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Push-up response of stonefly larvae in low-oxygen conditions

MOTOMI GENKAI-KATO,[1]* KENTARO NOZAKI,[1†] HIROMUNE MITSUHASHI,[2] YUKIHIRO KOHMATSU,[1] HITOSHI MIYASAKA[3] AND MASAMI NAKANISHI[1]

[1]*Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan,* [2]*Museum of Nature and Human Activities, Hyogo, 6 Yayoiga-oka, Sanda, Hyogo 669-1546, Japan and* [3]*Biodiversity Group, Institute of Low Temperature Science, Hokkaido University, Sapporo, Hokkaido 060-0019, Japan*

Under conditions of low oxygen availability, the larvae of the stonefly *Oyamia lugubris* McLachlan demonstrate a 'push-up' behavior that is thought to enhance respiratory efficiency. We conducted an experiment to investigate the effect of the oxygen supply on this behavior in winter and summer by using a lotic chamber and natural water. From the experiment, we determined the critical oxygen supply level below which the stonefly larvae are compelled to do push-ups. There was a small difference in the critical oxygen supply level between the seasons. This result emphasizes that a novel measurement of the oxygen availability, that is, the oxygen supply, could be an important determinant of the distribution of aquatic insects.

Key words: current velocity; dissolved oxygen concentration; oxygen supply; respiratory behavior; stonefly.

INTRODUCTION

In aquatic systems, there are several physical factors that affect the biota (Allan 1995). A limiting factor specific to the aquatic environment is oxygen availability, which may affect the distribution of animal species. When aquatic insects are stressed by low oxygen availability, they display peculiar behaviors that act to enhance respiratory efficiency (Eriksen *et al*. 1984; Ward 1992; Williams & Feltmate 1992). There have been studies describing the relationship between these behaviors and the oxygen availability (Knight & Gaufin 1963; Kamler 1969; Benedetto 1970; Kamler 1971; Nagell 1973; Murakami 1980), which showed that the frequency of the behavior increased with decreasing the oxygen availability. Many of these studies have addressed the effects of both an increased dissolved oxygen (DO) concentration and an increased water flow on the oxygen availability (Knight & Gaufin 1963; Kamler 1969, 1971; Murakami 1980), but have not been specifically designed to test their relative importance. In the present study, we define the oxygen supply, which is given by the product of the DO and the current velocity, as the oxygen availability. We conducted an experiment to determine the critical oxygen supply level below which the stonefly larvae were compelled to do push-ups.

In the present study, we focused on the push-up behavior displayed by the larvae of the stonefly *Oyamia lugubris* McLachlan (Perlidae) as an indicator of oxygen stress. The stonefly larvae were chosen because the push-up behavior is easy to observe, and thus the frequency is easy to determine. *Oyamia lugubris* is widespread in the mountain streams of Japan and under conditions of low oxygen availability, does push-ups where the body is rhythmically moved up and down (Murakami 1980). We investigated the effect of water flow under different DO levels on the frequency of the push-up behavior. So that the results could be applied to natural streams, our experimental design was such that the water temperatures and

*Author to whom correspondence should be addressed. Email: genkai@ecology.kyoto-u.ac.jp

†Present address: Limnological Laboratory, School of Environmental Science, University of Shiga Prefecture, Hikone, Shiga 522-8533, Japan.

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the DO levels reflected natural conditions. This was in contrast to the artificial conditions of previous studies in which, for example, the water temperature was fixed at room temperature or the DO was changed by nitrogen, oxygen or air bubbling.

METHODS

Larvae

The laboratory experiments were conducted in winter (16–17 December 1997) and summer (4–5 August 1998), at a laboratory of the Kiso Biological Station, Kyoto University in Kiso–Fukushima. Larvae of the stonefly (*O. lugubris*) were collected from the streambed of the Kuro-kawa River (35°51′ N, 137°40′ E) by using a hand net 2 h before the experiment. The Kuro-kawa river is a tributary of the Kiso River.

Experimental chamber system

The design of the chamber used is shown in Fig. 1. Larvae were put on the undersurface of a plastic plate (the plate was first rubbed with sand paper to make the surface coarse), because the larvae live under or between stones in nature. The water used for the experiment was drawn directly from a small natural mountain stream, Chigonosawa, beside the laboratory. The current velocity was easily altered by turning the tap that controlled the water flow. The mean current velocity was calculated by measuring the discharge from the experimental chamber (i.e. by measuring the time taken to fill a 1-l cylinder). The oxygen concentration of the water used for the experiment was determined by using the Winkler method.

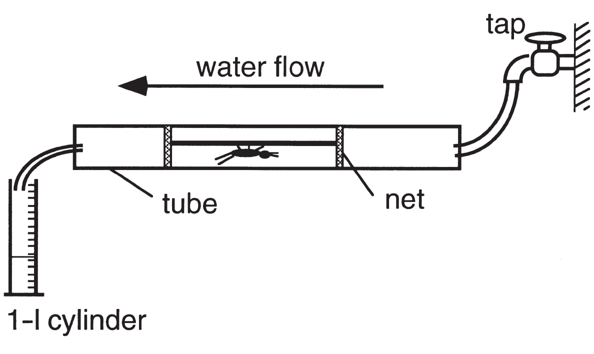

Fig. 1. Experimental apparatus used. The experimental chamber (50 cm in length and 3 cm in diameter) was made from a chloroethylene tube. Natural water from a stream was used for the experiment. Inside the tube, a stonefly larva was enclosed in a space 20 cm long by a 1-mm mesh net, which also contributed to making the water flow consistent.

The mean water temperatures were 8.1°C in winter and 19.5°C in summer. The dissolved oxygen concentrations in the water were 11.5 mg $O_2\ l^{-1}$ in winter and 8.05 mg $O_2\ l^{-1}$ in summer. These physical conditions changed very little over the course of the experiment in both seasons.

Experimental design and observation methods

In both seasons, the push-up behavior of the stonefly larvae at different current velocities was examined. The experiments were conducted separately for each season, with three different current velocities (0, 1 and 2 cm s^{-1}). Each treatment was replicated 13 times in winter and 18 times in summer in a random order for each current velocity. The larvae were acclimated to each experimental condition for 5 min in the chamber. The experiments in both seasons were conducted during the daytime (09.00–17.00 hours).

For each treatment, the number of push-ups completed by an individual was measured for 5 min at each of the three different current velocities. We excluded periods during which the larva moved about in the chamber from the measurements. To examine differences in behavior in different seasons and for different current velocities, the data were expressed as the number of push-ups per measurement period (hereafter termed the push-up frequency). To examine differences in the push-up frequency among body sizes, the stonefly larvae were divided into three size classes (Table 1).

To analyze the effect of water flow on the push-up behavior of stonefly larvae in different seasons, we examined the push-up frequency by using a repeated-measures three-way ANOVA with three current velocities as the repeated measures and season and body size as the main factors. The α-value was set at 0.05. All analyses were conducted using the statistical package StatView (version J5.0, Abacus Concepts Inc., Berkeley, California).

Table 1 Body length and head width of the stonefly larvae in winter and summer

Season	Body size	Body length (mm)	Head width (mm)
Winter	Large	27.6 ± 0.1 (4)	6.9 ± 0.1 (4)
	Intermediate	18.9 ± 1.3 (4)	5.0 ± 0.3 (4)
	Small	12.6 ± 1.0 (5)	3.3 ± 0.3 (5)
Summer	Large	24.8 ± 1.0 (6)	6.1 ± 0.2 (6)
	Intermediate	20.2 ± 1.1 (6)	4.9 ± 0.2 (6)
	Small	15.4 ± 0.6 (6)	4.0 ± 0.1 (6)

Data are mean ± SE; numbers in parentheses are *n*.

Table 2 Repeated measures ANOVA for the push-up frequency of the stonefly larvae under experimental conditions

Source	d.f.	Mean square	*F*	*P*
Between subjects				
Season	1	42 557.967	21.636	< 0.001
Size	2	308.948	0.157	0.856
Season × size	2	285.165	0.145	0.866
Error	25	1967.041		
Within subjects				
Velocity	2	8391.282	19.568	< 0.001
Velocity × season	2	3827.641	8.926	< 0.001
Velocity × size	4	34.907	0.081	0.988
Velocity × season × size	4	27.868	0.065	0.992
Error	50	428.836		

RESULTS

The relationship between push-up frequency and the water flow was different in summer and winter (Fig. 2). Treatments where size was a variable did not produce significant differences in push-up frequency (Table 2). The frequency was lower in winter than in summer, with a significant seasonal effect (Fig. 2, Table 2). An increased current velocity significantly lowered the frequency. The significant velocity–season interaction indicated that the effect of velocity on push-up frequency differed between summer and winter.

The relationship between the mean push-up frequency (f; push-ups min^{-1}) and the current velocity (v; cm s^{-1}) in Fig. 2 can be approximated by a hyperbolic curve: $f = a / (v + c) - b$. Using the three data points, we determined the parameters a, b and c for each season: $f_w = 4.97 / (v + 0.4) - 2$ for winter and $f_s = 383.95 / (v + 2.86) - 56.84$ for summer. Setting $f = 0$, we have the theoretical critical current velocity above which the stonefly larvae do

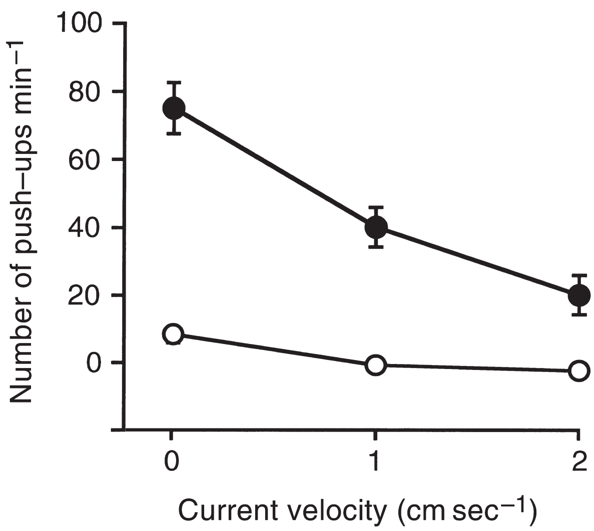

Fig. 2. Relationship between the push-up frequency (mean ± SE) and the water flow, measured in (○) winter (December 1997) and (●) summer (August 1998).

not do push-ups (because they have a sufficient oxygen supply). This is $v_w^* = 2.09$ for winter and $v_s^* = 3.89$ for summer.

For each season, we calculated a quantity called the oxygen supply (S; mg O_2 cm^{-2} s^{-1}), which is equal to $\rho \times v$, where ρ (mg O_2 l^{-1}) is the dissolved oxygen concentration. The critical level for the oxygen supply above which the stonefly larvae do not do push-ups can be calculated from the critical current velocity. The critical value for the oxygen supply is $S_w^* = 2.4 \times 10^{-2}$ for winter and $S_s^* = 3.13 \times 10^{-2}$ for summer.

The critical level for the oxygen supply in summer is higher than that for winter. Compared with the difference in the DO content or in the critical current velocity, the seasonal difference in the critical values for the oxygen supply is relatively small.

DISCUSSION

Our experiment using natural stream water revealed the effects of season and water flow on the push-up behavior of stonefly larvae. The push-up frequency was higher in summer than in winter. The seasonal differences in frequency could be attributed to the DO concentration of the water, which is dependent on the water temperature, and which has been implicated by many researchers as a factor causing oxygen stress (e.g. Benedetto 1970; Kamler 1971; Nagell 1973). The water flow was also shown to have an influence on the push-up behavior of the stonefly larvae. The effect of flow is to continually renew the water in contact with the gills of the larvae. The push-up frequency is considered to be dependent on the relationship of the water-renewal rate to the rate of oxygen depletion because of respiratory uptake, so the oxygen availability would increase as the current velocity increases. In the present experiment, the stonefly larvae did push-ups in considerably slower current velocities than those found in natural streams. This is, however, consistent with the report by Knight and Gaufin (1964) that stonefly live between and under stones where water flow is estimated to be reduced to velocities of less than 2 cm s^{-1}.

According to our definition, in theory, the oxygen supply must be zero when the DO or the current velocity is zero. In the latter case, however, the oxygen supply does not actually become zero because of the diffusion of oxygen.

The present experiment found that the critical level of oxygen supply for the stonefly larvae was higher in summer than in winter. This could be attributed to a change in the metabolic rate of the larvae in response to water temperature, which is a well-known phenomenon in animals (Schmidt-Nielsen 1990). An increase in the metabolic rate in summer can raise the oxygen consumption of the larvae. Moreover, there was a relatively small difference in the critical levels of oxygen supply in winter and summer, implying that the microhabitat distribution of the stonefly with respect to water flow in streams may change with season because DO levels change seasonally.

In conclusion, our results indicate that the respiration environments of some aquatic insects should be assessed in terms of the level of oxygen supply, not solely by the dissolved oxygen concentration or by water flow. It should also be considered that temperature may affect the metabolic rate of animals. Therefore, the thesis that water with lower temperature and faster flow is richer in oxygen is reinforced. Thus, a spatially and temporally changeable oxygen supply could be an important determinant of the seasonal microhabitat distribution of aquatic species in nature.

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REFERENCES

ALLAN J. D. (1995) *Stream Ecology*. Chapman and Hall, London.

BENEDETTO L. (1970) Observation on the oxygen needs of some species of European Plecoptera. *Internationale Revue der Gesamten Hydrobiologie* **55**: 505–510.

ERIKSEN C. H., RESH V. H., BALLING S. S. & LAMBERTI G. A. (1984) Aquatic insect respiration. In: *An Introduction to the Aquatic Insects of North America* (eds R. W. Merritt & K. W. Cummins) pp. 27–37. Kendall/Hunt Publishing Company, Dubuque, Iowa.

KAMLER E. (1969) A comparison of the closed-bottle and flowing-water methods for measurement of respiration in aquatic invertebrates. *Polskie Archiwum Hydrobiologii* **16**: 31–49.

KAMLER E. (1971) Reactions of two species of aquatic insects to the changes of temperature and oxygen concentration. *Polskie Archiwum Hydrobiologii* **18**: 303–323.

KNIGHT A. W. & GAUFIN A. R. (1963) The effect of water flow, temperature, and oxygen concentration on the Plecoptera nymph, *Acroneuria pacifica* Banks. *Proceedings of the Utah Academy of Sciences, Arts and Letters* **40**: 175–184.

KNIGHT A. W. & GAUFIN A. R. (1964) Relative importance of varying oxygen concentration, temperature, and water flow on the mechanical activity and survival of the Plecoptera nymph, *Pteronarcys californica* Newport. *Proceedings of the Utah Academy of Sciences, Arts and Letters* **41**: 14–28.

MURAKAMI N. (1980) Observation of the respiratory movement of *Oyamia gibba* Klapálek larva. *Biology of Inland Waters* **1**: 31–38 (in Japanese with English summary).

NAGELL B. (1973) The oxygen consumption of mayfly (Ephemeroptera) and stonefly (Plecoptera) larvae at different oxygen concentrations. *Hydrobiologia* **42**: 461–489.

SCHMIDT-NIELSEN K. (1990) *Animal Physiology*. Cambridge University Press, Cambridge, United Kingdom.

WARD J. V. (1992) *Aquatic Insect Ecology 1: Biology and Habitat*. John Wiley and Sons, New York.

WILLIAMS D. D. & FELTMATE B. W. (1992) *Aquatic Insects*. CAB International, Oxon.