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THE INFLUENCE OF HYPEROXIA, HYPOXIA AND TEMPERATURE ON THE RESPIRATORY PHYSIOLOGY OF THE INTERTIDAL ROCKPOOL FISH *GOBIUS COBITIS* PALLAS

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SUMMARY

The influence of hypoxia, hyperoxia and temperature on the oxygen consumption, heart rate and ventilation frequency of the intertidal rockpool fish *Gobius cobitis* Pallas were investigated to examine the respiratory adaptations of this species to intertidal conditions.

The standard mass-specific oxygen consumption ($\dot{M}_{O_2} \times m^{-1}$) during normoxia, calculated for a 50-g fish, averaged 1·27 mmol $O_2\,kg^{-1}\,h^{-1}$ at 12·5 °C and 3·62 mmol $O_2\,kg^{-1}\,h^{-1}$ at 25 °C. The Q_{10} value for oxygen consumption averaged 2·3.

During a stepwise reduction of oxygen partial pressure (P_{O_2}) the oxygen consumption was maintained down to a critical oxygen tension, P_c, of approximately 43 Torr (1 Torr = 133·3 Pa). Ventilatory frequency increased progressively while heart rate remained constant until the P_{O_2} was reduced below 16 Torr.

During hyperoxic exposure (P_{O_2} = 150–450 Torr), oxygen consumption remained constant at 12·5 and at 25 °C (Q_{10} = 2·3). Hyperoxia had no effect on heart rate, although ventilation frequency decreased with increasing P_{O_2} (to the same extent at both temperatures), indicating the overriding effect of hyperoxia on ventilatory frequency.

Gobius cobitis appears to be well-adapted to the respiratory stresses which occur on a daily basis within intertidal rockpools.

INTRODUCTION

Physicochemical parameters of intertidal rockpools have been shown to change rapidly over a daily tidal cycle (Ganning, 1971; Truchot & Duhamel-Jouve, 1980; Morris & Taylor, 1983; Bridges, Taylor, Morris & Grieshaber, 1984). Fishes living in this habitat may be exposed to hypoxic conditions with oxygen tensions as low as 2 Torr and to hyperoxic conditions with oxygen tensions up to 555 Torr, together with short-term changes in temperature from 12·5 to 25 °C, over a period of 2–8 h (Truchot & Duhamel-Jouve, 1980). These extreme environmental conditions may

Key words: oxygen consumption, Q_{10}, critical oxygen tension, ventilatory frequency, heart rate, blood pressure.

therefore influence the respiratory homoeostasis of the fish and create problems for gas transport.

Among intertidal fish, gobies represent the most diverse taxonomic group in North European waters (M. H. Horn & R. N. Gibson, in preparation) and little is known about the respiratory physiology of this group (reviewed by Bridges, 1987). The giant goby (*Gobius cobitis*) is found at levels higher than low-water neaps in the intertidal zone on the French Atlantic coast, in the Mediterranean (Gibson, 1970, 1972) and also in the Red Sea (Goren & Klausewitz, 1978). *Gobius cobitis* may attain a maximum length of about 30 cm and weigh up to 250 g.

The effect of hypoxia on oxygen consumption, heart rate and ventilation rate of fish has been described in a number of papers (Hughes & Saunders, 1970; Hughes, 1973; Butler & Taylor, 1975; Lomholt & Johansen, 1979; Randall, 1982; Hughes, Albers, Muster & Götz, 1983). Although there has been considerable work on the effect of hypoxia little is known about hyperoxia (Dejours, Toulmond & Truchot, 1977; Wilkes, Walker, McDonald & Wood, 1981). The present study was carried out to determine the response of *Gobius cobitis* to simulated environmental P_{O_2} and temperature changes. These simulated conditions consisted of exposure to hypoxia at 12·5 °C to simulate low tide at night and exposure to hyperoxia at 12·5 and 25 °C to simulate conditions experienced during low tides in daylight. This study therefore represents one of the first to look at the synergistic or antagonistic effects of environmental parameters on the respiratory physiology of intertidal fish.

MATERIALS AND METHODS

Specimens of *Gobius cobitis* Pallas were collected from intertidal rockpools near the Biological Station Roscoff, using a 3% quinaldine solution in acetone to anaesthetize the fish (Gibson, 1967). Overcollecting was avoided, as fish require approximately 10 years to attain a large size (>20 cm) (Gibson, 1970).

Fish were transported in seawater tanks to Düsseldorf where they were kept in a seawater aquarium at 13 ± 1 °C for periods up to 1 year. The aquarium water was aerated and filtered continuously and fish were fed weekly with squid, crab meat and sometimes mussels. The aquarium illumination was a 12 h:12 h, light:dark regime. Salinity, nitrite, pH and temperature of the water were measured regularly and kept within limits (salinity 35 ± 2‰, nitrite $<0{\cdot}1\ mg\ l^{-1}$, pH 8·0 ± 0·2, temperature 13 ± 1 °C).

Principle of measurements

In the experiments the following biotope conditions were simulated by the experimental conditions described in brackets: (1) high water (day or night) (12·5 °C, normoxia, 150–160 Torr); (2) low water (night) (12·5 °C, hypoxia, reduction of P_{O_2} from 150 to 15 Torr); (3) low water (daylight) (12·5 and 25 °C, hyperoxia, increasing P_{O_2} from 150 to 450 Torr). All experiments were carried out in constant light conditions, i.e. in darkened respirometers, to avoid changes in activity of the fish

induced by light changes. Hypoxic experiments were not carried out at 25 °C as this experimental regime rarely, if ever, occurs in the natural environment.

Statistical analysis was carried out using Student's *t*-test. The values are shown as the mean ± 1 S.D. unless otherwise stated.

Oxygen consumption measurements

Each fish was weighed and placed in a darkened Perspex respirometer chamber with a volume of 350, 950 or 2100 ml according to the size of the fish. When designing the system, the critical comments of Hughes *et al.* (1983), concerning flow-through systems for respiratory measurements, were taken into account. These include keeping the size of the respirometer small in comparison with the size of the fish and ensuring mixing within the respirometer. The whole system, including the reservoir tank (7 l), was thermostatically controlled. The flow rate through the respirometer was measured regularly and was maintained at a constant value, which varied according to the experimental set-up. Values between 50 and 200 ml min^{-1} were used. Water samples were taken through a bypass system to determine the oxygen tension of the water at the inlet (P_{O_2}in) and outlet (P_{O_2}out) of the respirometer chamber. Sampling was controlled by a three-way magnetic valve (PSV 100, Pharmacia, Freiburg, FRG) connected to a timer system. The P_{O_2} of the water samples was measured continuously with a P_{O_2} electrode (E 5047, Radiometer Copenhagen, Denmark) connected to a P_{O_2} monitor (PHM 72 c, Radiometer Copenhagen, Denmark). P_{O_2}in could be changed in steps using a gas mixing pump (Digamix, Type M 30/a, Wösthoff, Bochum, FRG) supplied with N_2 and O_2 (99·999 %) which were mixed with air and then equilibrated with sea water.

Standard oxygen consumption in relation to body mass and temperature

Animals were allowed to recover from handling after placing them in the respirometer for 14–16 h before commencing experiments. During a 7- to 9-h period P_{O_2}in and P_{O_2}out were measured alternately. P_{O_2}out was normally measured for a period of 45 min in each hour followed by measurements of P_{O_2}in for 15 min. During each P_{O_2}out measuring period approximately four values of ΔP_{O_2} (P_{O_2}in − P_{O_2}out) were determined at constant time intervals (10 min). Flow rate and chamber volume were adjusted such that a ΔP_{O_2} of approx. 6–30 Torr was reached in all experiments. From all ΔP_{O_2} values the oxygen consumption was calculated as described below. A mean value of oxygen consumption was then calculated for each individual.

After 12 h recovery from the first series of measurements at 12·5 °C, oxygen consumption at 25 °C was measured. Initially temperature was changed from 12·5 to 25 °C over a 2-h period and the animals were allowed to adapt to the new conditions for a further hour. During this time the P_{O_2} electrode was calibrated and the flow-through system adjusted to the new conditions. Measurements were then continued for approximately 5–7 h, in which P_{O_2}in and P_{O_2}out were measured as described above.

The oxygen consumption was calculated from the difference between inlet and outlet water P_{O_2} according to the following equation:

$$\dot{M}_{O_2} \times m^{-1} = \alpha \times \Delta P_{O_2} \times 1000 \times \dot{V},$$

where $\dot{M}_{O_2} \times m^{-1}$ is mass-specific oxygen consumption (mmol O_2 kg^{-1} h^{-1}), α is O_2 solubility coefficient (0·0013 and 0·0017 mmol l^{-1} $Torr^{-1}$ for 12·5 and 25°C, respectively, from Boutilier, Heming & Iwama, 1984), ΔP_{O_2} is P_{O_2}in−P_{O_2}out, m is mass (g) and $\dot{V}$ is flow ($l\,h^{-1}$).

Oxygen consumption during hypoxia and hyperoxia

Before starting experiments standard normoxic oxygen consumption was determined. P_{O_2}in was then reduced or increased in steps and at each step oxygen consumption was measured when a constant P_{O_2}out level had been reached. Each step determination lasted about 1 h, during which four values of ΔP_{O_2} were determined at constant time intervals as described above.

Ventilatory frequency (VF)

In a separate series of experiments, an experimental tank (7 l) was divided with a perforated screen into an experimental area containing the P_{O_2} electrode and the fish, and a manipulation area containing filtration and aeration equipment. The P_{O_2}in value of the sea water could be changed by equilibration with gas mixtures of N_2 or O_2 and air with the help of a gas-mixing pump (Wösthoff, Bochum, FRG). The determination of ventilatory rate was made using a modified impedance technique from Hoggarth & Trueman (1967). Two plastic-coated wires (diameter, 0·15 mm), with the ends stripped of their insulation, were fixed to the opercula of anaesthetized fish (MS 222, tricaine methanesulphonic acid, Sigma, St Louis, USA, 0·1 g l^{-1} sea water) by penetrating the epidermis with the pointed tip of the wire and applying histoacryl glue (Braun, Melsungen, FRG) over the incision. Excess wire was sewn onto the dorsal fin. The free ends of the wires were then connected to an impedance unit (Strathkelvin Instrumentation, Glasgow, Scotland). A small oscillating current was then provided between the electrodes by the impedance unit. Each movement of the opercula caused an impedance change between the electrodes and this produced a proportional voltage change which was amplified before being fed to a pen recorder (Washington, Bio Science, Sheerness, UK). After recovery from anaesthetization the fish were allowed to adapt to the experimental chamber for 16–20 h. Normoxic values for ventilatory frequency were then determined before measurements during hypoxia or hyperoxia. Water P_{O_2} was changed in steps as described above. At each step, ventilatory frequency (VF) was measured over a period of 15 min and VF of each minute in this measurement period determined. From these determinations a mean value was calculated. Each experiment lasted approximately 6–8 h and after each experiment the fish were allowed to recover for 12–16 h before changing the experimental conditions.

Heart rate (HF)

The experimental set-up was similar to that described under ventilatory frequency. Heart rate was measured with the help of electrodes (method A) but some experiments were carried out with both electrodes and a catheter (method B) to record blood pressure changes in the bulbus arteriosus. The two methods were compared to determine the relationship between electrode signal (ECG) and blood pressure changes.

Method A

Fish were anaesthetized with MS 222 ($0{\cdot}1\,g\,l^{-1}$ sea water). The main electrode consisted of a 5 cm long shellac-coated stainless steel wire (diameter, 0·1 mm). This was inserted 5–10 mm into the fish, near the pericardium, after making a small incision in the body wall with the help of a cannula, which also functioned as a guide for the electrode. The electrode was fixed in place with the aid of histoacryl glue. The free end of the wire was then stripped of insulation and soldered to a plastic-coated silver wire which was fixed with histoacryl glue onto the dorsal fin and sutured to a fin ray to relieve any tension. The soldered area was carefully insulated against surrounding water using a plastic sheath, histoacryl glue and silicon grease. A stainless steel plate 24 cm in length and 14 cm wide, placed in the manipulatory part of an experimental tank, functioned as the neutral electrode. Electrodes were connected to a differential amplifier (University of Düsseldorf) which gave a 1000-fold amplification of the signal. Amplified ECG signals were filtered through a bandpass (Type 3750 filter, Krohn-Hite Corporation, Avon, MA, USA) before recordings were made on a pen recorder (Gould 8188, Gould Ballainvillers, France).

Method B

A catheter of polyethylene (PE 10, Clay Adams, Parsippanny, USA) was inserted into the bulbus arteriosus of the fish. Implantation of the catheter took about 20–30 min, under anaesthetization with MS 222 ($0{\cdot}1\,g\,l^{-1}$) and cooling with ice. The fish was opened ventrally, anterior to the pelvic fin so that the bulbus was exposed. The catheter, which was filled with a fish Ringer–heparin ($200\,i.u.\,ml^{-1}$) solution, was inserted into the bulbus and fixed in place with histoacryl glue. A piece of Fibrospum (Promota, Heimberg, FRG) was placed in the wound to disinfect it and to prevent bleeding. The wound was then closed with histoacryl glue and the fish allowed to recover. The catheter was then connected to a pressure recorder (Gould P 231 D, Gould Instruments, OH, USA) and the signals were amplified and displayed using a pen recorder (Gould 8188, Gould Ballainvillers, France).

Experimental protocol

After fixing of electrodes or catheters, the fish were allowed to recover in the experimental chamber for 16–20 h at 12·5 °C. Initially a control value for heart rate at 12·5 °C and normoxia was determined before measurements during hypoxia and hyperoxia. The P_{O_2} of the water was changed in steps as described above. At each step, heart rate (HF) was measured over a period of 10 min and the heart rate for each

minute in this period determined. From these determinations a mean value was calculated. Each experiment lasted about 6–8 h after which the fish were allowed to recover at 12·5 °C and normoxia for 12–16 h before being exposed to a new experimental condition. Experiments with catheters were terminated by administering an overdose of MS 222 to the fish *via* the catheter.

RESULTS

Oxygen consumption in relation to body mass and temperature

In these experiments the oxygen consumption of 18 animals with a mass range from 9 to 240 g was measured at 12·5 and 25 °C. The P_{O_2} of inlet water was 155 ± 2 Torr in all experiments. Fig. 1 shows the logarithm of standard mass-specific oxygen consumption in relation to the logarithm of body mass.

The relationship between animal mass and O_2 consumption could be expressed as follows:

$$\dot{M}_{O_2} = a \times m^b,$$

and for mass-specific oxygen consumption:

$$\dot{M}_{O_2} \times m^{-1} = a \times m^{b-1},$$
$$\log \dot{M}_{O_2} \times m^{-1} = \log a + (b - 1) \log m,$$

where a is the coefficient equal to the total metabolism of an animal of unit mass, m is

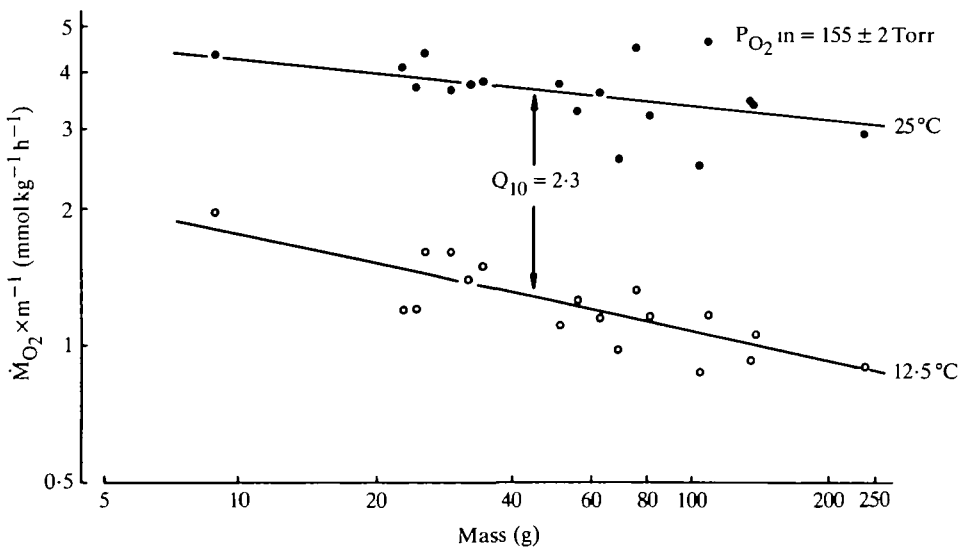

Fig. 1. The influence of body mass on standard oxygen consumption ($\dot{M}_{O_2} \times m^{-1}$) of *Gobius cobitis* at two different temperatures under normoxia. Each symbol represents the mean value for one individual measured at 12·5 °C (○) and 25 °C (●). The mean Q_{10} value was 2·3.

the mass of the animal and b−1 is the mass-specific regression coefficient (slope). From the data obtained for the oxygen consumption of *Gobius cobitis*, regression lines describing the relationship between $\dot{M}_{O_2} \times m^{-1}$ and body mass were calculated.

The equations are:

$$\dot{M}_{O_2} \times m^{-1} = 2{\cdot}87m^{-0{\cdot}21} \quad (r = -0{\cdot}79,\ 12{\cdot}5°C),$$
$$\dot{M}_{O_2} \times m^{-1} = 5{\cdot}49m^{-0{\cdot}11} \quad (r = -0{\cdot}51,\ 25°C).$$

The mass-specific regression coefficient, b, of $\log \dot{M}_{O_2}$ was −0·21 at 12·5 °C and −0·11 at 25 °C. There was no significant difference in the slopes of the regression lines but the elevations were significantly different ($P < 0{\cdot}01$).

The Q_{10} values of the measurements (12·5–25 °C) were 2·3 ± 0·3. There was no correlation between Q_{10} and mass ($r = 0{\cdot}11$). The deviations of the mean individual values from the regression line are greater at 25 °C than at 12·5 °C (see Fig. 1). This may be an effect of the acclimation of the fish to the temperature in the aquarium (13 °C) where they were kept, and an individual reaction to higher temperatures which resulted in higher activity and, therefore, in a higher oxygen consumption of the individual fish. The average standard deviation of individual mean values was 0·09 (12·5 °C) and 0·13 (25 °C).

Oxygen consumption during hypoxia

Six animals (mass 33–228 g) were used in the oxygen consumption experiments during hypoxia. The effect of a stepwise reduction of P_{O_2} on the oxygen consumption of individual fish is shown in Fig. 2. Oxygen consumption was relatively independent of P_{O_2} in above 40 Torr. The oxygen consumption of the fish became dependent upon the P_{O_2} of the water and decreased with decreasing oxygen tension at P_{O_2} in levels below 40 Torr. Two fish (135 g, 33 g) showed a slight reduction of oxygen consumption level with decreasing oxygen tension at P_{O_2} in levels above 40 Torr. This reaction could perhaps be explained by the presence of a high activity state in the fish above standard metabolic rate (SMR) at the beginning of the experiments, which decreased to SMR at lower oxygen tensions.

Fig. 3 summarizes the data on oxygen consumption and hypoxia for all six fish shown in Fig. 2. The ovals have no statistical significance since they only group data points for each experimental oxygen step and the order in which they were measured (see Materials and Methods). The two calculated regression lines represent the two distinct phases of oxygen consumption: a regulatory phase (ovals 1–4) in which the fish was able to maintain oxygen consumption relatively independently of the P_{O_2} of the surrounding water; and a dependent phase (ovals 4–6) in which oxygen consumption was linearly dependent on the P_{O_2} of the water. The regression line for the regulatory phase has a slope that is not significantly different from zero ($P < 0{\cdot}01$) and a low (0·43) correlation coefficient, indicating that oxygen consumption is independent of oxygen tension. The regression line for the dependent phase has a slope which is significantly different from zero ($P < 0{\cdot}001$) and a high correlation

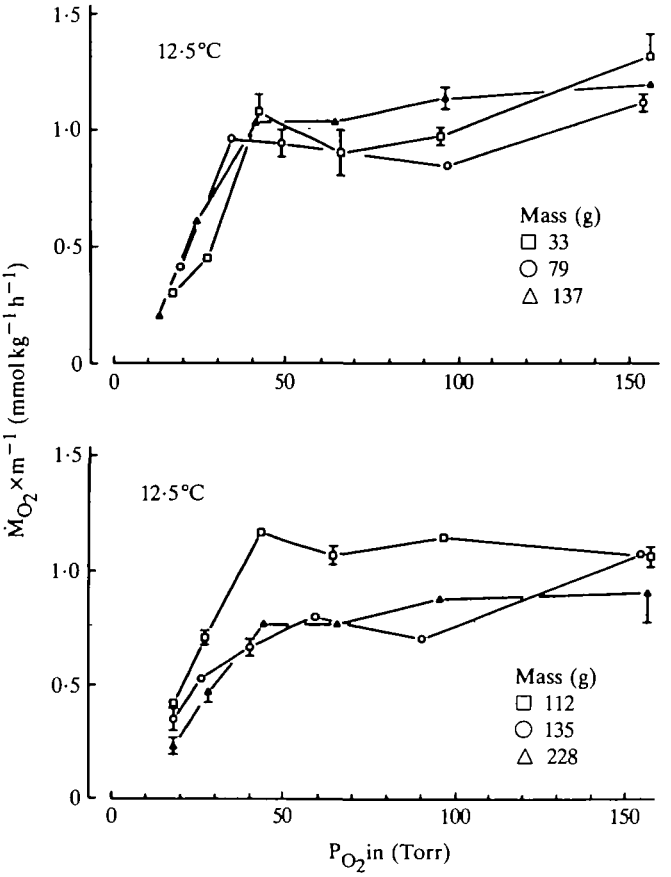

Fig. 2. The effect of declining oxygen tension in the respirometer inlet water (P_{O_2}in) on oxygen consumption ($\dot{M}_{O_2} \times m^{-1}$) of six specimens of *Gobius cobitis*. All values shown are means ± 1 S.D. wherever possible.

coefficient (0·89). The regression lines intersect at approximately 43 Torr and this represents the critical oxygen partial pressure, P_c, at which the oxygen consumption becomes dependent on the inspired P_{O_2}.

Hyperoxia

Seven animals (mass 88–228 g) were used to determine the oxygen consumption during hyperoxia: three animals were measured at both 12·5 and 25 °C, two animals at only 12·5 °C and two animals at 25 °C. During hyperoxic exposure, oxygen consumption remained independent of P_{O_2} from a P_{O_2}in of 150–450 Torr at both 12·5 and 25 °C (Fig. 4) as shown by the low correlation coefficients and slopes which were not statistically different from zero. Thus, oxygen tensions which occur in the biotope during the daylight ebb-tide had no effect on the oxygen consumption of the fish in these simulation experiments. Two animals showed a slight increase in oxygen consumption with increasing P_{O_2}in, but this may be the effect of increased activity.

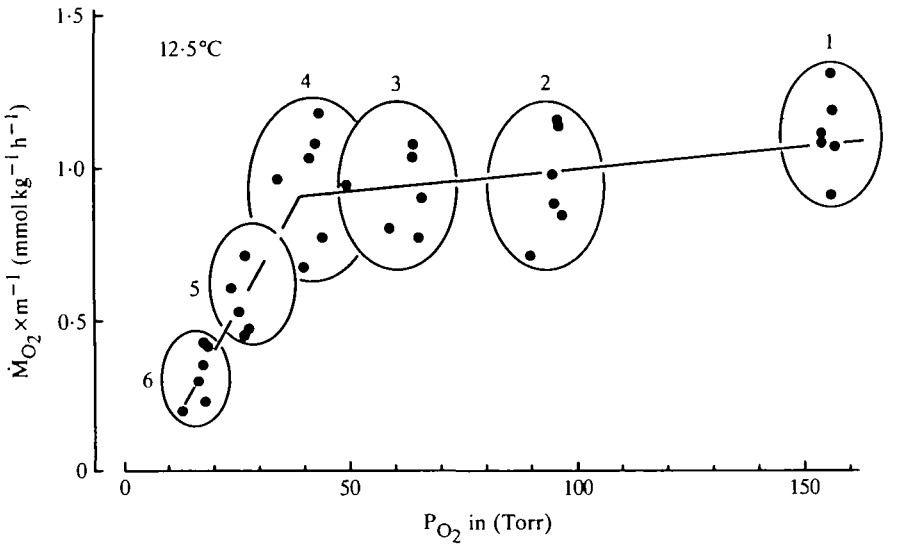

Fig. 3. The effect of declining oxygen tensions in the inlet water of the respirometer (P_{O_2}in) on $\dot{M}_{O_2} \times m^{-1}$. Combined results are shown for the six fish in Fig. 2. Each point represents a mean value for an individual at a particular oxygen tension. The ovals 1–6 group the data for various oxygen levels and the order in which measurements were made. Regression lines were calculated for the points enclosed in the ovals from 1 to 4 and from 4 to 6. Ovals 1–4: $\dot{M}_{O_2} \times m^{-1} = 0{\cdot}84 + 0{\cdot}001 P_{O_2}$in ($r = 0{\cdot}43$). Ovals 4–6: $\dot{M}_{O_2} \times m^{-1} = -0{\cdot}119 + 0{\cdot}025 P_{O_2}$in ($r = 0{\cdot}89$).

Ventilatory frequency (VF)

For measurements during hypoxia and hyperoxia (12·5 and 25 °C) 7–8 fish were used (mass 80–210 g). The ventilatory frequency (VF) of *Gobius cobitis* varied inversely with the ambient P_{O_2} (Fig. 5) at both 12·5 and 25 °C. At normoxia (155 Torr) the ventilatory frequency was $17{\cdot}3 \pm 5{\cdot}1$ beats min^{-1} and this increased sharply with the stepwise reduction of oxygen tension and averaged $41{\cdot}7 \pm 3{\cdot}9$ beats min^{-1} at a P_{O_2}in value of 13 Torr (hypoxia). With increasing oxygen tension VF decreased and averaged $9{\cdot}2 \pm 3{\cdot}3$ beats min^{-1} at 459 Torr (hyperoxia).

When exposed to higher temperatures (25 °C) VF rose from 17·3 beats min^{-1} at 12·5 °C to $50{\cdot}1 \pm 11{\cdot}6$ beats min^{-1} ($Q_{10} = 2{\cdot}3$) under normoxic conditions. VF decreased from this value with increasing P_{O_2} to 32 ± 10 beats min^{-1} at 427 Torr. As in the oxygen consumption measurements, the variability of ventilatory frequency increased with rising temperature, as indicated by the error bars. This variability may be due to different activity states of the animals.

To eliminate the individual differences between fish, ventilation frequency values were normalized with respect to the normoxic value for VF of each individual which was set at 100 %. Ventilation frequency at higher or lower P_{O_2} values was expressed as the percentage change (ΔVF) above or below the normoxic value. From these individual values, mean values for all fish were calculated (Fig. 6). All hypoxic and hyperoxic values were significantly different from the normoxic value and changes observed during hyperoxia at 12·5 °C are the same as those observed during

hyperoxia at 25°C, indicating that temperature has no effect on the hyperoxic reaction of VF.

Heart rate (HF)

Fig. 7 shows the results of control experiments in which ECG signals were compared with blood pressure recordings in the bulbus arteriosus. Pressure recordings show a direct correspondence with ECG recordings, as after the QRS peak an increase in blood pressure could be observed; this was due to systolic contraction of the heart. These control experiments were carried out to distinguish between other electrical signals which came from the ventilatory muscles and those from the heart. The extraneous signals could be partly filtered out (measuring bandwidth 60–100 Hz, −6 dB).

In contrast to ventilatory activity, heart rate (HF) was independent of declining oxygen tension. It was constant at about 26 beats min^{-1} over a P_{O_2} range from 450 to 16 Torr at 12·5°C and at about 79 beats min^{-1} between 155 and 450 Torr at 25°C (Fig. 8). Only two fish showed a decrease of HF below 16 Torr, indicating that the critical oxygen tension for heart rate is lower than the P_c of oxygen consumption. Temperature had a similar effect on heart rate as shown for oxygen consumption and ventilation rate, with a Q_{10} value of 2·6.

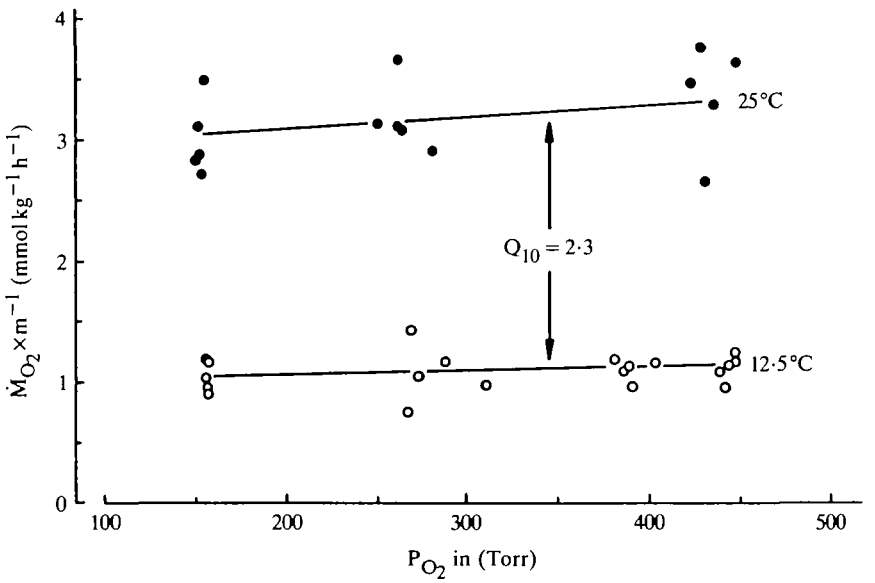

Fig. 4. Influence of normoxia and hyperoxia on the oxygen consumption ($\dot{M}_{O_2} \times m^{-1}$) of *Gobius cobitis*. P_{O_2}in indicates the oxygen tension of the inlet water. The graph shows the mean values of five animals measured at 12·5 and 25°C. From the mean values regression lines for 12·5 and 25°C were calculated. 12·5°C: $\dot{M}_{O_2} \times m^{-1} = 1{\cdot}04 + 0{\cdot}000P_{O_2}$in ($r = 0{\cdot}13$). 25°C: $\dot{M}_{O_2} \times m^{-1} = 2{\cdot}90 + 0{\cdot}000P_{O_2}$in ($r = 0{\cdot}32$). The slopes of the regression lines are not significantly different from a slope of 0 ($P < 0{\cdot}01$). The Q_{10} calculated from the regression lines at a P_{O_2} of 450 Torr is 2·3.

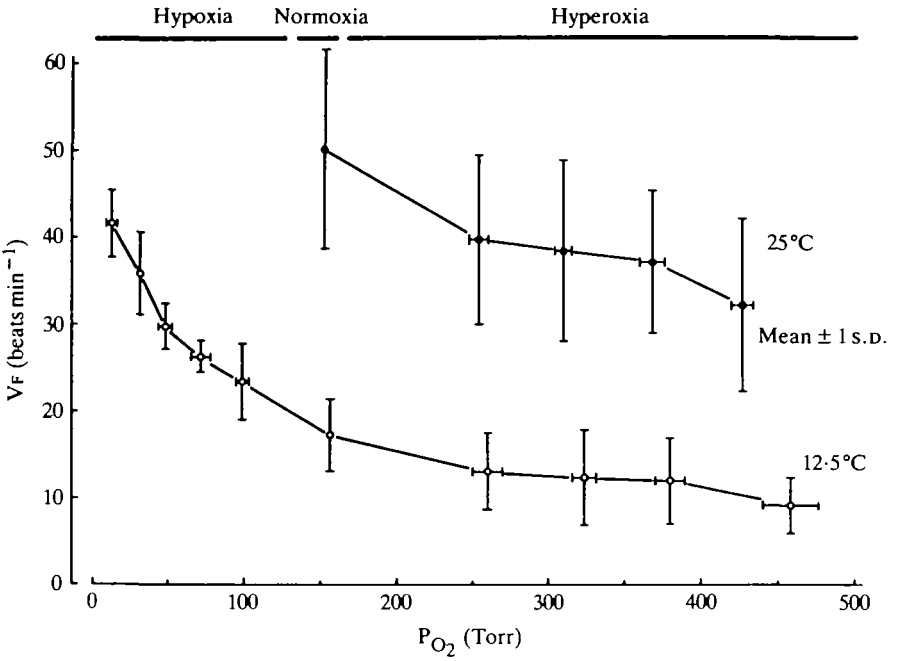

Fig. 5. Effect of hyperoxia at 12·5 and 25°C and hypoxia at 12·5°C on the ventilatory frequency (V_F) of *Gobius cobitis*. The experiment commenced at normoxic values (155 Torr). Individual data points represent mean values ± 1 S.D. for seven animals.

DISCUSSION

Intertidal fish represent a group of organisms which are exposed daily to environmental extremes of temperature and oxygen partial pressures. The use of laboratory simulations of hypoxia and hyperoxia, as in the present study, may therefore be particularly relevant in these species. Unfortunately, due to their small size and the difficulty of obtaining enough specimens within ecological constraints, our knowledge of the respiratory physiology of intertidal fish remains rudimentary (Bridges, 1987). The present study therefore seeks to provide some of the basic information on the response of the oxygen transport system of intertidal fish to environmental variables.

Effect of mass and temperature on oxygen consumption

The normal effect of size on $\dot{M}_{O_2} \times m^{-1}$ is a higher $\dot{M}_{O_2} \times m^{-1}$ per unit mass for smaller than for larger fish. This can be expressed in terms of the exponent $(b-1)$ relating $\dot{M}_{O_2} \times m^{-1}$ to body mass. For fish, the normal value is between $-0{\cdot}1$ and $-0{\cdot}2$ (Fry, 1957; Brett & Groves, 1979). Similar values ($-0{\cdot}21$ at 12·5°C and $-0{\cdot}11$ at 25°C) have been obtained for *Gobius cobitis*. An oxygen consumption of $1{\cdot}27\,\text{mmol}\,O_2\,kg^{-1}\,h^{-1}$ for a 50-g fish can be calculated from the regression line (Fig. 1). This value is similar to the range for animals labelled with a 'sluggish' living mode (Hughes & Knights, 1968). *G. cobitis* has a relatively herbivorous diet and Ralston & Horn (1986) have recently shown that in other herbivorous fish from

temperate zones a relatively low-activity life style has been adopted. In general, oxygen consumption is low in *G. cobitis* compared with that in other species of intertidal rockpool fish (see Table 1).

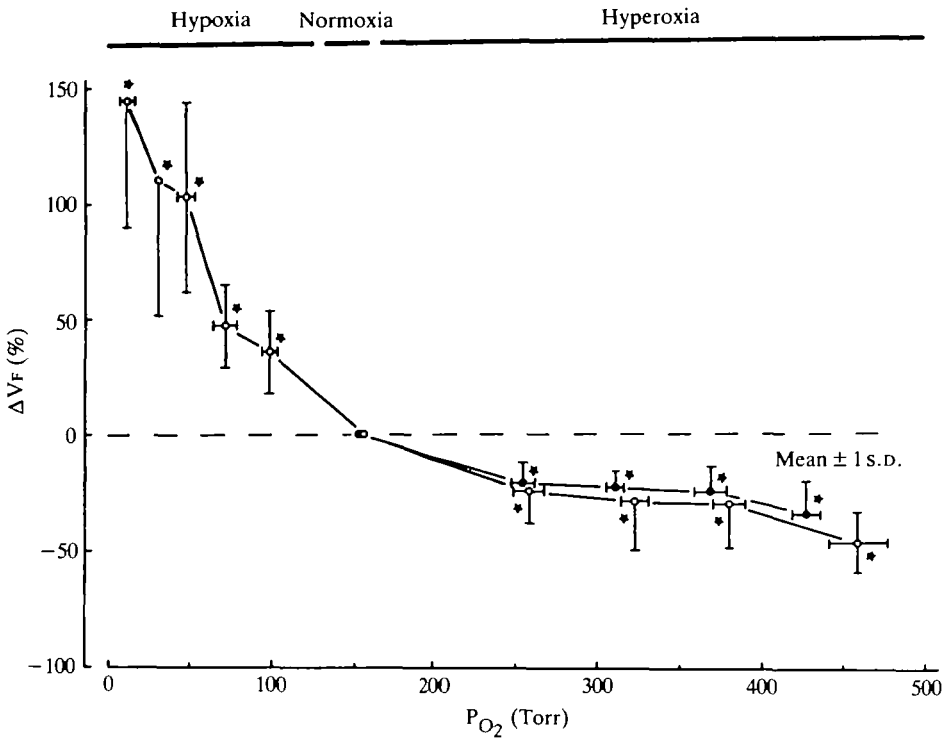

Fig. 6. Ventilatory responses to hyperoxia and hypoxia in *Gobius cobitis* expressed as the change (ΔV_F) in ventilatory frequency above or below the mean normoxic rate [23·4 beats min^{-1} at 12·5 °C (○) and 76 beats min^{-1} at 25 °C (●)]. An asterisk indicates a statistically significant difference from the normoxic value ($P<0{\cdot}01$). Error bars are ±1 S.D.

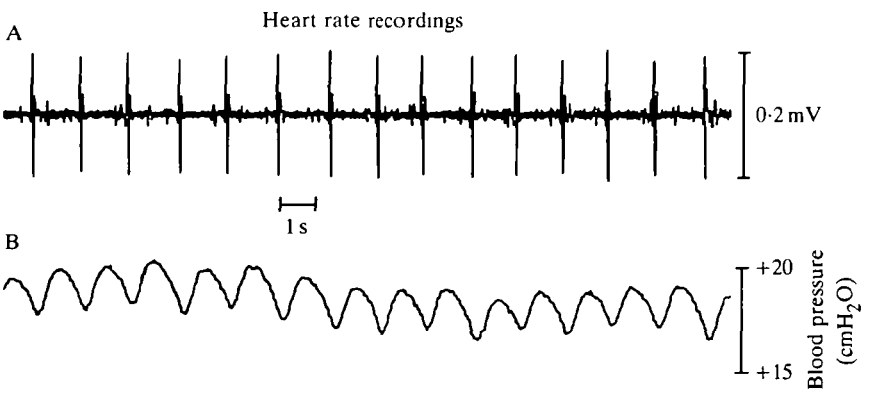

Fig. 7. Simultaneous original recordings of (A) ECG and (B) blood pressure in the heart and the bulbus arteriosus, respectively, of *Gobius cobitis* under normoxia at 12·5 °C. 1 cmH_2O = 98·1 Pa.

The influence of temperature on oxygen consumption, expressed as the Q_{10} value, averaged 2·3 for *Gobius cobitis*. Compared with Q_{10} values from other fishes (see Table 2) this indicates no special adaptation to temperature changes between 12·5 and 25°C. In *Blennius pholis* (Campbell & Spencer-Davis, 1975) Q_{10} values of 1·2 were reported between 2 and 5°C and 1·9 between 10 and 15°C and these authors

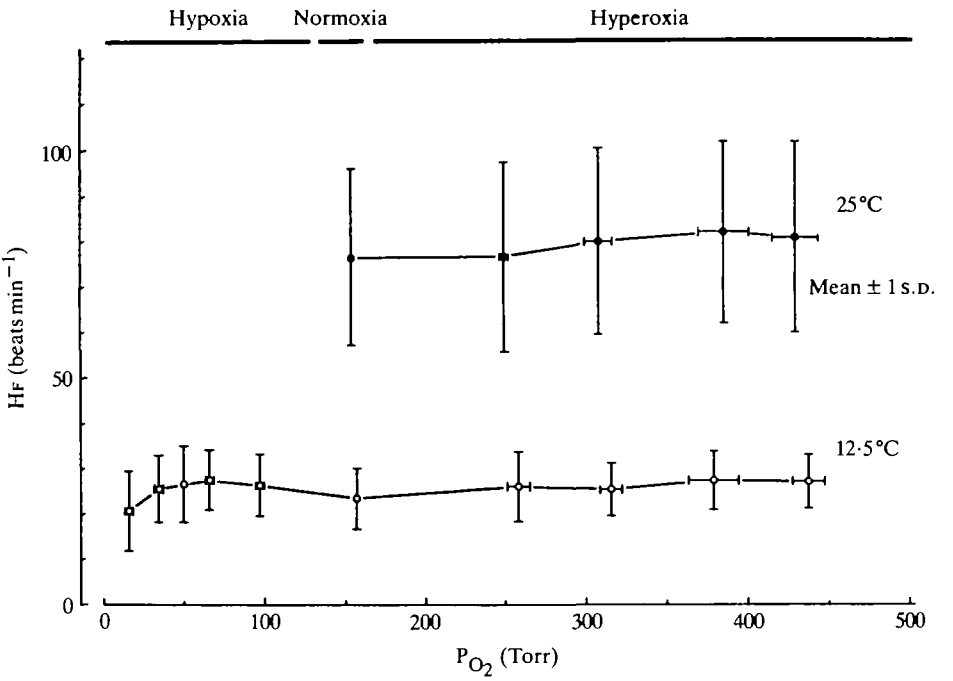

Fig. 8. Mean heart rate (H_F) of six specimens of *Gobius cobitis* exposed to hypoxia (12·5°C) and hyperoxia (12·5 and 25°C) commencing at normoxic values (155 Torr). Mean values are shown ±1 S.D.

Table 1. *Aquatic oxygen consumption for intertidal fish species found in rockpools near Roscoff, France*

Species	Mass (g)	Temperature (°C)	$\dot{M}_{O_2} \times m^{-1}$ (mmol kg^{-1} h^{-1})	Source
Blennius pholis	0·02–35	16	4·5	Milton (1971)
Blennius pholis	1–36	10	2·3	Campbell & Spencer-Davis (1975)
Blennius pholis	1–36	20	3·6	Campbell & Spencer-Davis (1975)
Ciliata mustela	51	13	4·4	Nonnotte & Kirsch (1978)
Cottus bubalis	10	15	4·4	D. F. Houlihan & D. J. Morrison (personal communication)
Gobius sp.	20	14	1·6	Altman & Dittmer (1971)
Gobius paganellus	10	25	6·9	Altman & Dittmer (1971)
Gobius cobitis	9–240	12·5	1·7	This study
Gobius cobitis	9–240	25	4·2	This study

Table 2. *Q_{10} values of different fish species*

Species	Q_{10}	Temperature range (°C)	Source
Blennius pholis[a]	2·2	2–25	Campbell & Spencer-Davis (1975)
Blennius pholis[b]	2·6	2–30	Campbell & Spencer-Davis (1975)
Clinocottus analis	2·9	15–25	Morris (1961)
Gillichthys mirabilis	2·5	10–17	Barlow (1961)
Gillichthys mirabilis	2·2	10–24	Barlow (1961)
Gillichthys seta	2·2	24–31	Barlow (1961)
Cottus bubalis	1·9	5–22	D. F. Houlihan & D. J. Morrison (personal communication)
Pholis gunnellus	2·3	5–22	D. F. Houlihan & D. J. Morrison (personal communication)
Cyprinus carpio	2·0	10–20	Hughes, Albers, Muster & Götz (1983)

[a] 10°C acclimation; [b] 20°C acclimation.

suggest that the low Q_{10} value is a special acclimation reaction to cold. Moffit & Crawshaw (1983), working on carp, observed that the Q_{10} value in experiments with acute temperature changes were similar to the Q_{10} values obtained using acclimated animals measured at the acclimation temperature, as long as the acute temperature changes were not stressful to the fish. Thus, in general, rockpool fish appear to show no specific adaptation of Q_{10} values when compared with the average value of Q_{10} for fish of 2·3 (value from Brett & Groves, 1979). Since hyperoxic conditions usually occur at high temperatures (daylight ebb-tide) a reduced sensitivity of oxygen consumption in this temperature range (12·5–25°C) may not be necessary.

Effect of hypoxia on oxygen consumption

Under moderate hypoxic conditions *Gobius cobitis* maintains oxygen consumption at a relatively constant level (regulatory phase). This regulatory phase can be observed both in individual measurements (Fig. 2) and in the combined results (Fig. 3). The occurrence of the regulatory phase, even when oxygen tensions are declining, has been discussed previously (Hughes, 1973; Steffensen, Lomholt & Johansen, 1982; Hughes *et al.* 1983). Oxygen consumption represents the sum of a number of processes taking place in the animal. During hypoxia, increased pumping will induce a rise in oxygen demand. Oxygen consumption can therefore only remain constant if the oxygen consumption of other tissues is reduced or the cost of increased ventilation is small. With declining oxygen tensions a point is reached, the critical oxygen tension, at which the oxygen demand of the respiratory pumps exceeds the extra oxygen supplied by hyperventilation and so oxygen consumption declines (Hughes, 1973). This P_c value averaged 43 Torr in experiments with *G. cobitis* (Fig. 3) and Hughes *et al.* (1983) point out that the true inspired P_{O_2} for the fish may be up to 10–15 Torr lower than that of P_{O_2} in for the respirometer. The values for *G. cobitis*, which are already low compared with other species (Steffensen *et al.* 1982; Hughes *et al.* 1983), would be even further depressed.

Low P_c values are common in intertidal rockpool species. Congleton (1980) reports a range of P_c values of 16–24 Torr in *Clinocottus analis*, 20–26 Torr for *Paraclinus intergripinnis* and 26–30 Torr for *Gibbonsia elegans*. In *Blennius pholis* a similar value to that observed in *G. cobitis* has been reported (Pelster, 1985). Innes & Wells (1985) report a value of 30–40 Torr in *Helcogramma medium*. At very low oxygen tensions (<20 Torr) intertidal rockpool fish may ventilate water from the surface layer (Congleton, 1980) or revert to air breathing (Pelster, 1985; Innes & Wells, 1985). *G. cobitis* has been observed to adopt the former strategy (unpublished observations).

Ventilatory and cardiac responses to hypoxia

In the present study only ventilatory frequency and heart rate were measured due to the technical difficulties of working with small fish. The conclusions drawn from such measurements must therefore be treated with some caution as stroke volume changes may also have occurred where frequency changes were not observed. Ventilatory frequency and heart rate are relatively low in *G. cobitis* under normoxic resting conditions, averaging 17 and 26 beats min^{-1}, respectively. These compare with ventilatory frequencies of 18 beats min^{-1} in *Callionymus lyra* (Hughes & Umezawa, 1968), 80 beats min^{-1} in *Pholis gunnellus* (Laming, 1983) and 63 beats min^{-1} in *Blennius pholis* (Pelster, 1985). It may therefore be assumed that stroke volume is relatively large in *G. cobitis*, in agreement with visual observations. The 'typical' response to hypoxia in fish is an increase in ventilation volume and a reflex bradycardia (Randall & Daxboeck, 1984; Taylor, 1985). This increase in ventilation is thought to compensate for the lower oxygen capacity of water. *G. cobitis* also shows this typical response, in that ventilatory frequency increases continuously with hypoxia (Figs 5, 6), reaching a value of approximately 150 % of the normoxic rate at a P_{O_2} of 20 Torr.

The mechanism by which an increase in ventilation volume is achieved varies among fish species. In *C. lyra* frequency decreases and stroke volume increases (Hughes & Umezawa, 1968). Kersten, Lomholt & Johansen (1979) observed a small change in frequency and a larger change in stroke volume in the flounder, *Platichthys flesus* and Steffensen *et al.* (1982) observed a decrease in ventilation frequency and an increase in stroke volume in the plaice, *Pleuronectes platessa*. In *Blennius pholis* only a small increase in frequency with hypoxic exposure is observed (Bridges *et al.* 1984; Pelster, 1985). In *G. cobitis* frequency appears to be the most important variable and it is interesting that the ventilatory frequency continues to increase (Fig. 6) even though oxygen consumption decreases at P_{O_2} values lower than 30–40 Torr (Fig. 3). This response may be adaptive for animals which continue to ventilate surface water under extreme hypoxic stress.

A reflex bradycardia, as shown by other fish, is absent in *G. cobitis* under hypoxic conditions (Fig. 8), heart rate being maintained relatively constant even at P_{O_2} values below the P_c. The bradycardia in other fish species is, however, offset by an increase in stroke volume (Randall & Daxboeck, 1984; Taylor, 1985), thereby maintaining perfusion of the respiratory surfaces at a high level. The bradycardia response

together with an increased stroke volume are thought to promote lamellar recruitment (Randall, 1982). Since heart rate is already low in *G. cobitis*, a bradycardia may be difficult to detect as in *Scyliorhinus canicula* (Taylor, 1985) or it may occur only under severe hypoxia at P_{O_2} values below 20 Torr, as in *Torpedo marmorata* (Hughes, 1978). In *G. cobitis*, which is often exposed to hypoxia, the maintenance of a constant heart rate may be energetically more efficient than changing heart rate and stroke volume.

Cardiorespiratory coupling has been proposed as a mechanism to increase the relative efficiency of gas exchange at the gill (Hughes, 1973; Taylor, 1985). In *G. cobitis* with a lower ventilation rate than heart rate this mechanism may be easier to maintain and indeed oxygen consumption only begins to decrease when ventilation rate and heart rate are mismatched (Figs 5, 8).

Temperature and hyperoxia

Temperature increases of water in intertidal rockpools during daylight usually have a slow time constant (2–6 h), due to the thermal capacity of the water. The subsequent decrease in temperature can, however, be rapid, as the rockpool is washed out by the incoming tide (Truchot & Duhamel-Jouve, 1980; Bridges *et al.* 1984). Simulated temperature changes under normoxic conditions induced a rapid change in ventilation rate (Fig. 5) and heart rate (Fig. 8) of *G. cobitis* with Q_{10} values of 2·4 and 2·6, respectively. These Q_{10} values are relatively high compared with values of 1·4 and 1·6, for ventilation and heart rate, respectively, in the trout (Heath & Hughes, 1973) and 1·4 for ventilation rate in another intertidal species, *Pholis gunnellus* (Laming, 1983). Moffit & Crawshaw (1983) have reported high Q_{10} values in the carp exposed to acute changes in temperature. It would appear that where cyclical changes in temperature occur no acclimation response is observed in terms of the Q_{10} value.

In previous studies on the influence of hyperoxia on fish respiration it has been shown that ventilation volume decreases with increasing P_{O_2} values as the ventilatory convection requirement decreases (Peyraud & Saferty, 1964; Dejours, 1973; Dejours *et al.* 1977; Wood & Jackson, 1980; Wilkes *et al.* 1981). Normally a decrease in ventilation will cause a concomitant increase in blood P_{CO_2} due to CO_2 retention (Dejours, 1973; Wood & Jackson, 1980; Wilkes *et al.* 1981). Under normoxic conditions this increase in CO_2 retention would stimulate ventilation. However, hyperoxia has an overriding effect on ventilation (Smith & Jones, 1982). These findings indicate the central role of O_2 compared with CO_2 in controlling the ventilatory drive (Dejours, 1973; Smith & Jones, 1982).

A similar decrease in ventilation rate has been observed in intertidal fish exposed to hyperoxia both in the laboratory (Bridges *et al.* 1984) and in the field (Bridges, 1987). In *G. cobitis*, a significant decrease in ventilatory frequency was observed at all P_{O_2} values. Changes in ΔV_F (Fig. 6), however, indicate that the ventilatory frequency decreased by approximately 40 % of the normoxic value at P_{O_2} values of >300 Torr in comparison with an increase of 150 % during hypoxia. As in hypoxia, changes in ventilation volume during hyperoxia appear to be mediated through

different mechanisms in different species. In the rainbow trout, Wood & Jackson (1980) found no change in frequency for the first 5 h of hyperoxic exposure but a marked change in stroke volume was observed. Wilkes *et al.* (1981) also observed no change in the ventilatory frequency but a change in stroke volume in the white sucker, *Catostomus commersoni*, exposed to hyperoxia. Since ventilatory frequency apparently decreases by 40% during hyperoxia (Fig. 6) and oxygen consumption remains the same (Fig. 4), this provides evidence either that there is an increase in oxygen consumption of certain tissues or that the cost of ventilation is small in *G. cobitis*.

When both temperature changes and hyperoxia are simulated together, the change in ventilatory frequency is of a similar order of magnitude at 12·5 and 25°C. This indicates that O_2 still plays a dominant role in controlling ventilation even at 25°C.

Cardiac responses to hyperoxia are little studied and Wilkes *et al.* (1981) reported a decrease in both dorsal aortic pressure and heart frequency in the white sucker exposed to hyperoxia, although some difficulties were experienced with low levels in control animals. Barrett & Taylor (1984) have also observed a bradycardia in the dogfish exposed to hyperoxia. In *G. cobitis* hyperoxia did not induce a change in heart rate in the P_{O_2} range 150–450 Torr. When both temperature and hyperoxic exposure were changed heart rate rose to the new temperature baseline but remained unaffected by hyperoxia.

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