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Behavioural responses of caddisfly larvae *(Hydropsyche angustipennis)* to hypoxia

H.G. van der Geest
Department of Aquatic Ecology and Ecotoxicology, Institute for Biodiversity and Ecosystem Dynamics, Faculty of Science, Universiteit van Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands, hgeest@science.uva.nl

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Abstract

The availability of aquatic oxygen can limit habitat suitability for benthic insects, and differences in hypoxia tolerance can therefore play a role in explaining distributions in the field. This study describes a behavioural test in which the trade off between different survival strategies after exposure to different oxygen concentrations is analyzed, using the caddisfly *Hydropsyche angustipennis* as a model organism. The impedance conversion technique was used to quantify patterns of behaviour for individual caddisflies at three levels of dissolved oxygen (100%, 50%, and 30% saturation) under controlled laboratory conditions. Exposure to hypoxia resulted in behavioural changes: under low-oxygen conditions, larvae increased their ventilation rate, which may increase oxygen uptake. However, they also increased the time spent on other activities, which may reflect avoidance behaviour.

Contents

Introduction

The oxygen concentration of the water and the upper sediment layer is of considerable importance to benthic communities (Ward, 1992; Chapman *et al*., 2004). Fluctuating oxygen levels are often observed in inland waters, as a result of complex diurnal and annual variations depending on both (a)biotic variables such as light intensity, current velocity or disintegration processes, as well as human activities like hydrological and geomorphological modifications or additional input of organic matter (e.g. Jacob and Walther, 1981; Paerl *et al.,* 1998). Minimal content of oxygen is an important factor limiting the distribution of benthic organisms and the ecological recovery of aquatic ecosystems. For example, Neumann (1994) and Becker (1987) demonstrated that re-colonization of the caddisfly *Hydropsyche contubernalis* in the River Rhine coincided with increasing oxygen levels.

This study aims to define a quantitative measure for sub-lethal effects of periods of low dissolved oxygen on aquatic insects, providing a more cost-effective and straightforward alternative to long-term chronic tests to reflect potential long term (life-cycle) effects. Since behavioural changes are often the first and most sensitive reactions to stress (Rand, 1985), and reduce the fitness of the organism (Blaxter and Ten Hallers-Tjabbes, 1992), it is expected that they predict potential effects on the population level, such as altered abundance of the species in the ecosystem (Gerhardt and Svensson, 1994). Therefore, the impedance conversion technique (Heinis and Swain, 1986) will be used to quantify patterns of behaviour at three levels of dissolved oxygen (100%, 50%, and 30% saturation) under controlled laboratory conditions. The caddisfly *Hydropsyche angustipennis* (a widely distributed case less net-spinning caddisfly) is selected as test organism because it has been demonstrated previously that a behavioural test with this species is a useful tool for analyzing effects of stress, in that case induced by the presence of toxicants (van der Geest *et al*., 1999).

Materials and methods

Test-species

All behaviour tests were performed with fifth instar *H. angustipennis* larvae, originating from a laboratory

culture. Originally, these cultures were started by collecting larvae or egg masses in the river Erft, a tributary of the river Rhine, in Germany, but prior to these experiments the caddisflies were reared in the laboratory already for ca. two years under normoxia conditions. The laboratory rearing procedure is described in detail by Greve *et al*. (1998). Under laboratory rearing conditions, the life cycle of *H. angustipennis* is completed in about three months, providing a continuous batch of larvae from which fifth instars were harvested for the experiments.

Experimental set-up

Behavioural responses of fifth instar *H. angustipennis* larvae were recorded using the impedance conversion technique in exactly the same experimental set-up as Heinis and Swain (1986). Prior to the measurements, larvae were placed individually in sealed nylon meshed tubes (7 cm length, 1.5 cm diameter) and a 24-hour acclimation period in Dutch Standard Water (DSW) was provided. DSW is a standardized synthetic analogue of common Dutch surface waters, containing 200 mg $CaCl_2{\cdot}2H_20$, 180 mg $MgSO4{\cdot}7H_20$, 100 mg $NaHCO_3$ and 20 mg $KHCO_3$ per L demineralized water (pH ~8.l, hardness 210 mg/L $CaCO_3$, alkalinity ~1.2 meq/L). During the acclimation period, *Urtica* powder was added as food. After the acclimation period, in which the larvae were able to construct their nets within the nylon tubes, they were divided over three different 5 L aquaria and exposed for 48-hour to the three different oxygen concentrations: 100%, 50% and 30% air saturation (ca. 9.2, 4.6 and 2.8 mg/L respectively). The oxygen concentration in the water in the aquarium was kept at a constant level by an oxystat that compared the actual oxygen concentration, measured by an oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH, USA), with the preset concentration. When the measured oxygen concentration was too high, an electrical gas valve was automatically opened to pass nitrogen into the water. When the measured oxygen concentration was too low, air was passed into the water and when the measured oxygen concentration was the same as the preset concentration, both valves were closed. The aquarium was covered with a glass plate and nitrogen and air were brought into the water by means of air stones. During the exposure time, no food was present, a 16:8-hour light:dark regime was provided and the temperature was maintained at 20°C.

For each concentration, behavioural patterns of twenty fifth instar larvae were recorded. At the end of the 48-hour exposure period, activity patterns of all larvae were recorded during 1-hour by placing the nylon tubes with the larvae in a measuring chamber (7 × 3 × 2.5 cm) which received re-circulating water from the exposure aquarium at a flow rate of 5 ml/min. After 5 minutes of acclimation, changes in the impedance of the system caused by the movement of the larvae were detected by two stainless steel electrodes placed at either side of the measuring chamber connected to the impedance converter and assimilated with 53 ms-seconds time intervals with the computer program Aqualand (Augustijn Onderzoek) on a MSDOS 486 microcomputer. From the activity signals, three different types of behaviour were defined according to the relative frequencies and amplitudes: undulatory movements or ventilation (mono-frequent with a relative high and constant amplitude), inactivity (signals below background noise) and other activity (multi-frequent with different amplitudes). Examples of the different types of behaviour as recorded with the impedance conversion technique are given in figure 1.

Data analysis

For each larva, the time spent on these different types of behaviour were determined and expressed as percentages of the total registration time (1-hour). Per treatment, the average time spent on the different types of behaviour was calculated based on the observations on all 20 larvae. In addition, for each treatment and

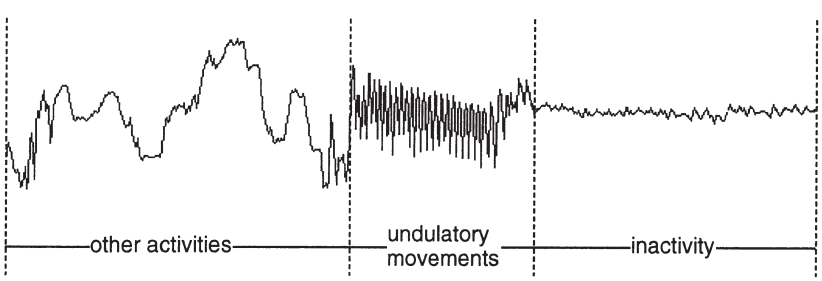

Fig. 1. Example of different types of behaviour of fifth instar *Hydropsyche angustipennis* larvae recorded with the impedance converter and the computer program Aqualand.

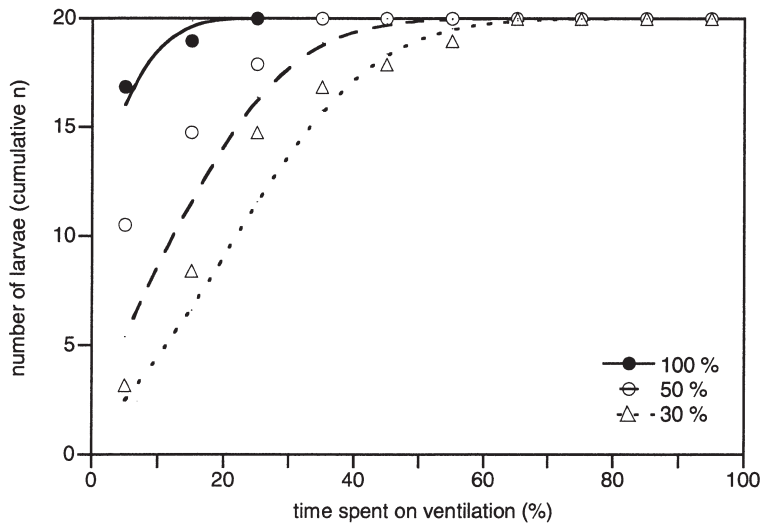

Fig. 2. Cumulative frequency distributions of time spent on ventilation by fifth instar *Hydropsyche angustipennis* larvae (n = 20) after 48-hour exposure to different oxygen concentrations (100, 50 and 30% air saturation), measured during one hour with the impedance converter technique. The lines indicate the cumulative normal distribution fitted through the data.

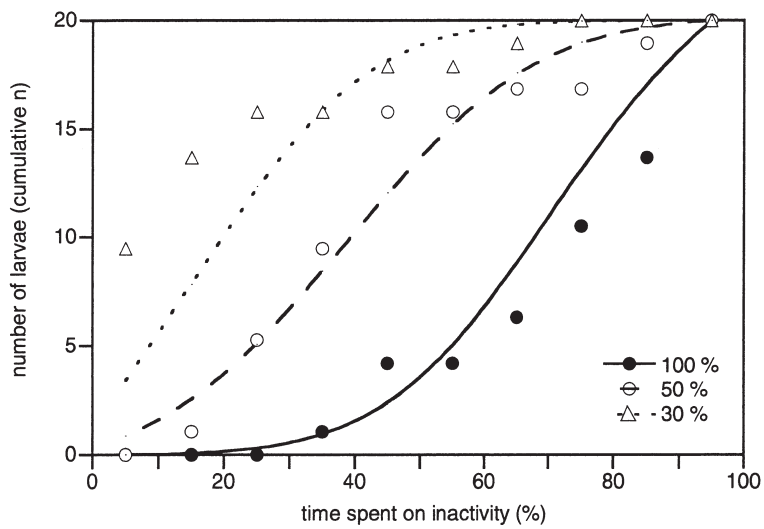

Fig. 3. Cumulative frequency distributions of time spent on inactivity by fifth instar *Hydropsyche angustipennis* larvae (n = 20) after 48-hour exposure to different oxygen concentrations (100, 50 and 30% air saturation), measured during one hour with the impedance converter technique. The lines indicate the cumulative normal distribution fitted through the data.

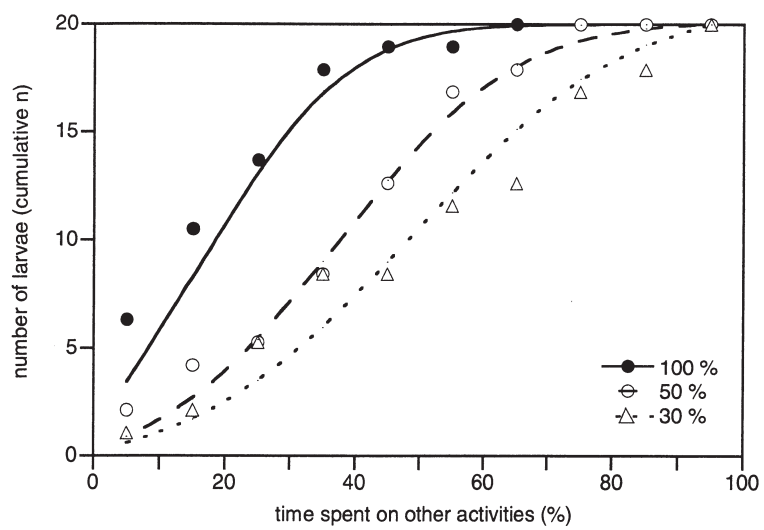

Fig. 4. Cumulative frequency distributions of time spent on other activities (other than ventilation or inactivity) by fifth instar *Hydropsyche angustipennis* larvae (n = 20) after 48-hour exposure to different oxygen concentrations (100, 50 and 30% air saturation), measured during one hour with the impedance converter technique. The lines indicate the cumulative normal distribution fitted through the data.

each type of behaviour, cumulative frequency distributions were calculated. An example for ventilation at 100% air saturation is given below: All observations on times spent on a ventilation at 100% air saturation (n = 20) were ranked from low to high and the number of larvae that spent between 0 and 10% of their on time on ventilation were counted. Next the number of larvae that spent between 10-20% of their on ventilation, between 20-30%, 30-40% *et cetera* until 90-100% were counted. These counted numbers of larvae were cumulatively plotted against the averages of the corresponding times classes (5, 15, 25% *et cetera*). Based on the average time spent on ventilation and the corresponding standard deviation, a cumulative normal distribution curve was calculated using the NORMDIST statistical function in Microsoft Excel 2000, and plotted in the obtained frequency distribution plot. This procedure was also performed for the other two types of behaviour (inactivity and other activities) recorded with the impedance converter technique. Differences in behavioural responses between the various exposure regimes were tested using one-way analysis of variance followed by Tukey post hoc tests with the computer program SYSTAT (SPSS, Chicago, IL, USA).

Results

For time spent on ventilation, inactivity and other activities at 100, 50 and 30% air saturation, significant differences were observed among the treatments ($p<0.05$; Figs. 2-4). Under normoxia conditions, all larvae spent less than 20% of their time on ventilation. When the oxygen concentration was reduced to 30% air saturation, however, the average time spent on ventilation increased significantly ($p<0.05$) from 3 to 25% (Table 1) and individual larvae spending more than 60% of their time on ventilation were observed (Fig. 2). Simultaneously, the number of inactive larvae was strongly reduced at 30% air saturation when compared to control conditions: almost one third of the larvae were more than 90% of their time inactive under normoxia conditions, while this was reduced to none of the larvae at 30% air saturation (Fig. 3).

The increased time spent on activity was not only spent on ventilation, but also on other activities: the average time spent on other activities in the lowest oxygen treatment (30%) was twice as high as in the control (100%) (Table 1). At 100% air saturation, half of the test population (10 larvae) spent less then 20% of their time on behaviour other then resting or ventilation, while at 50 and 30% air saturation this was reduced to 4 and 2 larvae respectively (Fig. 4).

Table 1. Average time (as percentage ± standard errors, n = 20) spent on different types of behaviour (ventilation, inactivity or other activities) by fifth instar *Hydropsyche angustipennis* larvae after 48-hour exposure to different oxygen concentrations (100, 50 and 30% air saturation).

Behaviour	100%	50%	30%
ventilation	3 (± 2)	14 (± 3)	25 (± 3)
inactivity	77 (± 5)	44 (± 5)	21 (±5)
other	20 (± 4)	42 (± 5)	54 (± 6)

Discussion

It was clearly demonstrated that exposure to lowered oxygen concentrations changed the behavioural patterns of *Hydropsyche angustipennis* larvae. Herewith this study corroborates the observations of amongst others Philipson (1977), Philipson and Moorhouse (1974), Becker (1987) and Engels (1997) who described changes in ventilatory and net-spinning activities of hydropsychid larvae in relation to oxygen content under experimental conditions.

The effects of low oxygen very much depend on environmental conditions (such as current velocity and temperature (Philipson and Moorhouse, 1974)), or experimental conditions (such as oxygen concentration during culturing or size and design of the test chamber) making it difficult to compare different studies. In general, however, the specific tolerance towards lowered oxygen concentrations differs greatly between aquatic insects. In figure 5, the sensitivity distribution based on effect concentrations published in literature shows that effect concentrations vary among species, ranging from almost anoxic to concentrations little less than saturated. Also, it can be seen that no specific sensitive or tolerant insect orders can be distinguished. Furthermore, Philipson and Moorhouse (1974) demonstrated that even between closely related hydropsychid species differences could be found in their sensitivity towards low oxygen.

In order to compare such differences it is argued that behavioural responses such as studied here could be important in identifying the species that are more susceptible to indirect impact of hypoxia in the field. In general, the changed behavioural patterns observed here and in the above mentioned studies result from respiratory stress in response to a reduced oxygen availability and aim to increase oxygen uptake (Ho-

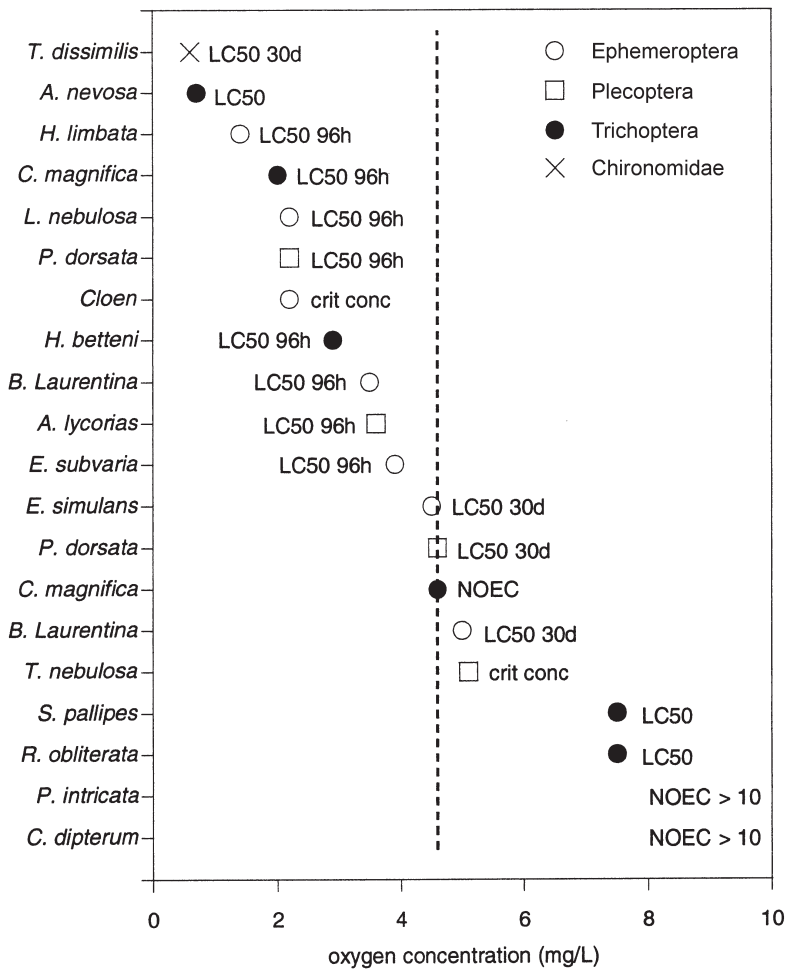

Fig. 5. Sensitivity distribution of several aquatic insects exposed to different oxygen concentrations (data collected from literature). LC50-values represent the oxygen concentrations at which 50% of the population does not survive. NOEC-values represent the No Observed Effect Concentrations. The dotted line indicates the 50% oxygen level at which significant changes in the behaviour of the caddisfly *Hydropsyche angustipennis* were observed in this study.

back and Stanley, 2001). Simultaneously, ventilatory movements also costs energy thereby being no longer available for growth or maintenance. Aquatic insects could therefore apply two different strategies when subjected to (periods of) hypoxia. Firstly, by reducing all activities an organism could minimize its oxygen demand and simply wait until the conditions improve. This type of behavioural response was for example demonstrated for *H.* angustipennis exposed to an industrial effluent containing toxic compounds (Gerhardt, 1996). Secondly, an organism could actively try to avoid hypoxia and look for more favorable conditions by increasing its locomotion behaviour. This is described for many species, including for e.g. damselflies (Apodaca and Chapman, 2004) and blue crabs (Bell and Eggleston, 2005). The latter, however, also requires increasing the oxygen intake to provide enough energy for the increased locomotion behaviour. In this study, indeed it was observed that both locomotion and respiration behaviour significantly increased when exposed to lowered oxygen concentrations. The behavioural frequency distributions thereby provide a quantitative measure of the avoidance response exhibited by *H. angustipennis*. In a detailed study by Bell and Eggleston (2005), it was demonstrated that avoidance behaviour of animals to hypoxia is influenced by many biotic and abiotic factors and therefore difficult to understand. But on the same hand, many studies suggest that such responses are linked to distribution patterns in the field of species subjected to periods of low oxygen (e.g. Becker 1987; Jacob and Walther, 1981; Chapman *et al*., 2004). The quantitative description of the strategies of aquatic insects to cope with hypoxia as studied here could therefore serve as an important variable when relating field distributions to variations in oxygen concentrations and species specific differences in tolerance towards low oxygen. To validate this conclusion, however, further

interdemic or interspecific comparisons, and comparisons to other physiological or biochemical indices of hypoxia are required.

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