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Intertidal respiration of *Anurida maritima* (Collembola: Neanuridae)

Dietmar ZINKLER[1], Raimund RÜSSBECK[1], Marc BIEFANG[1] and Horst BAUMGÄRTL[2]

[1]Lehrstuhl für Tierphysiologie, Ruhr-Universität Bochum, D-44780 Bochum, Germany; e-mail: dietmar.zinkler@ruhr-uni-bochum.de
[2]Max-Planck-Institut für molekulare Physiologie, D-44202 Dortmund, Germany

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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 O_2-needle electrode technique which allowed recordings of pO_2 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 O_2 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 pO_2 changes in the watery medium close to the animal.

MATERIAL AND METHODS

Specimens of *A. maritima* were collected on the incoming tide from a sandy flat situated in the Mokbaai area of the Frisian Wadden Island of Texel, The Netherlands. Hundreds of animals inhabit so-called "nests" (Joosse, 1966) under stones scattered on the surface of the tidal beach. The "nests" were accessible by removing stones. *A. maritima* were collected in summer. The collembolans were transported to Ruhr-University Bochum. They were placed in moistened plastic boxes at an acclimation temperature of 18°C and a 12L : 12D photoperiod. The animals were fed with pieces of frozen polychaete worms, *Hediste diversicolor*. Individuals ranging from 410–620 µg in body mass were used in the experiments, which were performed within 4 weeks after collection.

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 O_2/N_2-mixtures at declining ambient O_2 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 O_2-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

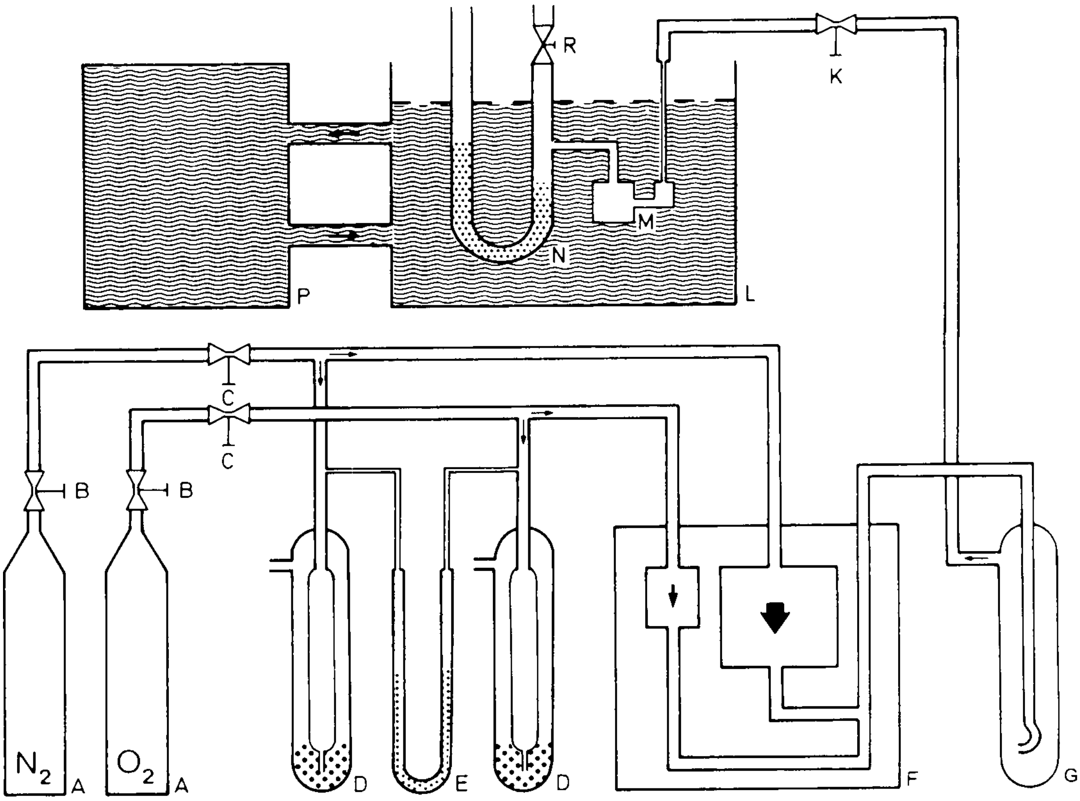

Fig. 1. Schematic diagram of the experimental setup to determine O_2 uptake from air in Collembola during normoxic and hypoxic conditions. Gas cylinder and outlet valves (A, B, C), gas-mixing pump (D, E, F, G, K), temperature controlled water-bath (L) of the Warburg apparatus, conical microflask with 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 pO_2 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 pO_2 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 O_2 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 pO_2 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 pO_2 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 submergence (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 submergence 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,

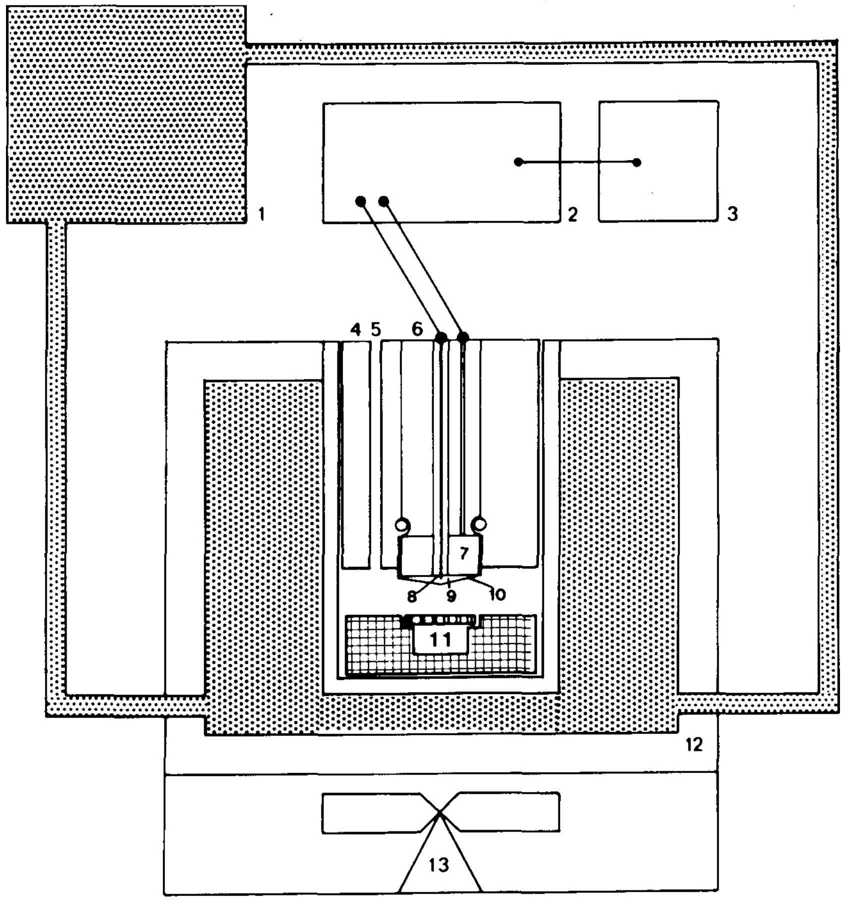

Fig. 2. Schematic diagram of the experimental setup for polarographic measurements of O_2 uptake in *A. maritima* during submergence. Cooling thermostat (1), oxygen meter (2), recorder (3), lucite plunger with slot (4, 5), oxygen electrode (6–10), modified stirring bar with animal chamber (11), temperature controlled bath (12) and magnetic stirrer (13).

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).

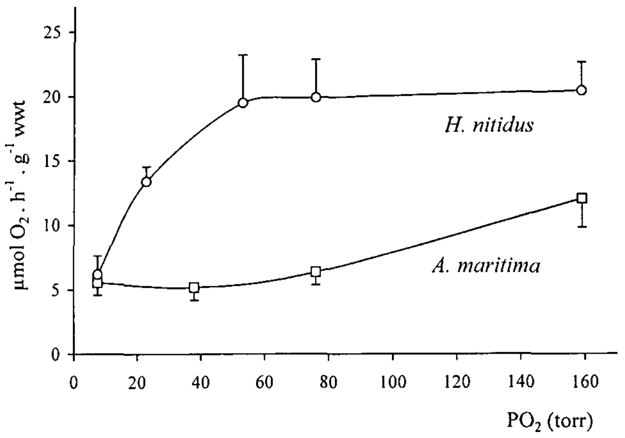

Fig. 3. Oxygen uptake rate of *A. maritima* and *H. nitidus* during decreasing ambient O_2 partial pressures in air (T = 18°C; mean values ± SD; n = 10 and n = 6, respectively).

Spatio-temporal pO_2 changes in seawater

pO_2 profiles obtained during the insertion of the microneedle electrode into initially air-saturated seawater along the length of the animal chamber are shown in Fig. 5. A gradual emergence of the pO_2 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 pO_2 of 150.8 torr. After 15 min typical symmetrical V-shaped profiles are seen. Declining ambient oxygen tensions were first obtained close to the collembolan surrounded by an air bubble. The animal is always located in the "pO_2 dip". After 60 min the shape of the pO_2 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 collembolan *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-

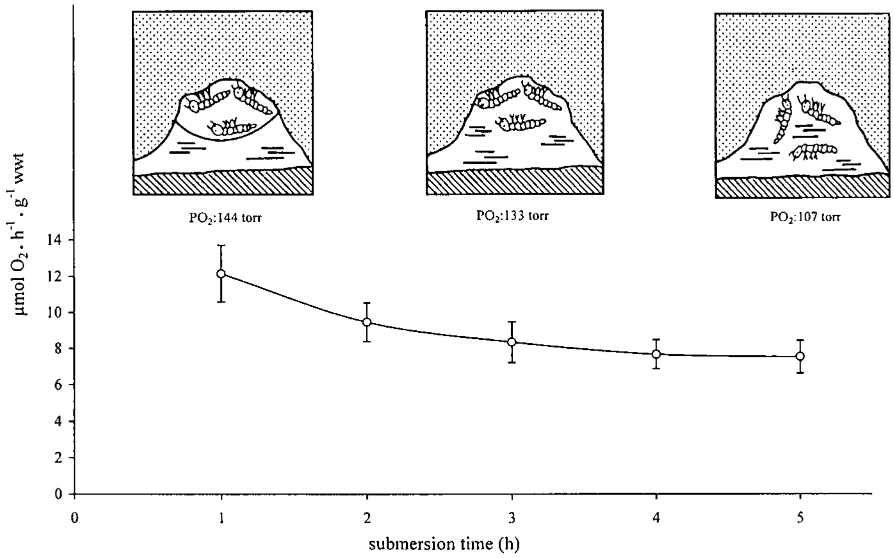

Fig. 4. Oxygen uptake rate of *A. maritima* during varying periods of submergence (T = 18°C; mean values ± SD; n = 6). Oxygen availability after one, three and five hours of experimental submersion is indicated by pO_2 data. Animals were initially surrounded by an air bubble which disappears after prolonged 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 submergence. The animals are able to breath air, though of decreasing ambient pO_2.

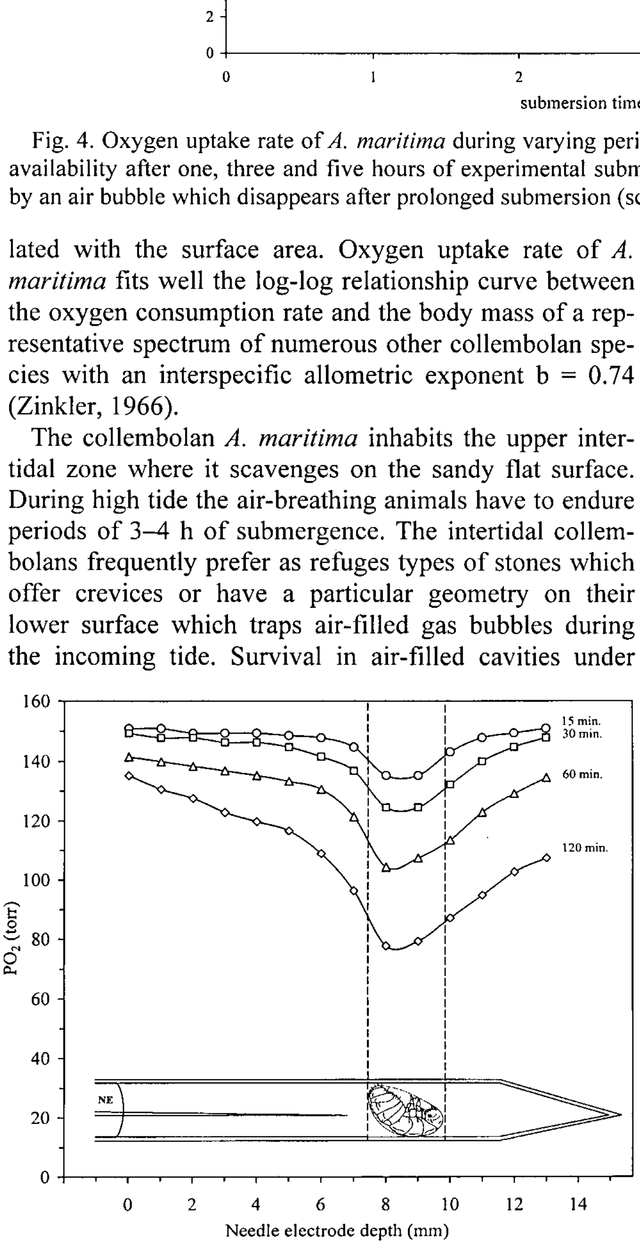

Fig. 5. pO_2 profiles close to submerged specimen of *A. maritima* in relation to needle electrode (NE) distance and submersion time. Measurements were taken in steps of 500 µm. The figure displays the pO_2 profiles obtained during a single experiment, representative for three other experiments each slightly differing with regard to the animal position within the capillary.

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 pO_2 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 pO_2 below which the O_2 uptake of *A. maritima* declines drastically was not really achieved in our experiments but must be remarkably low. In comparison, the critical pO_2 value of the soil-inhabiting collembolan species *H. nitidus* is much higher. The animal exhibits a steeply decreasing rate of oxygen uptake at pO_2 levels below 50 torr. The critical pO_2 of two other hemiedaphic species, namely *Folsomia candida* and *Tomocerus flavescens,* 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. *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 referring to gas diffusion efficiency (Crisp, 1964) that the macroplastron of *A. maritima* supports respiration as a passive gas gill. They also pointed out that the characteristic cuticle ornamentation of the intertidal collembolan species should be considered as a preadaptation possessed by many soil dwelling microarthropods.

Our recordings of pO_2 changes in the seawater close to a submerged animal demonstrate for the first time by direct measurements, that the air bubble not only acts as an oxygen store but also as a compressible gas gill. Since oxygen is continually consumed by the animal, the oxygen content of the bubble decreases. As a result, an inward diffusion of oxygen from the water into the bubble takes place; however, nitrogen diffuses out. The bubble gradually shrinks as nitrogen is lost and finally collapses (Rahn & Paganelli, 1968).

Hitherto, little was known about the efficiency of a gas gill acting below stones in small seawater bodies during still water conditions. We conclude from our experiments that the life time of such a bubble containing an inactive individual of *A. maritima* is extended by nearly three-fold.

Although the support of the compressible gas gill lasted only approx. 3 h a 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 *A. maritima*, held in place by cuticular minor tubercles may act as an incompressible gas gill during prolonged periods of submergence. 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 submergence, 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 submergence 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.

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