Intertidal respiration of Anurida maritima (Collembola: Neanuridae)

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Key words. Collembola, Anurida, intertidal respiration, hypoxia, oxyregulation, pO₂ needle electrode

Abstract. The intertidal collembolan Anurida maritima can endure periods of twice-daily submergence by seawater. The air-breathing terrestrial apterygote insect has developed specific adaptations to prevent respiratory failure during hypoxic stress. When submerged, the animal is initially enclosed by an air-bubble. This bubble lasts three times longer than the small amount of stored oxygen would allow. The air bubble acts not only as an oxygen store but also as a compressible gas gill. This was demonstrated by an O₂-needle electrode technique which allowed recordings of pO₂ changes in the watery medium close to the animal. Oxygen uptake in A. maritima follows an aerial mode of respiration during the first three hours of submergence. Oxygen consumption rate at decreasing ambient O₂ partial pressures showed oxyregulating behaviour during severe hypoxia.

INTRODUCTION

The littoral collembolan Anurida maritima is a terrestrial colonizer of intertidal habitats of the Atlantic Ocean. The animal is frequently exposed to pronounced environmental fluctuations. One characteristic feature of its physiologically stressful environment is the occurrence of short, regular periods of low and high tide. As a result, the main problems for this terrestrial collembolan species during submersion are maintenance of position, osmoregulation and oxygen supply (Cheng, 1976; Witteveen et al., 1987; Witteveen & Joosse, 1988).

The air-breathing apterygote insect relies on cuticular respiration. A tracheal system is lacking. Anurida shows a circatidal endogenous rhythm (Foster & Moreton, 1981). During low tide periods, part of the population can be found wandering over the mud flats in search of food. Of course, oxygen concentration of their respiratory medium air is plentiful to sustain aerobic ATP production. One hour before the incoming tide the animals return to their underground refuges, where they endure progressive submergence clumped together in air-filled cavities or small air bubbles. Since the oxygen content of seawater is at least 30 times lower than that of air, oxygen rapidly becomes a limiting factor. Additionally, oxygen supply may be constrained by low seawater velocity or even still-water conditions in the environment close to the animals. Moreover, the oxygen demand of such a great number of animals within their refuge may deplete the oxygen partial pressure of the surrounding seawater. Obviously, the air-breathing A. maritima has to cope with environmental hypoxia. It can be hypothesised that ecophysiological adaptations exist as a mechanism to survive periods of oxygen deficiency while submerged. The major aim of this study was to measure oxygen consumption rates during aerial and aquatic modes of respiration and to demonstrate for the first time the presence of an effective compressible gas gill by recording pO₂ changes in the watery medium close to the animal.

MATERIAL AND METHODS

Specimens of Heteromurus nitidus were selected from stock cultures which have been reared in a climate room of 18°C at a 12L : 12D photoperiod. The stock of our current laboratory population was derived from a culture maintained at the University of Kiel, Department of Ecology, Germany. Field collected individuals formed the original stock 8 years before. A standard mixture of powdered carrots, lucerne meal, Baker’s yeast, agar and water was provided for food (Spahr, 1981). Animals under test had a body mass ranging from 200–350 µg.

A schematic diagram of our experimental setup for manometric measurements of oxygen uptake from air is shown in Fig. 1. The respiratory rates of A. maritima and H. nitidus were determined with a Warburg microrespirometer (B. Braun, Melsungen, Germany). The experimental technique under normoxic conditions was as described by Zinkler (1966). Different O₂/N₂-mixtures at declining ambient O₂ partial pressures were delivered by a gas mixing pump (Wösthoff Digamix SA 27/3). Each conical reaction flask and the connected micromanometer were flushed with the O₂-concentration to be tested (7 min, flow rate 210 ml/min). All experiments were performed at 18°C. Each result came from groups of 6–18 individuals owing to increasing hypoxia. Volumes of oxygen were converted to STP (standard temperature and pressure) conditions. Data are presented as mean ± SD. Data sets have been compared with ANOVA to determine significant differences. Oxygen uptake of A. maritima in seawater was measured using a thermostated (± 0.01°C) glass vessel with an animal chamber of 1.5 ml (Model 53, microconversion Y 5304, Yellow
Fig. 1. Schematic diagram of the experimental setup to determine O₂ uptake from air in Collembola during normoxic and hypoxic conditions. Gas cylinder and outlet valves (A, B, C), gas-mixing pump (D, E, F, G, K), temperature controlled water-bath (L) of the Warburg apparatus, conical microflask with one side arm and vent valve (M), micromanometer (N, R) and cooling thermostat (P).

Springs Instruments, USA). The animal chamber was filled with air-saturated seawater (salinity 25 mg·ml⁻¹). The animals (usually n = 4 individuals) were placed in the small recess of the modified stirring bar and covered by a perforated lid made of lucite. The stirring speed was 150 rpm. All experiments were made at 18°C. The decrease in pO₂ owing to respiration was registered with a Clark-style electrode and, after amplification, displayed continuously on a flatbed chart recorder. A schematic drawing of the experimental setup for polarographical measurements of oxygen uptake rates is shown in Fig. 2.

Local and temporal pO₂ changes in the close environment of submerged individual A. maritima were measured polarographically with very thin microcoaxial needle electrodes. The experimental setup was as described by Baumgärtl et al. (1994). In order to mimic small, secluded water-filled spaces which prevail close to the “nest” of submerged Anurida, a thin glass capillary (5 mm in diameter) was used as an animal chamber. The capillary was filled with 238 μl air-saturated seawater (salinity 25 mg·ml⁻¹). Electrode and capillary were kept horizontal in order to hold the air-bubble and the enclosed collembolan in a consistent position. The experiments were carried out at 21°C.

RESULTS

Oxygen uptake from air

Oxygen uptake from air in two collembolan species, the intertidal A. maritima and the soil-inhabiting Heteromurus nitidus, was measured during decreasing ambient O₂ partial pressures (Fig. 3). The experimental data showed oxyregulating behaviour in both collembolan species. The oxygen consumption rate of A. maritima dropped in response to moderate hypoxia but at a lower pO₂ range between 75 and 7.5 torr, was maintained at a steady reduced rate of approx. 46% of the normoxic metabolic activity. The metabolic response of H. nitidus to limiting oxygen was quite different from the oxyregulating behaviour of A. maritima. Its oxygen consumption rate was independent of varying hypoxic partial pressures down to 53 torr. Below this critical pressure, further reduction in environmental pO₂ caused the oxygen uptake rate of the animals to decline rapidly.

Oxygen uptake during submergence

Oxygen uptake in A. maritima was measured during different periods of submersion (Fig. 4). Although seawater provides a much poorer source of oxygen than air, the oxygen consumption rates of inundated animals were at least nearly as high as they were in air. During the first hour of submersion with initially air-saturated seawater, no significant difference between the oxygen consumption rate in normoxic air and seawater occurred. After the first hour of submersion oxygen consumption rate dropped significantly (ANOVA; P < 0.01) in response to reduced oxygen availability. Nevertheless, aerobic metabolic activity remained at a comparatively high level during this short-term submersion experiment.

At the beginning of submersion the animals were enclosed by a small air bubble which vanished after a few hours (Fig. 4, insets). The oxygen consumed by the animals during an inundation period of five hours considerably exceeds the oxygen store of the air bubble. In theory,
the animals could draw on the air bubble's oxygen for less than one hour. In practice, the air bubble did not disappear until three hours had elapsed. Obviously, two different oxygen stores, namely the air bubble and the seawater in the close environment seem to be responsible for the submerged animals' oxygen supply. The existence of such an inward diffusion of oxygen from the seawater into the bubble was proved by measurements of oxygen partial pressure profiles close to submerged *A. maritima* (see below).

**Spatio-temporal pO₂ changes in seawater**

pO₂ profiles obtained during the insertion of the micronneedle electrode into initially air-saturated seawater along the length of the animal chamber are shown in Fig. 5. A gradual emergence of the pO₂ profile takes place in relation to the submergence period of an individual animal. Clearly, *A. maritima* is able to exploit oxygen even from still seawater. At the beginning of the experiment the body of seawater had a uniform pO₂ of 150.8 torr. After 15 min typical symmetrical V-shaped profiles are seen. Declining ambient oxygen tensions were first obtained close to the collemobolan surrounded by an air bubble. The animal is always located in the "pO₂ dip". After 60 min the shape of the pO₂ profiles became asymmetric, indicating that the oxygen store at the conical part of the animal chamber is much more limited than that at the opposite end.

**CONCLUSIONS AND DISCUSSION**

The intertidal collemobolan *A. maritima* is exposed to periods of regular, twice-daily submergence. As a terrestrial invader the animal is adapted to respiring in air. Gas exchange occurs over the general body surface. As a result, the metabolism rate of the animal is closely corre-
Fig. 4. Oxygen uptake rate of *A. maritima* during varying periods of submersion (T = 18°C; mean values ± SD; n = 6). Oxygen availability after one, three and five hours of experimental submersion is indicated by pO2 data. Animals were initially surrounded by an air bubble which disappears after prolonged submersion (schematic insects).

...lated with the surface area. Oxygen uptake rate of *A. maritima* fits well the log-log relationship curve between the oxygen consumption rate and the body mass of a representative spectrum of numerous other collembolan species with an interspecific allometric exponent b = 0.74 (Zinkler, 1966).

The collembolan *A. maritima* inhabits the upper intertidal zone where it scavenges on the sandy flat surface. During high tide the air-breathing animals have to endure periods of 3–4 h of submergence. The intertidal collembolans frequently prefer as refuges types of stones which offer crevices or have a particular geometry on their lower surface which traps air-filled gas bubbles during the incoming tide. Survival in air-filled cavities under stones seems to be comparatively risk-free for intertidal collembolans which have to cope with twice-daily submersion. The animals are able to breath air, though of decreasing ambient pO2.

*A. maritima* seems to be well adapted to exploit even low oxygen concentrations from small air-filled cavities. Although oxygen consumption rates of the collembolans decrease during moderate hypoxia the animals are able to maintain a reduced but regulated oxygen uptake down to 7.5 torr. The critical pO2 is a suitable value to characterize the transition from an oxygen independent to an oxygen dependent pattern of oxygen uptake (Grieshaber et al., 1994). The critical pO2 below which the O2 uptake of *A. maritima* declines drastically was not really achieved in our experiments but must be remarkably low. In comparison, the critical pO2 value of the soil-inhabiting collembolan species *H. nitidus* is much higher. The animal exhibits a steeply decreasing rate of oxygen uptake at pO2 levels below 50 torr. The critical pO2 of two other hemiedaphic species, namely *Folsomia candida* and *Tomocerus flavescent*, were reported to be 50 and 75 torr, respectively (Zinkler & Rüssbeck, 1986). *Folsomia* also showed a marked increase in heart frequency during severe hypoxia. The adaptive changes in blood flow rate should help to maintain partial pressure differences between medium, blood and tissue (Paul et al., 1997). The existence of similar circulatory changes in *A. maritima* has yet to be tested. Another explanation for the maintenance of a reduced but regulated oxygen uptake in this intertidal species over a wide range of severe hypoxia is assumed to be a conspicuous decrease of the overall energy expenditure in response to limiting oxygen.

Air provides a much richer source of oxygen than seawater. Additionally, common aquatic respiratory systems like gills are lacking in *A. maritima*. Therefore, the main problem for flooded animals seems to be the maintenance of respiration. Unexpectedly, the inundated animals not...
oxygen uptake rates, comparable with those recorded during aerial respiration. This conformity is not accidental. \textit{A. maritima} has developed structural adaptations allowing aerial respiration during submersion. The collembolans show cuticular macro- and microplastrons while submerged. Initially, the animal is enclosed in a small air bubble, held in place by setae, appendages and a characteristic cuticular arrangement of wax coated macrotubercles (King et al., 1990; Wichard et al., 1995).

The air bubble shrinks or even disappears after prolonged submersion (Witteveen & Joosse, 1988). The bubble exists conspicuously longer than the small amount of stored oxygen would allow. For this reason, several authors have suggested that such an air bubble or film may also act additionally as a gas gill (Thorpe & Crisp, 1949; Paulus, 1971; Joosse, 1976). This implies that the water body in the close environment should serve as a second store for the animal’s oxygen demand. King et al. (1990) calculated from their structural data and equations that the air bubble not only acts as an oxygen store but also as a compressible gas gill. Since the support of the compressible gas gill lasted remarkably high oxygen uptake rate remained for 5 h during submersion. The occurrence of this second strategy to maintain aerobic ATP production was not demonstrated in the present study.

Although the support of the compressible gas gill lasted only approx. 3 h remarkably high oxygen uptake rate continued for up to 5 h (Fig. 4). Joosse (1976) suggested that a very thin microplastron on the surface of \textit{A. maritima}, held in place by cuticular minor tubercles may act as an incompressible gas gill during prolonged periods of submersion. The occurrence of this second strategy to maintain a functioning aerobic ATP production was not demonstrated in the present study.

Of course, oxygen supply is much poorer in unstirred seawater than in stirred media. After two hours of still water submersion, severe hypoxia (77.2 torr) occurred in the environment close to the animal (Fig. 5), whereas a remarkably high oxygen uptake rate remained for 5 h during submersion with stirred seawater. In the natural habitat of submerged animals the slope of the oxygen diffusion gradient from seawater to the air bubble is related to the local geometry of their refuge, the position of the animals and the velocity of the surrounding seawater.

ACKNOWLEDGEMENTS. The technical assistance of T. Behning and H. Degel is greatly appreciated.

REFERENCES


Received September 28, 1998; accepted January 25, 1999

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