

**Effects of anoxia
on the behaviour,
haemolymph lactate and
glycogen concentrations
in the mud crab
Rhithropanopeus harrisii ssp.
tridentatus (Maitland)
(Crustacea: Decapoda)***

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KEYWORDS

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Abstract

The ability to accumulate lactate as result of laboratory exposure to anoxia was determined in the mud crab *Rhithropanopeus harrisii tridentatus*. Haemolymph lactate levels rose from $0.232 \pm 0.13 \mu\text{mol ml}^{-1}$ to $25.337 \pm 1.6 \mu\text{mol ml}^{-1}$ during 32 h of anoxia. High haemolymph lactate levels resulting from anoxia were associated with a number of behavioural responses. The response of *R. harrisii tridentatus* to very low levels of oxygen < 2 Torr was relatively slow ($\text{LT}_{50} = 24.3$ h). Glycogen was found in normoxic concentrations of $15.21 \pm 4.68 \mu\text{mol g}^{-1}$ d.w.

These decreased after 6 h exposure to anoxia. The results are discussed in relation to the metabolic requirements for the survival of other crustaceans under low oxygen conditions.

1. Introduction

The xanthid crustacean *Rhithropanopeus harrisii* (Gould) ssp. *tridentatus* (Maitland) was accidentally introduced into Baltic waters during the Second World War. The home range of this species lies in the brackish

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waters off the North American Atlantic coast (Buitendijk and Holthuis, 1949). In the southern Baltic the eastern Polish coastal zone) the mud crab inhabits the Dead Vistula, the Vistula Lagoon. This benthic decapod occurs at depths of 0–5 m in littoral pools and in muddy-sandy sediments (Żmudziński, 1961) and is able to survive the unfavourable environmental oxygen conditions during winter, when the water is frozen.

Many animals may regularly be exposed to hypoxic or even anoxic conditions; indeed, they are known to be very tolerant of hypoxia or anoxia. The isopod crustacean *Saduria entomon* is able to survive prolonged anoxia of up to 300 h (Hagerman and Szaniawska, 1990). Decapod crustaceans may survive low oxygen conditions for periods of many hours or even days (Lowery and Tate, 1986; Taylor and Spicer, 1987; Stickle *et al.*, 1989). When conditions become hypoxic, decapods are known to show a number of behavioural responses. A primary reaction would be to move into shallow regions of the pools and adopt a raised posture (Taylor and Butler, 1973). If the escape reaction is impossible, the animals must utilise anaerobic pathways of energy metabolism.

In many organisms, the metabolic requirements for survival at low oxygen levels are met by anaerobic glycolysis, where glycogen, used for energy storage, is catabolised to the level of lactate (Hochachka *et al.*, 1973). In decapods, lactate is the major end-product during anaerobiosis (Bridges and Brand, 1980), which is accumulated in the tissues and haemolymph. Hill *et al.* (1991) showed that the increase in lactate concentration in the tissue of *Carcinus maenas* is paralleled by that of the haemolymph. Although the fermentation products succinate and alanine, besides lactate, have been found to accumulate in the shrimp *Callinassa californiensis*, the stone crab *Menippe mercenaria* and the crab *Potamonautes warreni*, the amounts accumulated during exposure to anoxia were insignificant in comparison with that of lactate (Zebe, 1982; Albert and Ellington, 1985; Van Aardt and Wolmarans, 1987). Gnaiger (1983) has suggested that the metabolic pathway with end-products such as succinate or propionate are utilised by animals frequently exposed to long periods of severe environmental hypoxia because this pathway is energetically more efficient than the lactate pathway.

The purpose of this investigation was to study the behavioural reactions of the mud crab *R. harrisii tridentatus* under anoxic conditions (< 2 Torr). In addition, the aim of the study was to determine whether the glycogen lactate pathway common in decapod crustaceans also occurs in *R. harrisii* ssp. *tridentatus*. This paper presents the results of metabolic reactions and survival of the mud crab during anoxia.

2. Material and methods

The material for this study was collected from fishing nets on the Dead Vistula in the vicinity of the Gulf of Gdańsk, Poland in September 1995. Specimens of *R. harrisii tridentatus* were immediately transported in small tanks of brackish water to the Department of Marine Biology and Ecology, University of Gdańsk, where they were stored in tanks containing aerated water. The bottom of all the tanks was covered with a 15 mm layer of muddy-sandy sediment. The crabs were maintained at 8.8°C in brackish water (3.6 PSU). They were fed twice weekly with fish meat, but immediately prior to experimentation, they were starved. For behaviour and survival experiments at least 15 crabs were used per oxygen combination (141 Torr, < 2 Torr).

2.1. Anoxia experiment

Two plastic tanks containing 2 litres of brackish water were maintained at $6 \pm 0.6^\circ\text{C}$ in a thermostatically controlled water bath. The tanks were covered with plastic, which helped to prevent the diffusion of oxygen back into the water. Individuals of *R. harrisii tridentatus* (fresh weight range 0.7 g to 1.6 g) were introduced into each tank and left undisturbed for 24 h under aerobic conditions. After this time, the P_{O_2} (oxygen tension) in the water was reduced by bubbling O_2 – free N_2 through air-stones in one of the tanks and monitored *via* an OXI 96 Microprocessor Oximeter. The pH of the water was maintained at 7.47 throughout the experiment. The anaerobiosis condition (< 2 Torr) was achieved after 4 h. The mud crabs were kept under anoxic conditions for up to 32 h. Crabs were carefully removed from the tank and samples of either haemolymph or the whole animal were taken as described below. Each sample represents one mud crab.

The control individuals maintained at the normoxic level (141 Torr) were also sampled.

2.2. Biochemical analyses

Lactate

Haemolymph samples (50 μl) were removed with a 250 μl syringe through the arthrodistal membrane of one of the pereopods and mixed immediately with ice-cold perchloric acid (0.6 mol l^{-1}) in a centrifuge tube. The mixture was placed in an ice-bath for 10 min and centrifuged. The concentration of L-lactate in the haemolymph was analysed with a Boehringer-Mannheim Test Kit (Cat. No. 139084).

Glucose

Circulating glucose was analysed in 20 μ l haemolymph samples according to the enzymatic and colorimetric method (Cat. No. EM 95001) described by Teuscher and Richterich (1971).

Glycogen

At each sampling interval, mud crabs were immediately frozen in liquid N₂. The crabs were dried at 60° C, pulverised, and subsamples of 10 mg taken. The glycogen content was determined using the method described by Dubois *et al.* (1956), involving extraction with 15% trichloroacetic acid. The results were calculated from the standard curve prepared for glucose. Glycogen was expressed as a weight, including carapace weight.

3. Results

3.1. Behavioural observations

The control crabs were relatively quiescent throughout the experiment. The majority of *R. harrisii tridentatus* crabs under normoxic conditions buried themselves in the sand or, if they stayed on the surface, tried to hide under stones. The crabs were not very lively, although, when fed, they moved towards food.

When the oxygen concentration was reduced to the level of < 2 Torr, the crabs unburied themselves and moved away from places of nitrogen input. Anoxic conditions were achieved after 4 h (pre-anoxia). After several hours of exposure to such conditions the crabs lifted the anterior parts of their bodies and the majority of them gathered in groups of 3 or 4 and crawled onto each other. After 20 h of anoxic conditions the majority of the touching crabs responded very slowly as they were in a moribund state. This behaviour was correlated with the highest haemolymph lactate levels. It was also noted that a large percentage of the moribund crabs died.

3.2. Survival under normoxic and anoxic conditions

Under normoxic conditions > 141 Torr all mud crabs survived the experiments. The crabs were declared dead when they did not move after 30 min in well-oxygenated water. The response of *R. harrisii tridentatus* to very low oxygen levels of < 2 Torr is relatively slow when compared with the other crabs that were tested. This is best illustrated by the LT₅₀ for *R. harrisii tridentatus*, which was 24.3 h (Fig.1). After 48 h of exposure under anoxic conditions all the crabs died.

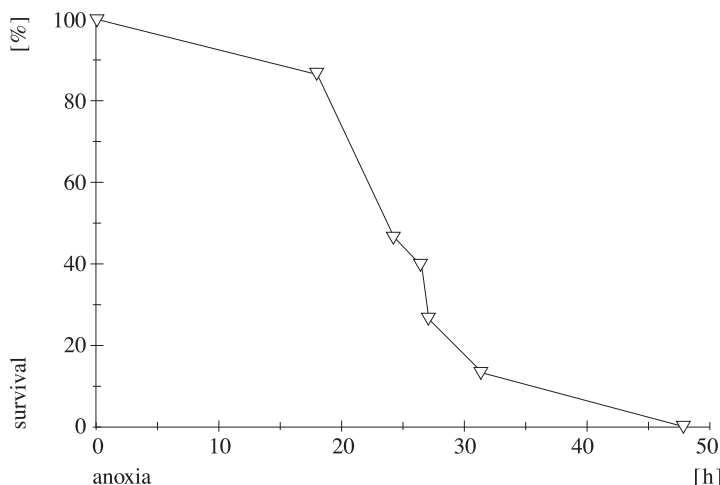


Fig. 1. *R. harrisii tridentatus*. Survival as a function of time on exposure to anoxia

The concentrations of substrates (glycogen, blood glucose) and metabolites (blood lactate) after various exposure times to anoxia are summarised in Tab. 1.

Table 1. *R. harrisii tridentatus*. Mean concentration ($\pm$ SD) of glycogen, haemolymph lactate and glucose during anoxia (< 2 Torr). In parentheses: sample size

Time [h]	Glycogen [$\mu\text{mol g}^{-1}$ d.w.]	Glucose [$\mu\text{mol ml}^{-1}$ blood]	Lactate [$\mu\text{mol ml}^{-1}$ blood]
0	$15.37 \pm 3.6(7)$	$0.232 \pm 0.1 (3)$	$0.236 \pm 0.13(5)$
2	$15.3 \pm 4.5(6)$	$0.323 \pm 0.04(3)$	$0.656 \pm 0.54(4)$
4	$14.98 \pm 3.4(7)$	$0.371 \pm 0.07(3)$	$1.582 \pm 1.02(3)$
6	$15.11 \pm 4.5(7)$	$0.394 \pm 0.09(2)$	$5.28 \pm 1.59(4)$
8	$14.33 \pm 3.8(6)$	$0.634 \pm 0.16(3)$	$8.679 \pm 1.23(3)$
10	$13.05 \pm 4.5(5)$	$0.843 \pm 0.12(3)$	$11.143 \pm 1.24(3)$
20	$7.33 \pm 1.3(5)$	$1.543 \pm 0.15(4)$	$21.22 \pm 0.84(3)$
24	$4.15 \pm 1.7(5)$	$1.663 \pm 0.03(3)$	$21.98 \pm 1.55(3)$
28	$3.1 \pm 1.2(4)$	$1.613 \pm 0.1 (2)$	$23.743 \pm 1.25(3)$
32	$1.79 \pm 0.5(4)$	$1.445 \pm 0.06(4)$	$25.337 \pm 1.6 (3)$

3.3. Effect of lactate accumulation

Because of the anoxic conditions, the blood lactate level in *R. harrisii tridentatus* increases rapidly. The results shown in Fig. 2 indicate that the lactate concentration increases with the beginning of anoxia from 0.236

$\mu\text{mol ml}^{-1}$ to $21.22 \mu\text{mol ml}^{-1}$ after 20 h (16 h full anoxia). During this experiment the accumulation rate was approximately linear ($1.049 \mu\text{mol ml}^{-1} \text{ h}^{-1}$). After such a significant increase in the haemolymph lactate concentration, the lactate concentration increased during the subsequent hours only slightly to $25.337 \mu\text{mol ml}^{-1}$. The highest blood lactate concentration ($27.451 \mu\text{mol ml}^{-1}$) was found in the mud crabs which died during the experiment. The blood lactate level in crabs kept under normoxic conditions (control crabs) was at rather a constant low level, $0.283 \pm 0.2 \mu\text{mol ml}^{-1}$.

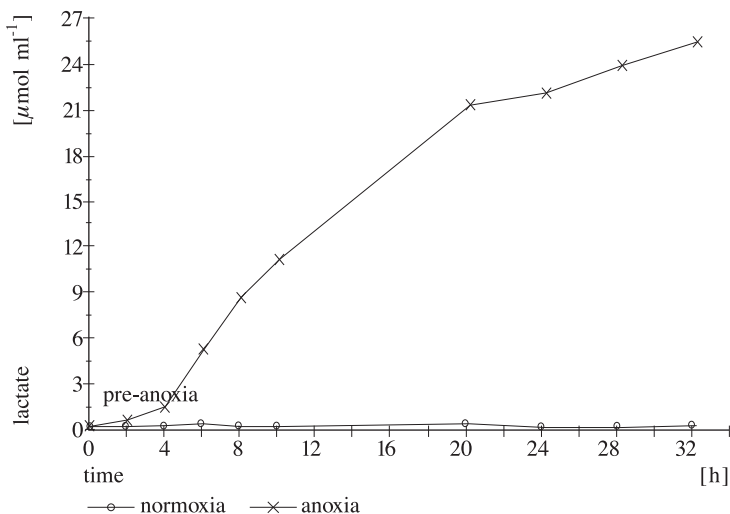


Fig. 2. *R. harrisii tridentatus*. Mean concentrations of haemolymph lactate under anoxic and normoxic conditions

3.4. Changes in blood glucose and glycogen concentrations

The level of circulating glucose under normoxic conditions remained unchanged at $0.209 \pm 0.1 \mu\text{mol ml}^{-1}$. During the first 4 h of pre-anoxia the concentration of glucose in *R. harrisii tridentatus* blood increased slightly. After 4 h there was a rapid increase in concentration, reaching a maximum value at $1.663 \mu\text{mol ml}^{-1}$ (Fig. 3). After 24 h of the experiment the glucose concentration decreased; this corresponds strongly to a glycolytic flux, which reflects a decrease in glycogen from its maximum value of $15.114 \mu\text{mol ml}^{-1}$ to $1.795 \mu\text{mol ml}^{-1}$. Under normoxic conditions the glycogen level, expressed as dry weight including carapace weight, levelled out at $15.206 \pm 4.6 \mu\text{mol g}^{-1} \text{ d.w.}$ (Fig. 4). The lowest glycogen levels ($1.4 \pm 0.4 \mu\text{mol g}^{-1} \text{ d.w.}$) were determined in dead crabs.

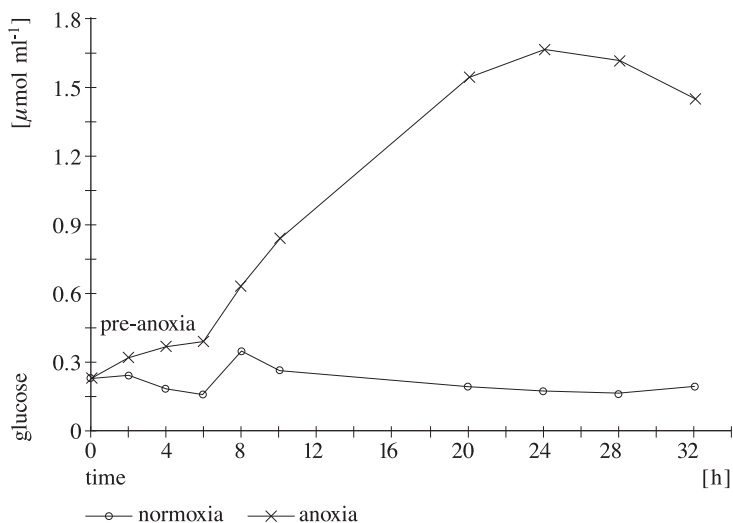


Fig. 3. *R. harrisii tridentatus*. Changes in mean haemolymph glucose concentrations during exposure to anoxic and normoxic conditions

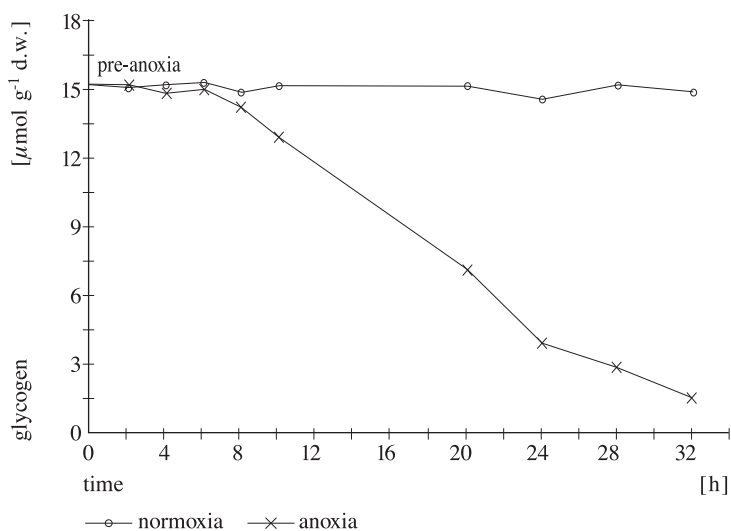


Fig. 4. *R. harrisii tridentatus*. Total mean glycogen concentrations under anoxic and normoxic conditions

4. Discussion

The mud crab *R. harrisii tridentatus*, which lives in the Dead Vistula and the Vistula Lagoon, periodically faces deficiencies of oxygen or its total lack in the near-bottom layer because this basin freezes over during

winter (Łomniewski, 1958). During periods of decreased oxygen levels, many organisms escape, the more sensitive ones die, while a few have the ability to adjust (Theede *et al.*, 1969; Oeschger *et al.*, 1992). The significant behavioural reactions to anoxic conditions observed in the mud crab – unburying, escape towards water with more oxygen, raising the anterior part of body – were also observed in other decapod crustaceans (Taylor and Butler, 1973; Taylor and Spicer, 1987; Hill *et al.*, 1991). A characteristic reaction to anoxia stress not described in the literature was the gathering of crabs *R. harrisii tridentatus* in groups.

This research reveals that *R. harrisii tridentatus* is able to survive under conditions of total anoxia for more than 24 h. A similar resistance to the lack of oxygen in water was observed by Pritchard and Eddy (1979), Zebe (1982) for Thalassinidea crustacea, *Upogebia pugettensis* and *C. californiensis*, which were also kept under anoxic conditions for 24 h. *Carcinus maenas* was slightly less resistant, the LT_{50} being 15.8 h (Hill *et al.*, 1991). Lowery and Tate (1986) revealed that a large percentage of moribund crabs *Callinectes sapidus* (6 h anoxia) would have died if the normoxic conditions had not been restored within 10–15 minutes.

Earlier studies on crustacea metabolism showed that anaerobic metabolism in Decapoda almost completely depends on anaerobic glycolysis, with L-lactate as a major, final product (Hochachka *et al.*, 1973; De Zwaan and Skjoldal, 1979; Zebe, 1982; Gäde, 1983; Taylor and Spicer, 1987; Hill *et al.*, 1991). Studies on *R. harrisii tridentatus* confirmed the increase of glycogenolysis (during 32 h: $0.424 \mu\text{mol ml}^{-1} \text{ h}^{-1}$) and the consistent accumulation of lactate (during 32 h: $0.784 \mu\text{mol ml}^{-1} \text{ h}^{-1}$). An interesting phenomenon is related to the lack of a significant glycogen decrease during the first 6 h of experiment. This indicates that there must have been a different source of lactate production up to $5.28 \mu\text{mol ml}^{-1}$. The most probable source are oligosaccharides, the rapid decrease of which from 6.98 to $3 \mu\text{mol g}^{-1}$ was observed by Hill *et al.* (1991) in the crab *C. maenas* under similar experimental conditions. The above conclusions are supported by the steady increase in circulating glucose from the normoxia level $0.232 \pm 0.1 \mu\text{mol ml}^{-1}$ to $0.4 \pm 0.09 \mu\text{mol ml}^{-1}$ in this study.

The maximum concentration of lactate in *R. harrisii tridentatus* blood under anoxic conditions occurred over 20 h of total anoxia, the accumulation rate being $1.04 \mu\text{mol ml}^{-1}$. Higher values were obtained for the blue crab, *C. sapidus*, in which the maximum haemolymph lactate concentration was $42.6 \mu\text{mol ml}^{-1}$, and the lactate accumulation rate was $15\text{--}18 \mu\text{mol ml}^{-1} \text{ h}^{-1}$ (Lowery and Tate, 1986). Similarly high concentrations of lactate were determined in freshwater crabs *Potamon warreni* after six hours of exposure to anoxic conditions. The total lack of oxygen caused the lactate

concentration in the haemolymph of these crabs to rise from a pre-exposure value of $0.55 \mu\text{mol ml}^{-1}$ to $34.78 \mu\text{mol ml}^{-1}$ (Van Aardt and Wolmarans, 1987).

During the experiment glycogen levels were also determined for *R. harrisii tridentatus* after their death. Therefore, a reasonable conclusion is that their death was did not result directly from the lack of a metabolic substrate, but was due rather to lactate accumulation in the haemolymph, which was determined. According to Hill *et al.* (1991), exposure of *C. maenas* to anoxic conditions significantly influences the acidity of its haemolymph acid-base balance, which is caused by lactate accumulation. Other research on acid-base disturbance in the haemolymph of the prawns *Palaemon elegans* and *P. serratus* indicate the direct influence of lactate accumulation on the decrease in acid-base regulation (Taylor and Spicer, 1991). Therefore, it can be assumed that the crabs died as a result of a high lactate concentration.

This paper shows that, as in other crustacea which are facultative anaerobes, when *R. harrisii tridentatus* crabs are subjected to anoxic conditions, anaerobic glycolysis occurs, and glycogen decomposes to lactate. Among the decapod Branchyura described here, the mud crab shows the highest tolerance to a total lack of oxygen in water.

5. Conclusions

- When conditions became anoxic, mud crabs *R. harrisii tridentatus* showed a number of behavioural responses like unburying, escape towards more oxygenated water, raising the anterior part of a body, and gathering in groups of three or four.
- Under anoxic conditions LT_{50} in *R. harrisii tridentatus* was 24.3 h.
- The haemolymph lactate level in *R. harrisii tridentatus* kept under anoxic conditions increased from 0.232 ± 0.13 to $25.337 \pm 1.6 \mu\text{mol ml}^{-1}$.
- After 6 h exposure to anoxia a decrease in glycogen concentration from the normoxic level to $1.79 \pm 0.5 \mu\text{mol g}^{-1} \text{ d.w.}$ was recorded.
- In *R. harrisii tridentatus*, the metabolic requirements to survive under low oxygen conditions were met by anaerobic glycolysis, where glycogen was catabolised to lactate.

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