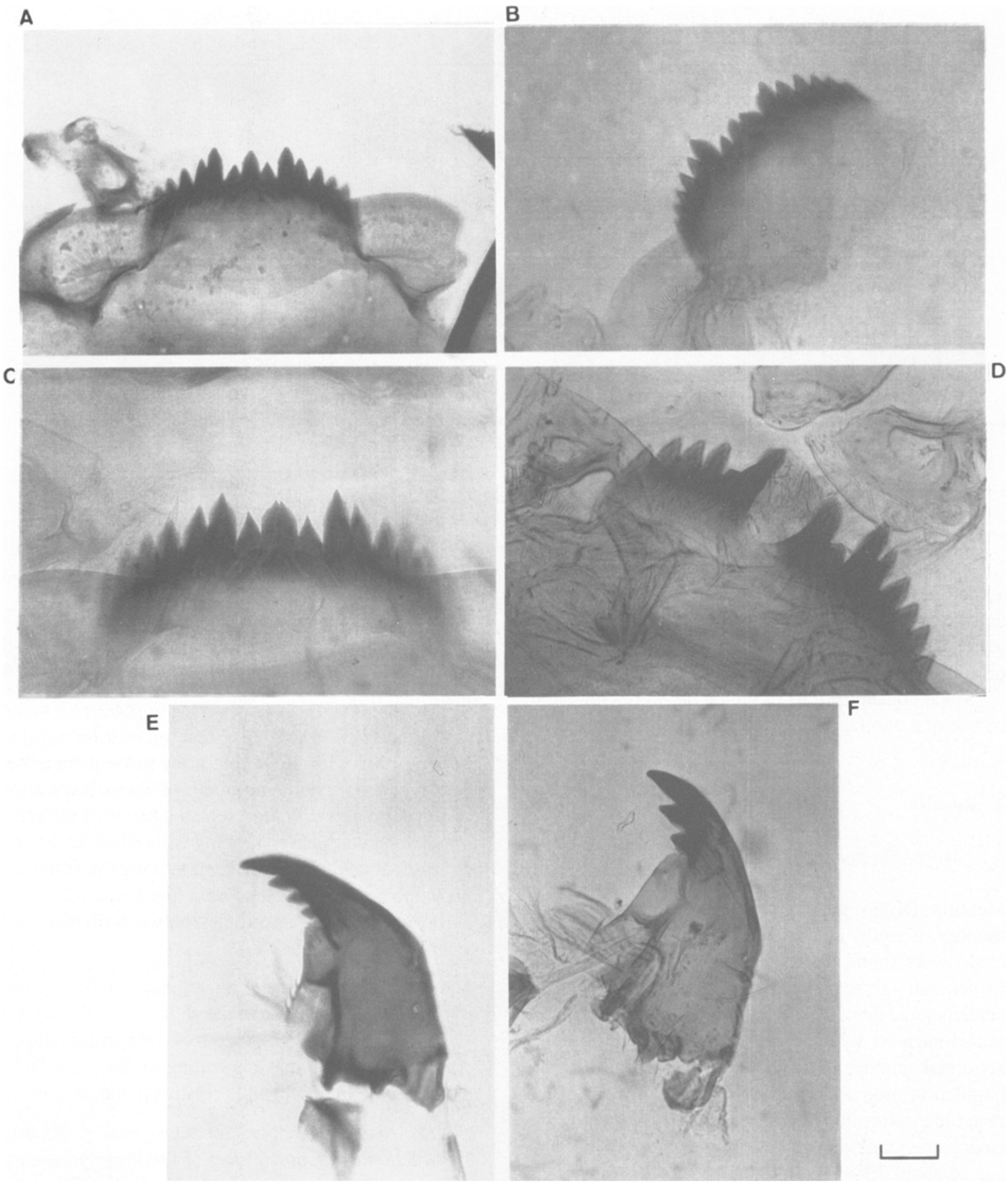

Fig. 1. *Chironomus* gr. *thummi* instar 4, (A) normal mentum, (B) mentum medial tooth fused to first inner lateral tooth, (C) mentum split medial tooth, (D) mentum Köhn gap, (E) normal mandible and (F) mandible missing an inner tooth. Scale line = 43 μm (A, B) and 28 μm (C–F).

Table 1. Characteristics of the sampling sites

River watershed coordinates	Ijse (NEI) tributary of Dijle, Schelde basin 4°37′25″ E, 50°48′46″ N		Dommel (DO) Meuse basin 5°25′23″ E, 51°14′21″ N	
Water quality parameters(1)				
pH	7.6		6.6	
Cond.(μS/cm)	726		344	
NH_4^+ (mg/l)	2.95		0.66	
NO_2^- "	0.35		0.15	
NO_3^- "	8.72		3.21	
o-P "	0.82		0.09	
Metals(2)	*Water ($\mu g/l$)*	*Sediment ($\mu g/kg$)*	*Water ($\mu g/l$)*	*Sediment ($\mu g/kg$)*
Cd	n.d.	0.4	7.2	25.4
Cu	6.0	22.0	7.0	9.0
Pb	12.0	48.0	13.0	25.0
Zn	20.0	155.0	270	450.0
As	1.5	3.2	12.5	24.0
Hg	0.15	0.09	0.75	1.0

(1) From IHE (1985); (2) from Vlaamse Milieumaatschappij (unpubl.).

The cumulative data on the number of emerged adults per day and tank were calculated for the deformed and non-deformed larvae and compared pairwise with the Student's t-test for dependent samples.

Metal concentrations in the larvae were compared by use of a one-factor ANOVA (site).

4. Results

4.1. *Morphological responses*

Mentum (Köhn gap, split medial tooth, changed number of teeth) and mandible-deformities (missing teeth) were found in larvae from both sites (Fig. 1). In general, larvae from both sites showed a similar percentage of deformed larvae, about 7–14% for deformities such as Köhn gaps, split mentum tooth and abnormal number of teeth (the last two only in the population used for the behaviour experiment), and mandible deformities. In the population used for the emergence experiment a tendency to a higher percentage of about 30–43% was noted for deformities such as split medial teeth and abnormal number of teeth. No deformities were found in the antennae, the premandibles and the pecten epipharyngis.

4.2. *Characterising the behavioural pattern of* Chironomus *gr.* thummi

With the help of the impedance conversion method the following types of behaviour were specified and quantified (Fig. 2):

(1) Locomotion consisted either of swimming, i.e. wriggling with the whole body at frequencies of 2.4 to 4.5 Hz and amplitudes of about 200 mV or of looping, which represented a series of different movements such as looking for a place to set the head, attaching the head and moving the abdomen to the new place. This was a highly multifrequent behaviour with amplitudes between 50 and 100 mV.

(2) Ventilation consisted of regular almost monofrequent undulations of the abdomen of the sessile larva within a range of 1.8 to 4.5 Hz and an amplitude of 50–100 mV, depending on the location of the larvae in the chamber relative to the electrodes.

(3) Inactivity was defined for all signals $\leq$ 20 mV, which was the noise level of the impedance converter.

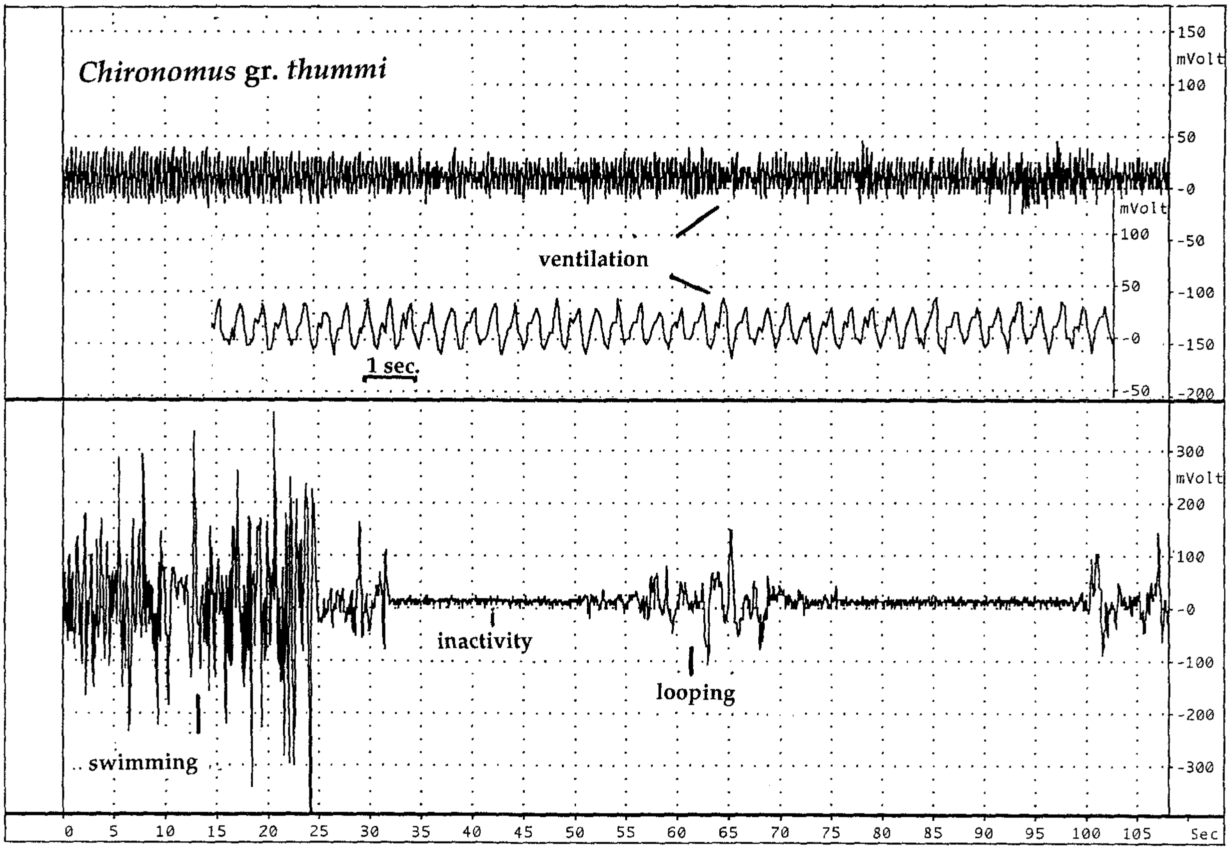

Fig. 2. Examples of different behaviours of *Chironomus* gr. *thummi* larvae measured with impedance conversion technique.

4.3. *Comparisons of the behaviour of deformed and non-deformed larvae*

The behaviour of larvae from NEI differed significantly from that of the larvae from DO, independently of mouth part deformities by more locomotion and less inactivity of the DO-larvae ($p = 0.025$) (Fig. 3).

In NEI, larvae with morphological deformities of the mentum showed significantly less activity ($p < 0.05$) and spent significantly more time on ventilation ($p < 0.025$) with more ventilation phases ($p = 0.057$) than non-deformed larvae. In DO, no differences in the behaviour of deformed and non-deformed larvae were found (Fig. 3).

4.4. *Emergence and metal concentrations*

In NEI, non-deformed larvae emerged significantly better than deformed larvae ($p < 0.001$). In DO, however, no significant differences were found in the emergence curves of both larval groups, though deformed larvae tended to emerge better than normal larvae ($p = 0.060$) (Fig. 4). The overall number of emerged imagos from deformed larvae in NEI was significantly less ($p < 0.001$) than in DO, whereas the non-deformed larvae in both sites emerged equally well ($p = 0.410$).

In DO, the concentrations of Cd and Zn in the larvae were significantly higher than in NEI (Cd: $p = 0.003$, $F = 10.9$; Zn: $p = 0.030$, $F = 5.2$). Lead concentrations in both populations were similar, whereas copper concentrations, were elevated in organisms from NEI compared to DO ($p = 0.040$, $F = 10.1$; Table 2).

5. Discussion

The deformity percentages of fourth instar *Chironomus* gr. *thummi* larvae from the Ijse and the Dommel can be considered as medium to high, as compared to data on *Chironomus* larvae from the literature (e.g. Warwick, 1980; Cushman, 1984: <5%, respectively

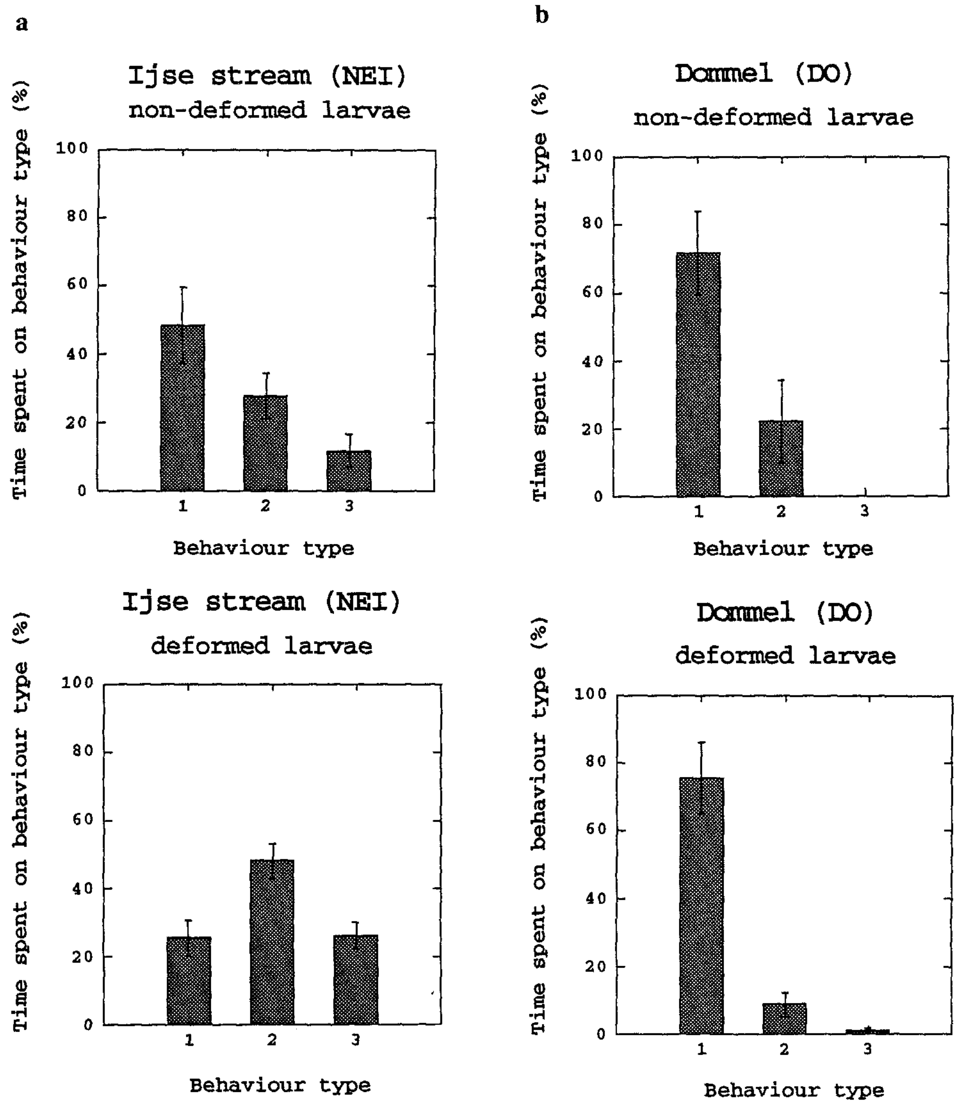

Fig. 3. Behavioural pattern (1. locomotion, 2. ventilation, 3. inactivity) of non-deformed and deformed larvae of *Chironomus* gr. *thummi* in (a) NEI and (b) DO. The values represent means (n = 5 to 8) and standard errors.

Table 2. Metal concentrations (means ± sd) in non-deformed instar 4 larvae of *Chironomus* gr. *thummi*. n = number of samples, each sample consisting of 3 to 5 pooled animals

Stream	Site	n	Cd $x \pm$ sd	Cu $x \pm$ sd	Pb $x \pm$ sd	Zn $x \pm$ sd
			---mg kg^{-1} DW---			
Ijse	NEI	14	0.9 ± 0.8	81.3 ± 10.4	32.7 ± 5.9	440.7 ± 38.2
Dommel	DO	14	6.8 ± 1.7	44.6 ± 4.5	27.5 ± 5.6	681.6 ± 98.5

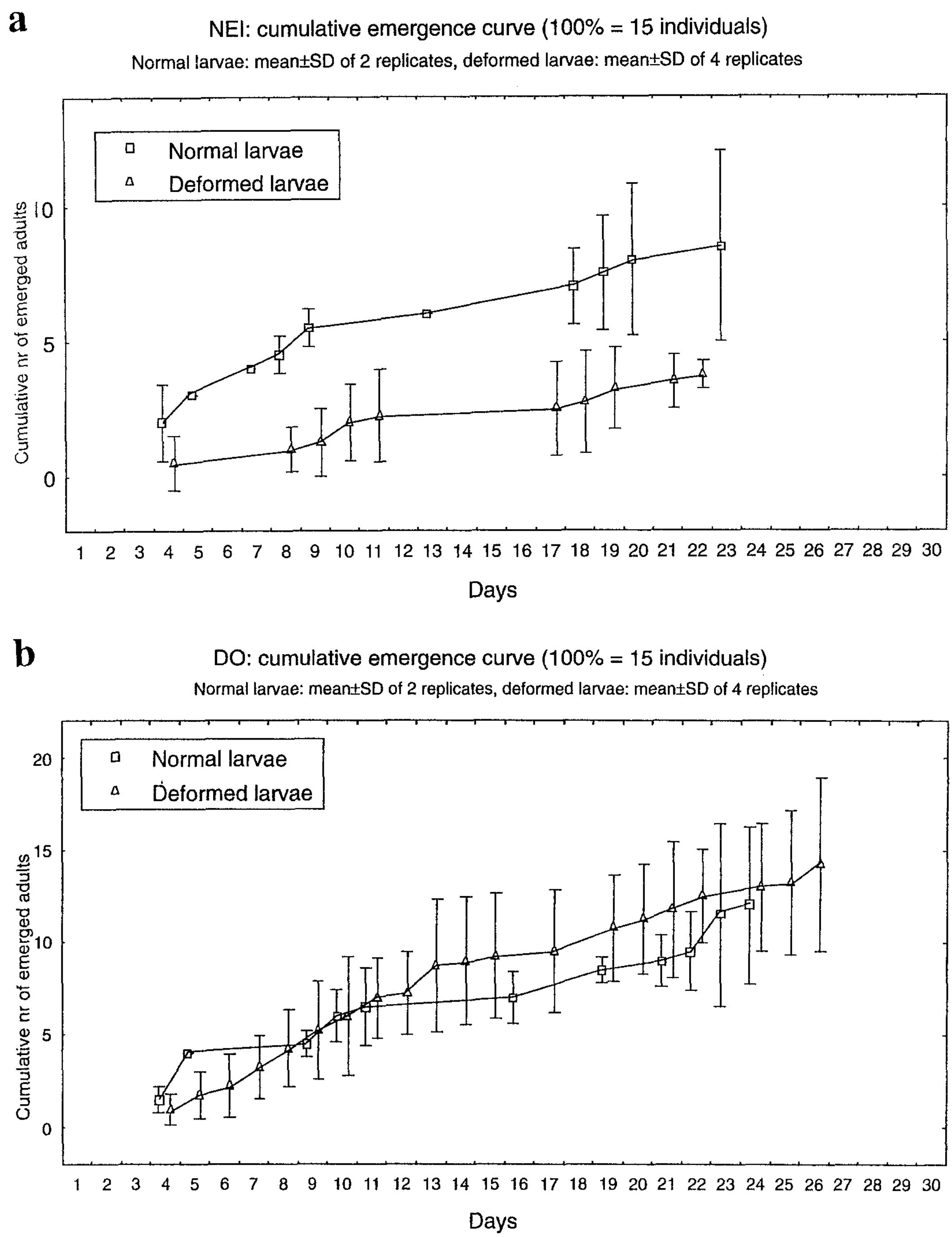

Fig. 4. Emergence of non-deformed and deformed *Chironomus* gr. *thummi* larvae in (a) NEI and (b) DO.

heavy metals, pesticides and eutrophication; coal liquid; Köhn & Frank, 1980: <40%, industry, heavy metals). The *in situ* deformity results showed that the sediments of the two sites contained sublethal concentrations of toxicants which have acted chronically on the larvae prior to sampling. Field relations between micropollutants and deformities in Belgian lowland rivers, including the Ijse and the Dommel are reported in Janssens de Bisthoven *et al.* (1995). The combined use of deformities in the field and *in vitro* assessment of a condition endpoint (emergence) and behaviour measurements allowed to further refine the response of the species to aquatic pollution. As Reynoldson & Metcalfe-Smith (1992) pointed out, functional tests tend to be more sensitive than community-based measurements, as they largely operate at the level of the individual organism.

Chironomus gr. *thummi* showed three different types of behaviour in the electrode chambers with remote electrodes allowing free movements: ventilation, locomotion (swimming, looping) and inactivity. Other studies on the behavioural pattern of chironomids using an impedance conversion technique were performed with *Glyptotendipes pallens*, filter-feeding chironomids sitting in their tubes with the electrodes placed close to the tubes (Heinis *et al.*, 1990). *Chironomus riparius* larvae are tubiculous collector-gatherers of detritus (Rasmussen, 1984). They feed by extending the head and posterior parts of the body outside the tube while using the posterior prolegs to maintain contact with the inner surface of the tube (Berg, 1995). Their excellent swimming ability has been used by several authors as an indicator of sublethal response to stress (visual observation): Cushman & McKamey (1981) found decreasing activity of *Chironomus tentans* upon exposure to acridine and quinoline. Similarly, Detra & Collins (1991) categorised 4 degrees of swimming activity in *Chironomus riparius* and were able to find a relation between exposure time to parathion, choline esterase inhibition and decreased activity. Pascoe *et al.* (1989) reported an increased time spent on ventilation and a decreased time spent on feeding in *Chironomus riparius* upon exposure to 10 mg Cd/l.

Larvae from DO generally showed a higher locomotory activity than larvae from NEI, which could either be a sign of increased fitness due to adaptation to the pollution (Klerks & Weis, 1987) or a sign of increased "escape"-behaviour to avoid the polluted site. This should be tested by drift experiments on the respective sites.

In water from NEI, a stream polluted by agriculture, domestic pollution and metals (Cu), larvae with a deformed mentum spent less time on locomotion and more time on ventilation than non-deformed larvae. Less activity may imply a lower fitness, with respect to colonizing new substrates, or inter- and intraspecific competition (Krantzberg, 1992). Vuori (1994) found that Cd-stressed larvae of *Hydropsyche* spp. were less active and spent less time on fighting in competition situations. Lower locomotory activity due to metal stress was also found for *Gammarus pulex* exposed to Pb and Cu (Gerhardt, 1995), and for *Leptophlebia marginata* in response to Cd, Fe and Pb (Gerhardt, 1992 and 1994) and for *Glyptotendipes pallens* due to Cd (Heinis *et al.*, 1990). Decreased activity may be a compensation for the higher energy expenditure caused by pollution stress.

Increased ventilation can be seen as a means of increased oxygen consumption for metabolic processes. Coping with pollution stress requires additional energy demanding physiological processes, e.g. metallothionein production.

From both the emergence and behaviour experiments it appeared that deformed larvae in NEI are less fit than non-deformed larvae, which implies that they are more affected by sublethal toxic stress in the aquatic ecosystem. Deformed larvae often tend to show higher metal concentrations (Pb, Cu, Cd) than normal larvae (Janssens de Bisthoven *et al.*, 1992; Janssens de Bisthoven, 1995), which could explain their lower fitness. Impaired emergence has been related to pollution stress by Wentsel *et al.* (1978), Pascoe *et al.* (1989), Hatakeyama (1988) and Van Urk *et al.* (1992). Normal and deformed larvae have been selected into separate groups in order to assess eventual emergence differences (including mortality) within one population by Janssens de Bisthoven & Ollevier (1989). In the present study, where the cumulative emergence curves only differed for their maxima, the results indicate different mortality rates of instar 4 larvae between both populations (deformed larvae) or in deformed and normal larvae within one population (NEI). That means that morphological deformities are indicative of other sublethal effects of toxicants. Kosalwat & Knight (1987) found a delayed adult emergence, combined with a morphological response (in the pecten epipharyngis) upon exposure of *Chironomus decorus* to substrate-bound copper.

In the water from DO, a stream with elevated metal pollution (chronic point source pollution of zinc and cadmium), reflected in the larvae (Cd, Zn, Table 2), no difference in behaviour between non-deformed and deformed larvae was found. This finding was supported by the emergence data, where non-deformed larvae emerged at least equally well. Deformed and non-deformed larvae seem to be equally fit, even though the deformities may be a sign of metal stress. As this site has been polluted for decades, the midges could have developed detoxification mechanisms (e.g. metallothionein synthesis: Yamamura *et al.* (1983);

Roesijadi *et al.* (1989)) to prevent harmful effects of the metals on the animal's metabolism, which are otherwise reflected in the behaviour and instar 4 emergence rate. Metal exposure of invertebrates is known to induce metal tolerance (long term: Wentsel *et al.* (1978); Krantzberg & Stokes (1989); Bengtsson *et al.* (1992); short term: Postma & Davids (1995)). We cannot exclude the possibility that larvae in DO may survive better than those in NEI in spite of the pollution because of less inter-specific competition or better food quality (higher organic matter content in the sediment). Postma *et al.* (1994) stressed that the availability of food has also a crucial effect on the toxicity of Cd in *Chironomus riparius* larvae.

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