

## THE EFFECT OF TEMPERATURE ON THE MUSCLE OXYGEN LEVELS IN ANTARCTIC FISH\*

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**Abstract:** Direct measurements of the available oxygen in the muscle were carried out by the implanting of polarographic sensors in living Notothenidae (Pisces, Teleostei). Curves for normal oxygen levels corresponding to air-saturated water at 0° and under the effect of gradual increasing of the temperature were obtained.

### 1. Introduction

The measurement of standard oxygen consumption by Antarctic fish, using both closed and open circuit respirometers, has been the subject of several papers (for a review, see CLARKE, 1983). Oxygen consumption was measured by HEMMINGSEN *et al.* (1969) in *Pagetopsis macropterus*, an Antarctic hemoglobinless fish, and in three species of *Notothenia*. The fish were kept in an aquarium maintained at about -1°C, the oxygen determinations carried out by a microoxygen electrode calibrated with a tonometer and against a gasometric method (SCHOLANDER and VAN DAM, 1956). RALPH and EVERSON (1968) measured the metabolic rates of Antarctic fish, including the ice-fish *Chaenocephalus aceratus*, using a respiration chamber, the oxygen consumption of the fish expressed as mg of oxygen consumed per hour. A somewhat more elaborated system was used by HEMMINGSEN and DOUGLAS (1970) who measured oxygen consumption of the ice-fish *Chaenocephalus aceratus* and *Pseudochaenichthys georgianus* in a closed and continuous flow respirometer at 1°C. In the closed respirometer system, the fish were allowed to reduce the oxygen content of the water to levels critical for survival. HOLETON (1970) assayed the oxygen consumption of Antarctic fishes by measuring  $P_{O_2}$  of the water filling a respirometer in which cannulated specimens were placed. Oxygen tension of the water and blood samples was measured with a macrooxygen electrode and a physiological gas analyzer.

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In order to better define the available oxygen in fish tissue, a special set consisting of a microelectrode for the determination of  $O_2$ , coupled with a system of simultaneous measurement of pH, salinity, temperature and oxygen content of the water in the test chamber, has been developed by LUCCHIARI *et al.* (1984) and initially used for the determination of the available oxygen in living carp muscle.

This method can be considered adequate for the measurement of oxygen in living tissues and has been used for the study of tissue oxygen metabolism in living Antarctic fish.

Thus, the present paper shows the results of experiments planned to establish the effect of temperature on the muscle oxygen levels in Antarctic fish. Since the species diversity of inshore Antarctic fish communities is relatively low (JOHNSTON, 1985), our research was concentrated on the Nototheniids which dominate the fish fauna and are highly endemic to Antarctic waters.

A preliminary account of this paper was given at the Tenth Symposium on Polar Biology, Tokyo, Japan.

## 2. Materials and Methods

Specimens of *Notothenia coriiceps neglecta*, *N. rossii marmorata* and *Trematomus bernacchii* (Nototheniidae, Teleostei, Pisces), with a total length of 22–35 cm, were caught off Admiralty Bay (King George Island, South Shetlands) during the 1984/85 and 1985/86 summers. The depth of capture was around 40 m using bottomset gill net (ROSMAN, 1980). The fish were transported to the station and maintained in a sea-water circulating bath at outdoor temperature.

### 2.1. Oxygen sensor

The oxygen sensor used was the platinum cathode with a silver-silver chloride reference electrode, both built with 0.12 mm diameter wires. Both wires were welded to a thin flexible and insulated conductor cable and then, perpendiculary fixed at a distance of 3–6 mm from each other to a thin  $4 \times 7$  mm plastic base. The weld was covered with self-polymerizing acrylic and the wires with a layer of epoxy patch resin. Immediately before the insertion of the sensor into the fish tissue, the tips of the silver and platinum wires were cut and exposed.

### 2.2. Calibration of the oxygen sensor

Calibration of the sensor was carried out by first dipping the electrodes into a 3% water solution of sodium dithionite ( $P_{O_2}=0$ ) and then in air-saturated water ( $P_{O_2}=160$  mmHg). Among 10 microelectrodes, 2 were randomly chosen to study the correlation between the polarographic current and the temperature, maintaining the same  $O_2$  concentration. Variation of temperature was established within the range of 0–10°C.

### 2.3. Implantation of the oxygen sensors

The animal were held without anesthesia in a tray containing sufficient sea-water (0°C) to maintain the fish respiration. For the insertion of the oxygen sensor, the scales were taken off latero-dorsally, just in front of the dorsal fin, and a 2-cm cut was made

in the skin with scissors. The skin was separated from the underlying tissues, the muscle surface being exposed. A thin layer of super bonder glue was applied to the base of the sensor before its implanting in the muscle, in order to fix the electrode in the animal body. The microelectrode was implanted perpendicularly to the dry muscular surface, covered immediately with the two skin flaps, and fixed by means of two surgical stitches. Immediately after, the animal was placed in an experimental chamber, similar to the one previously used for carp (LUCCHIARI *et al.*, 1984), but adapted to match the typical shape of the Nototheniids. The fish assumed immediately a normal body position, the respiratory frequency being recovered some hours after. The animals were allowed to rest for 12–24 h before the beginning of the experiments.

The correlation between the polarographic current and the temperature of the sensor in a constant concentration of oxygen was satisfactorily linear within the range of 0–10°C, the average slope being equal to 0.0098  $\mu\text{A}/^\circ\text{C}$ .

By using this coefficient, the level of oxygen in the muscle was obtained by the equation

$$P_{\text{O}_2} = \frac{i_{m,t}}{i_0 + 0.0098t} \times 160 \text{ (mmHg)},$$

where  $i_0$  is the polarographic current at 0°C and  $i_{m,t}$  the polarographic current at temperature  $t$ . All measurements were carried out in air-saturated water.

For the temperature maintenance and controlled raising, a circulating refrigerating unit, specially built by Refrigeração Paraná S/A (to whom we are indebted) was used.

### 3. Results

Values of muscular  $P_{\text{O}_2}$  within the range of 0 to 1°C were obtained for 5 individuals of *N. coriiceps neglecta*, one *N. rossii marmorata*, and one *T. bernacchii*. The relationship between temperature, within the range of 0–1°C, and the residual available oxygen in the muscle of those Notothenidae is shown in Fig. 1.

Under normal environmental conditions, which correspond to the water temperature of 0–1°C, the muscular available oxygen, measured as  $P_{\text{O}_2}$ , gives an average of 15.4 with a standard deviation of 2.1 mmHg. The individual measurements give fairly constant values among the different species (Fig. 1), but in two individuals of *N. coriiceps neglecta* gave values of 8.4 and 26.4 which might be either extreme values due to a variety of causes or even to technical problems, as for example the proximity of the microelectrodes to a capillary.

The values found, in mmHg, were as follows: for *N. coriiceps neglecta*, 16.4, 16.1, 13.1, 8.4, and 26.4; for *N. rossii marmorata*, 15.2; and for *T. bernacchii*, 15.4.

Some peculiar features can be expected among the Notothenidae when the effect of temperature on the levels of muscular residual oxygen is analyzed (Fig. 1).

In *T. bernacchii* the residual oxygen is maintained in reasonable constant levels when the environmental temperature is raised from 0 to 8°C. The fish seems to possess cardiorespiratory mechanisms adequate to supply suitable levels of oxygen to the tissues. Above 8°C, however, the levels of muscular residual oxygen fall to 11 and 9 mmHg at 9 and 10°C respectively. *N. coriiceps neglecta* shows a stable profile of muscular

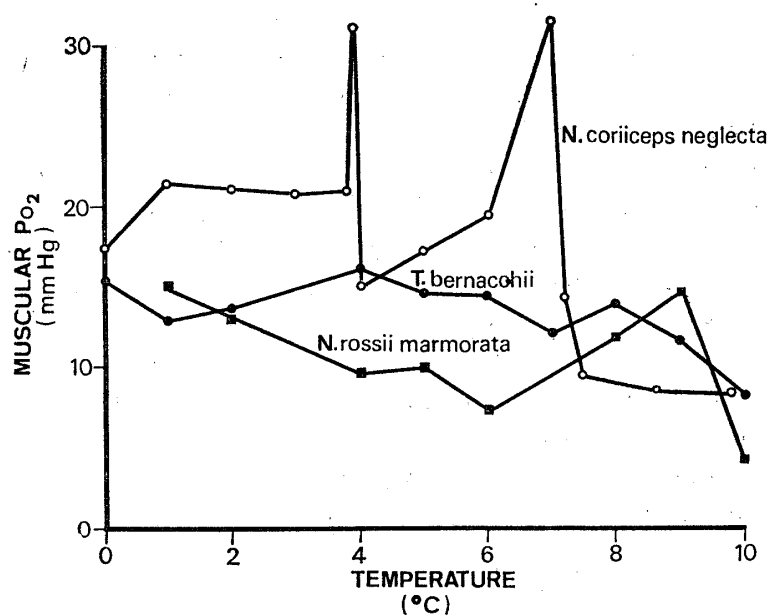


Fig. 1. Measurements of the available oxygen in the muscle of three Antarctic fish at different temperatures. The raising of the temperature was at a rate of 1°C/h. The available oxygen was recorded continuously along the whole experiment. Values shown are the average measurements of 5 individuals of *N. coriiceps neglecta*. For *N. rossii marmorata* and *T. bernacchii* only one each individual was assayed.

residual oxygen up to 3.8°C. Above 3.8°C there is a sudden rise of the available muscular oxygen ( $P_{O_2}=31$  mmHg), falling, immediately after, to 15 mmHg. From 4° up to 7.5°C the residual oxygen increases to 31.6 mmHg to fall, soon after, to 9.6 mmHg. In *N. rossii marmorata*, the levels of muscle available oxygen fall steadily between 1 and 6°C,  $P_{O_2}$  reaching a minimum of 7.3 mmHg. From 6 to 9°C, the  $P_{O_2}$  value increases up to 14.4 mmHg falling at 10°C to 4 mmHg.

#### 4. Discussion

In the present paper a technical device was used in order to analyze the fish muscular oxygenation in regard to the environmental increase of the temperature.

The linear correlation found for the electrodes immersed in water containing constant concentration of oxygen as well as the average angular coefficient thus obtained, allowed the determination of the available oxygen in the muscle of the different species of fish, at each specific temperature, within the range of 0–1°C.

It has to be emphasized that in the present paper no correlation has been yet established between blood  $P_{O_2}$  and muscle  $P_{O_2}$  although it is desirable to obtain such data due to the representativeness of blood  $P_{O_2}$  in the assessment of the physiological state of fishes. However, the measurements of muscular  $P_{O_2}$  alone have shown to be valuable data in order to follow up the behavior of individual fish subjected to different environmental conditions (LUCCHIARI *et al.*, 1984).

Some consideration has to be done in regard to the results herein obtained, mainly

in the case of *N. coriiceps neglecta*. As it was shown, for this species,  $P_{O_2}$  at 0–1°C gave two extreme values (8.4 and 26.4 mmHg), the normal value being 15.4 mmHg. This variation, particularly for the case of the extreme values, could be due either to individual variations or to the position of the implanted electrodes.

However, significant in *N. coriiceps neglecta* are the two peaks shown in Fig. 1, which appear which the temperature reaches around 3.8 and 7.5°C. Independently of the initial  $P_{O_2}$  values, the behavior shown in Fig. 1 has been obtained in all experiments with *N. coriiceps neglecta*.

Some pertinent observations were the subject of several authors. Thus, according to ELLIOT (1981), when the water temperature changes, the rate of thermal equilibration of the body is usually rapid, the stress response of the fish being divided in progressive phases of the respiratory movements: the first one with some external indication of abnormal behavior and rapid ventilatory movements; the second showing increasing ventilatory movements; at the third, the respiratory movements being only opercular. The raising of environmental temperature (CRAWSHAW, 1977) causes an increase in the gills ventilation of the fish which is controlled by the anterior brainstem.

In fact, the physiological effect caused by raising the environmental temperature to fish is being the subject of several investigators (for reviews see RANDALL, 1970; FRY, 1971).

On the other hand, GRIGG (1967) and SACHELL (1971) stated that hemoglobins of *T. bernacchii* and *T. borchgrewincki* are adapted to function at –1.4 to –2°C, the blood oxygen affinity being greatly diminished by even small increases of the temperature and the oxygen capacity reduced, being only 70% of the normal at 4.5°C.

FISHER and SULLIVAN (1958) showed that by raising the temperature from 4 to 28°C brook trout (*Salvelinus fontinalis*) reacts by increasing the random activity, followed by decrease between 12–20°C, reaching a new peak before the decrease to the lethal levels. These results seem to parallel the behavior showed by *N. coriiceps neglecta* in regard to muscular available oxygen measured at different temperatures.

It seems that different Antarctic species, even sharing the same environment, are differently adapted to sudden environmental temperature changes. Among the species herein studied, *T. bernacchii* seems to have the most effective cardiovascular system to minimize the stressing effect of temperature increase.

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#### References

- CLARKE A. (1983): Life in cold water; the physiological ecology of marine ectotherms. *Oceanogr. Mar. Biol. Ann. Rev.*, **21**, 321–353.
- CRAWSHAW, L. I. (1977): Physiological and behavioral reactions of fishes to temperature change. *J. Fish. Res. Bd. Can.*, **34**, 730–734.
- ELLIOT, J.M. (1981): Some aspects of thermal stress on freshwater teleosts. *Stress and Fish*, ed. by A. D. PICKERING. London, Academic Press, 209–245.
- FISHER, K. C. and SULLIVAN, C. M. (1958): The effect of temperature on the spontaneous activity of

- speckled trout before and after various lesions of the brain. *Can. J. Zool.*, **36**, 49–63.
- FRY, F. E. J. (1971): The effect of environmental factors on the physiology of fish. *Environmental Relations and Behavior*, ed. by W. S. HEAR and D. J. RANDALL. Academic Press, 1–98 (Fish Physiology, Vol. 6).
- GRIGG, G. C. (1967): Some respiratory properties of the blood of four species of Antarctic fishes. *Comp. Biochem. Physiol.*, **23**, 139–148.
- HEMMINGSSEN, E. A. and DOUGLAS, E. L. (1970): Respiratory characteristics of the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol.*, **33**, 733–744.
- HEMMINGSSEN, E. A., DOUGLAS, E. L. and GRIGG, G. C. (1969): Oxygen consumption in an Antarctic haemoglobin free fish, *Pagetopsis macropterus* and three species of Notothenis. *Comp. Biochem. Physiol.*, **29**, 467–470.
- HOLETON, G. F. (1970): Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* LÖNNBERG) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol.*, **34**, 457–471.
- JOHNSTON, I. A. (1985): Temperature, muscle energetics and locomotion in inshore Antarctic fish. *Oceanics*, **11**(2), 125–142.
- LUCCHIARI, P. H., FANTA-FEOFILOFF, E., BOSCARDIM, A. T. and BACILA, M. (1984): A technique for the determination of the available oxygen in living carp (*Cyprinus carpio*) muscle. *Comp. Biochem. Physiol.*, **78A**(4), 675–679.
- RALPH, R. and EVERSON, I. (1968): The respiratory metabolism of some Antarctic fish. *Comp. Biochem. Physiol.*, **27**, 299–307.
- RANDALL, D. J. (1970): Gas exchange in fish. *The Nervous System, Circulation and Respiration*, ed. by W. S. HEAR and D. J. RANDALL. Academic Press, 253–292 (Fish Physiology, Vol. 4).
- ROSMAN, I. (1980): Fishing with Bottom Gillnets. Rome, FAO Training Series, **3**, 30 p.
- SATCHELL, E. H. (1971): *Circulation in Fishes*. Cambridge, Cambridge Univ. Press, 131 p.
- SCHOLANDER, P. F. and VAN DAM, L. (1956): Microgasometric determination of oxygen in fish blood. *J. Cell. Comp. Physiol.*, **48**, 529–532.

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