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THE EFFECT OF PROGRESSIVE HYPOXIA ON RESPIRATION IN THE DOGFISH (*SCYLIORHINUS CANICULA*) AT DIFFERENT SEASONAL TEMPERATURES

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SUMMARY

1. Dogfish were acclimated to 7, 12 or 17 °C and exposed to progressive hypoxia at the temperature to which they had been acclimated. During normoxia, the Q_{10} values for oxygen uptake, heart rate, cardiac output and respiratory frequency over the full 10 °C range were: 2·1, 2·1, 2·1 and 2·5 respectively. Increased acclimation temperature had no effect on cardiac stroke volume or systemic vascular resistance, although there was a decrease in branchial vascular resistance, pH_a and $pH_{\bar{v}}$.

2. Progressive hypoxia had no effect on heart rate or oxygen uptake at 7 °C, whereas at 12 °C and 17 °C there was bradycardia, and a reduction in O_2 uptake, with the critical oxygen tension for both variables being higher at the higher temperature. Cardiac stroke volume increased during hypoxia at each temperature, such that cardiac output did not change significantly at 12 and 17 °C. Neither pH_a nor $pH_{\bar{v}}$ changed significantly during hypoxia at any of the three temperatures.

3. The influence of acclimation temperatures on experimental results from poikilotherms is pointed out. Previously-published results show quantitative differences.

4. The significance of the present results with respect to the functioning and location of oxygen receptors is discussed. It is argued that as the metabolic demand and critical oxygen tension of the whole animal are increased at high acclimation temperatures the same must be the case with the oxygen receptor. This would raise the stimulation threshold and could account for the bradycardia seen during hypoxia becoming manifest at higher values of P_{I,O_2}, P_{a,O_2} and $P_{\bar{v},O_2}$ as the acclimation temperature is raised.

INTRODUCTION

Many fish are exposed to seasonal variations in temperature in their natural environment, and as far as teleosts are concerned, a rise in environmental temperature causes an increase in metabolism, as indicated by oxygen uptake (Fry & Hart, 1948; Beamish, 1964; Heath & Hughes, 1973). This increase in oxygen requirement places demands on the respiratory and cardiovascular systems which are met, at least partly, by increases in ventilation volume and heart output (Hughes & Roberts, 1970; Stevens *et al.* 1972; Watters & Smith, 1973). The availability of oxygen is another

environmental variable whose effects on teleosts have been studied by a number of investigators (Saunders, 1962; Randall & Shelton, 1963; Holeton & Randall, 1967*a*, *b*; Marvin & Heath, 1968), and there are indications that the responses to hypoxia are influenced by environmental temperature. For example, Fry & Hart (1948) and Spitzer, Marvin & Heath (1969) have shown that the critical oxygen tension (the point at which the rate of oxygen uptake begins to fall during progressive hypoxia) increases in teleosts as environmental temperature rises. However, information on the response of the respiratory and cardiovascular systems of teleosts to hypoxia at different temperatures is sparse (Watters & Smith, 1973).

To date, it appears that there have been no systematic studies on the influence of environmental temperature on respiration and circulation in elasmobranchs, although the effects of hypoxia on these animals have been studied in some detail (Satchell, 1961; Piiper, Baumgarten & Meyer, 1970; Butler & Taylor, 1971). These investigations were, however, carried out at different temperatures and on different species of dogfish. The present study is an investigation of the response to hypoxia of the dogfish, *Scyliorhinus canicula* at different acclimation temperatures.

These fish are found mostly at a depth of 50–60 m, but can be in water as shallow as 3–5 m or as deep as 100–200 m around the coast of Britain (Wheeler, 1969). In the English Channel, off the coast of Plymouth where our fish were caught, seasonal temperature variations of 7–15 °C have been reported at a depth of 10 to 50 m, whereas in the upper 1–2 m, the annual variations can be from 5–19 °C (Armstrong, Butler & Boalch, 1970; Cooper, 1958). We chose, therefore, to work over the mean seasonal range between 7 and 17 °C.

MATERIALS AND METHODS

Dogfish (*Scyliorhinus canicula*) of either sex and weighing between 0·56 and 1·05 kg were used. They were obtained from the Marine Biological Association, Plymouth, and were kept in tanks containing circulated, filtered sea water at 7, 12 or 17 °C for at least 18 days prior to experiments to allow them time to acclimate to the experimental temperature (Baslow, 1967). The fish were collected at the time of the year when the sea temperature at the collecting site in the English Channel was close to the subsequent acclimation temperature (Armstrong *et al.* 1970).

All surgical operations were performed in the constant temperature room where the animals had been acclimated. The surgical techniques were as described by Butler & Taylor (1971). The fish were anaesthetized in MS 222. A cannula was inserted into the caudal artery and advanced forward until its tip was in the dorsal aorta just behind the iliac arteries. This cannula was used for measuring dorsal aortic blood pressure as well as for sampling arterial blood. Ventral aortic blood pressure and mixed venous blood were obtained from a cannula in the first right afferent branchial artery. Any unused blood was injected back into the animal via a cannula in the caudal vein. A cannula was also placed in the orobranchial cavity and in a few fish, a cannulating blood flow transducer (Biotronex Ltd) was inserted into the ventral aorta between the 2nd and 3rd afferent branchial vessels (Hanson & Johansen, 1970). The animal was then placed into the experimental apparatus and left for at least 16 h in fully aerated, circulating sea water to recover from the anaesthetic and handling. The

fish was clamped by its tail (Butler & Taylor, 1971) in a 'Perspex' respirometer tube. This tube was itself submerged in sea water in a blackened 'Perspex' box, and the top of the box was three-quarters covered with black 'Perspex'. Thus the fish was in dim light and yet could not see the experimenters. The sea water was filtered and pumped from the blackened box by two pumps. Each pump sent water through glass cooling coils, but one pump returned water directly to the box, while the other sent water to a constant-head, gas exchange column. From here the water entered the respirometer via a flow-meter and a bubble trap. The flow rate through the respirometer tube was 450 ml min^{-1} at 7 °C and 12 °C, and 600 ml min^{-1} at 17 °C. Water leaving the respirometer overflowed into the blackened box. During the course of an experiment the temperature of the sea water in the respirometer was maintained within 0·5 °C of the acclimation temperature of the experimental animal.

After the recovery period, with the animal in normoxic sea water, samples were taken from water entering and leaving the respirometer and from the orobranchial cavity of the fish. The oxygen tension (P_{O_2}) of these samples was measured immediately using a Radiometer PHM 71 acid-base analyser and an oxygen electrode which was at the same temperature as the sea water (Butler & Taylor, 1971). Seawater samples taken via the cannula in the orobranchial cavity were used to determine the oxygen tension of the inspired water (P_{I,O_2}). From the P_{O_2} values of the water entering and leaving the respirometer, the solubility coefficient for O_2 in sea water at the experimental temperature, and the flow rate through the respirometer, it was possible to calculate the oxygen uptake of the animal ($\dot{V}_{O_2}$). Arterial and mixed venous blood samples (0·75 ml) were taken from the dorsal and ventral aortae and immediately analysed for their P_{O_2} and pH with the Radiometer PHM71, and for their oxygen content (C_{O_2}) with a Lex–O_2–Con oxygen analyser (Lexington Instrument Corporation). Haematocrit was also measured and any remaining blood was injected back into the animal via the caudal vein.

After the sea water and the blood samples had been analysed, the P_{O_2} of the water entering the respirometer was reduced in a series of steps by bubbling fixed ratios of air and nitrogen through the gas exchange column for 30 min. Tests showed that it took at the most 20 min for the respirometer to equilibrate and for $\dot{V}_{O_2}$ to stabilize after the P_{O_2} had been reduced. At the end of the 30 min equilibration period blood and water samples were taken and analysed as described previously, and the P_{O_2} of the water entering the respirometer was reduced again; and so on. It was intended that specific values of P_{I,O_2} should be obtained at each reduction, but this proved impossible without continuous monitoring of water from the orobranchial cavity. In fact P_{I,O_2} was reduced in 4–6 steps of 15–30 mmHg down to a value of approximately 40 mmHg. Values of any variable at 10 mmHg intervals of P_{I,O_2} between 130 mmHg (normoxia) and 40 mmHg were then obtained for each animal by linear interpolation. From these values a mean curve was plotted showing the effect of progressive hypoxia on the chosen variable at any one temperature.

Pressures (and when recorded, blood flow) were monitored continuously. Dorsal and ventral aortic blood pressures were measured using strain gauge transducers (Type 4-327-L 221, Devices Ltd), orobranchial pressure was measured by a S.E. Laboratories S.E. 4-82 transducer, and blood flow was measured by a Biotronex BL-610 pulsed logic electromagnetic flow meter set to an upper frequency response

of 50 Hz. Zero flow was assessed from the flow baseline reached during bradycardia. At the end of the experiment the cannulating transducer was removed from the fish and calibrated with the fish's own blood. Stroke volume was obtained by integrating the area under the flow curve, but because of the position of the probe in the animal, the flow obtained by this method only represents the blood going to the first two pairs of gill arches. Total cardiac output ($\dot{Q}$) was calculated using the formula:

$$\dot{Q}(\text{ml kg}^{-1}\text{ min}^{-1}) = \frac{\dot{V}_{O_2}(\text{ml kg}^{-1}\text{ min}^{-1}) \times 100}{C_{a,O_2} - C_{\bar{v},O_2}(\text{vol \%})}.$$

Resistance to blood flow through the branchial or systemic regions of the vascular system were obtained from the formula:

$$\text{Resistance (dynes sec}^{-1}\text{ cm}^{-5}) = \frac{\text{Drop in mean pressure across the vascular bed (mmHg)} \times 13{\cdot}6 \times 981 \times 60}{\dot{Q}(\text{ml min}^{-1}) \times 10}.$$

Venous pressure was assumed to be zero at all times.

The word 'normoxic' means the highest P_{I,O_2} to which the animals were exposed (130–140 mmHg). Student's *t*-test was used to test the significance of any difference between two mean values and the word 'significant' in the present report means significant at the 95% confidence level ($P < 0{\cdot}05$).

RESULTS

Normoxia

The normoxic values of all the measured variables at the three experimental temperatures are given in Table 1, and it can be seen that oxygen uptake, heart rate, cardiac output and respiratory frequency all show an increase with a rise in acclimation temperature. Over the full 10 °C temperature range, the Q_{10} values for these variables are: 2·1, 2·1, 2·1 and 2·5 respectively. It is clear that as heart rate and cardiac output have a similar Q_{10}, that stroke volume did not increase from 7 to 17 °C. Also, with oxygen uptake and cardiac output having the same Q_{10}, it is evident that the difference in arterial and mixed venous oxygen content did not change with a rise in temperature. Haematocrit was significantly higher in animals acclimated to 17 °C compared with those acclimated to 7 °C but this was not accompanied by a significant rise in C_{a,O_2}, although P_{a,O_2} was similar at the two extreme temperatures. The lack of a statistical correlation between C_{a,O_2} and haematocrit is likely to be the result of a relatively greater degree of inaccuracy in the technique used to measure blood oxygen content. In fact the mean values do show an increase in C_{a,O_2} at 17 °C compared with 7 °C and it is probably safe to conclude that at higher temperatures there is a rise in oxygen carrying capacity of the blood as a result of an increase in haematocrit. Accompanying the increase in cardiac output at higher acclimation temperatures were increases in both ventral and dorsal aortic blood pressures. Over the full 10 °C range, mean dorsal aortic pressure increased by 72%, whereas mean ventral aortic pressure rose by 50%. These changes in blood pressure, together with the changes in cardiac output, indicate that resistance to blood flow remained substantially unchanged in the systemic vascular bed at the two extreme temperatures, although there was a 25% increase in

Table 1. *Mean values,* ± S.E. *of mean, of measured variables during normoxia at the three different acclimation temperatures*

(n = number of animals.)

Temperature	7 °C (n = 7)	12 °C (n = 8)	17 °C (n = 7)
P_{I,O_2} (mmHg)	140 ± 2·7	140 ± 1·8	131 ± 1·9
P_{a,O_2} (mmHg)	90·9 ± 8·7	114·4 ± 1·9	97·6 ± 2·8
C_{a,O_2} (vol %)	4·0 ± 0·2	4·7 ± 0·4	4·7 ± 0·6
$P_{\bar{v},O_2}$ (mmHg)	21·3 ± 3·4	34·5 ± 3·4	32·9 ± 3·2
$C_{\bar{v},O_2}$ (vol %)	2·1 ± 0·2	2·7 ± 0·4	2·7 ± 0·6
pH_a	7·885 ± 0·075	7·813 ± 0·060	7·743 ± 0·033
$pH_{\bar{v}}$	7·829 ± 0·071	7·770 ± 0·065	7·684 ± 0·024
Respiratory frequency (no. min^{-1})	24·4 ± 1·1	39·4 ± 1·6	60·7 ± 2·6
Oxygen uptake (ml kg^{-1} min)	0·346 ± 0·038	0·465 ± 0·042	0·735 ± 0·043
Cardiac frequency (beats min^{-1})	19·5 ± 0·8	28·7 ± 1·1	40·8 ± 2·5
Cardiac stroke volume (ml kg^{-1})	1·01 ± 0·16	0·81 ± 0·06	1·00 ± 0·13
Cardiac output (ml kg^{-1} min^{-1})	19·2 ± 2·5	23·2 ± 1·9	39·8 ± 6·2
Vascular resistance in the gills ($\times 10^4$ dynes sec^{-1} cm^{-5})	3·49 ± 0·49	3·09 ± 0·39	2·39 ± 0·39
Vascular resistance in the body ($\times 10^4$ dynes sec^{-1} cm^{-5})	9·25 ± 1·55	11·48 ± 1·09	9·01 ± 1·71
Mean pressure in the ventral aorta (mmHg)	23·1 ± 1·0	31·8 ± 1·7	34·8 ± 1·4
Mean pressure in the dorsal aorta (mmHg)	16·8 ± 1·3	24·8 ± 1·2	28·9 ± 2·0
Haematocrit	16·0 ± 1·1	17·2 ± 1·0	20·5 ± 1·6
Wt. (kg)	0·857 ± 0·046	0·788 ± 0·027	0·712 ± 0·037

systemic resistance at 12 °C. Resistance in the branchial vascular bed showed a progressive decrease with a rise in acclimation temperature. Both arterial and mixed venous pH decreased with a rise in acclimation temperature and in each case $\Delta pH/\Delta T$ was −0·014 unit/°C over the full 10 °C range.

Hypoxia

The effect of a progressive decline in P_{I,O_2} on cardiac function at the three different temperatures is shown in Fig. 1. The chronotropic response was somewhat different at each temperature. At 7 °C there is no significant change in heart rate throughout the hypoxic period whereas at 12 °C heart rate was 70% of its normoxic value at a P_{I,O_2} of 42 mmHg (Table 2). The first significant change in heart rate from the normoxic value was recorded at a P_{I,O_2} of 60 mmHg. From Fig. 3(*b*) it can be seen that at this P_{I,O_2}, P_{a,O_2} was 30 mmHg and $P_{\bar{v},O_2}$ was 12 mmHg. At 17 °C heart rate showed a steady decline during progressive hypoxia to 50% of the normoxic value at a P_{I,O_2} of 39 mmHg (Table 2). The first significant reduction from the normoxic level was at a P_{I,O_2} of 90 mmHg, which is equivalent to a P_{a,O_2} of 55 mmHg and a $P_{\bar{v},O_2}$ of 22 mmHg (Fig. 3*c*). In contrast, the cardiac inotropic response to hypoxia was basically similar at each temperature. Stroke volume increased as P_{I,O_2} fell, more so at 7 and 17 °C than at 12 °C, but the net result was that at 12 and 17 °C the rise in stroke volume compensated for the fall in heart rate so that cardiac output showed no significant change during hypoxia at the upper two temperatures. At 7 °C, however, the increase in stroke volume gave rise to an increase in cardiac output of similar magnitude, the first significant increase above the normoxic value being noted at a P_{I,O_2} of 60 mmHg. The cardiac inotropic changes just described have been obtained using an indirect method, viz. the Fick equation, but direct measurement of blood

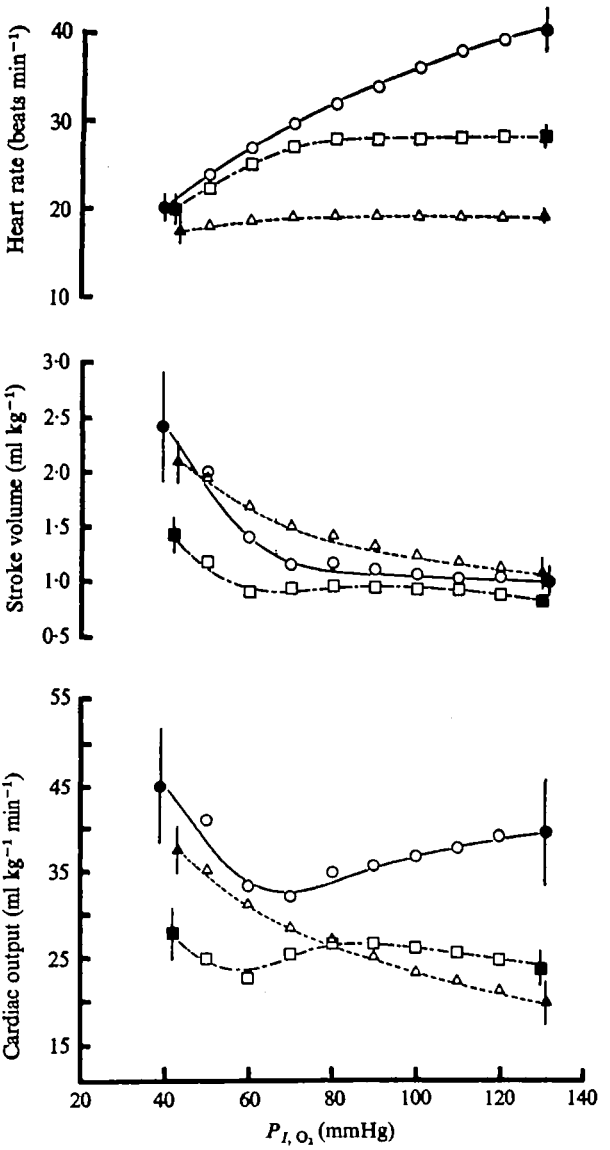

Fig. 1. Changes in heart rate, cardiac stroke volume and cardiac output in dogfish during progressive hypoxia at 7 °C (△), 12 °C (□) and 17 °C (○). The number of animals contributing to each curve were: 7 at 7 °C, 8 at 12 °C and 7 at 17 °C. The curves were obtained by linear interpolation of the original data (see Materials and Methods). For each curve, the first and last points were plotted with vertical lines which indicate ±S.E. mean.

flow to the first two pairs of gills also indicates that stroke volume rises during hypoxia (Fig. 2).

The difference between arterial and mixed venous blood oxygen content ($a-\bar{v}_{O_2}$ content difference) was similar during normoxia at all three temperatures and decreased during hypoxia. At 7 °C, C_{a,O_2} declined faster than $C_{\bar{v},O_2}$, so that the $a-\bar{v}_{O_2}$ content difference had been significantly reduced by the time P_{I,O_2} had reached 90 mmHg (Fig. 3*a*). Oxygen uptake did not change significantly throughout the period of

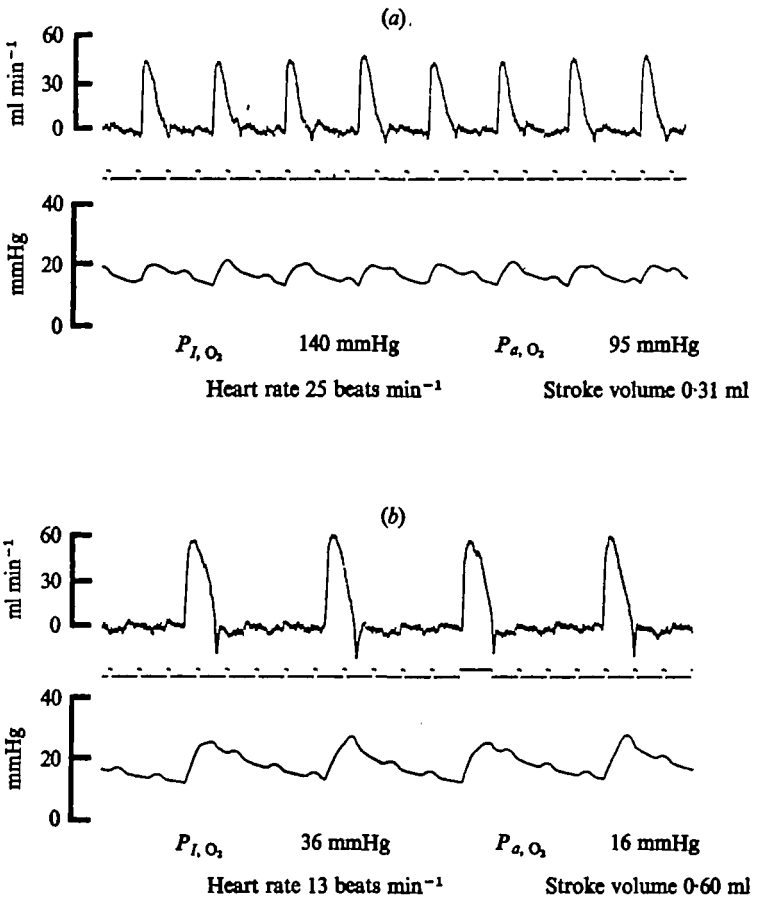

Fig. 2. Traces from an unanaesthetized female dogfish at 12 °C and weighing 0·85 kg, showing blood flow to the first two pairs of branchial blood vessels during (*a*) normoxia and (*b*) hypoxia. In each series, the traces from top to bottom are: pulsatile blood flow, time marker (sec) and ventral aortic blood pressure. Values of P_{I,O_2}, P_{a,O_2}, stroke volume and heart rate are given beneath each series.

progressive hypoxia (Fig. 4). At 12 °C the $a-\bar{v}_{O_2}$ content difference was maintained down to a P_{I,O_2} of 60 mmHg, and was reduced as P_{I,O_2} fell below this value (Fig. 3*b*). Oxygen uptake also fell, and was significantly below the normoxic value at P_{I,O_2} of 48 mmHg (Fig. 4) which corresponds to a P_{a,O_2} of 20 mmHg and a $P_{\bar{v},O_2}$ of 9 mm Hg (Fig. 3*b*). At 17 °C, $a-\bar{v}_{O_2}$ content difference did not significantly decrease below the normoxic value until P_{I,O_2} fell below 60 mmHg. At this temperature however, oxygen uptake fell significantly below the normoxic value at a P_{I,O_2} of 63 mmHg (Fig. 4), which is equivalent to a P_{a,O_2} of 31 mmHg and a $P_{\bar{v},O_2}$ of 12 mmHg (Fig. 3*a*). At a P_{I,O_2} of approximately 40 mmHg, oxygen uptake was 78% of the normoxic value at 7 °C, 69% of normoxic at 12 °C, and 56% of normoxic at 17 °C (Table 2).

Blood pressure in the ventral aorta was maintained at its normoxic values during progressive hypoxia at 7 and 12 °C, but fell continuously and was significantly below the normoxic value at a P_{I,O_2} of 80 mmHg at 17 °C. In the dorsal aorta, pressure was maintained throughout the exposure to hypoxia at 7 °C, was only significantly below

Table 2. *Mean values, ± S.E. of mean, of measured variables at the end of progressive hypoxia ($P_{I,O_2} \simeq 40$ mmHg) at the three different acclimation temperatures*

Temperature	7° C ($n = 7$)	12 °C ($n = 8$)	17 °C ($n = 7$)
P_{I,O_2} (mmHg)	43 ± 1·0	42 ± 1·3	39 ± 2·2
P_{a,O_2} (mmHg)	13·8 ± 0·7	16·5 ± 1·5	16·8 ± 1·7
C_{a,O_2} (vol %)	1·3 ± 0·04	1·6 ± 0·12	1·2 ± 0·17
$P_{\bar{v},O_2}$ (mmHg)	6·6 ± 1·4	7·0 ± 0·9	6·0 ± 0·6
$C_{\bar{v},O_2}$ (vol %)	0·5 ± 0·1	0·4 ± 0·1	0·2 ± 0·05
pH_a	7·909 ± 0·069	7·741 ± 0·064	7·685 ± 0·037
$pH_{\bar{v}}$	7·840 ± 0·069	7·660 ± 0·061	7·639 ± 0·33
Respiratory frequency (no. min^{-1})	26·3 ± 1·9	44·0 ± 1·7	56·3 ± 2·6
Oxygen uptake (ml kg^{-1} min^{-1})	0·265 ± 0·007	0·320 ± 0·017	0·410 ± 0·034
Cardiac frequency (beats min^{-1})	17·7 ± 1·5	20·1 ± 1·7	20·6 ± 1·5
Cardiac stroke volume (ml kg^{-1})	2·09 ± 0·21	1·43 ± 0·16	2·42 ± 0·49
Cardiac output (ml kg^{-1} min^{-1})	37·4 ± 5·3	27·9 ± 2·9	45·0 ± 6·9
Vascular resistance in the gills ($\times 10^4$ dynes sec^{-1} cm^{-5})	3·28 ± 0·79	3·07 ± 0·32	1·68 ± 0·35
Vascular resistance in the body ($\times 10^4$ dynes sec^{-1} cm^{-5})	3·46 ± 0·57	7·61 ± 1·06	4·53 ± 0·70
Mean pressure in the ventral aorta (mmHg)	24·8 ± 1·9	27·5 ± 2·3	23·3 ± 1·8
Mean pressure in the dorsal aorta (mmHg)	13·5 ± 1·2	18·8 ± 2·1	17·1 ± 1·4

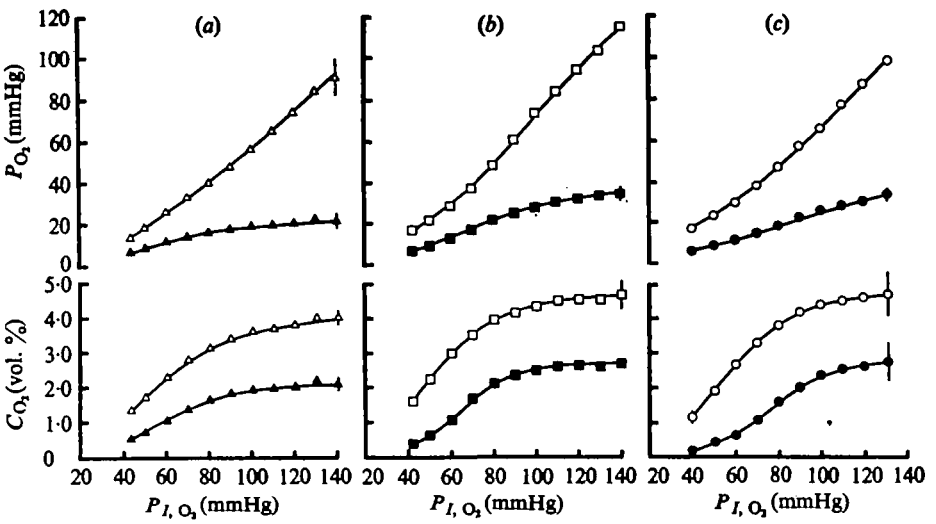

Fig. 3. Changes in P_{a,O_2} and C_{a,O_2} (open symbols) and $P_{\bar{v},O_2}$ and $C_{\bar{v},O_2}$ (closed symbols) during progressive hypoxia at (*a*) 7 °C, (*b*) 12 °C and (*c*) 17 °C. For further details see Fig. 1.

the normoxic value at a P_{I,O_2} of 45 mmHg at 12 °C, but fell steadily and was significantly below the normoxic level at a P_{I,O_2} of 80 mmHg at 17 °C (Fig. 5). Computations from the data on cardiac output and blood pressures indicate that resistance to blood flow through the branchial vascular bed did not change significantly during hypoxia, but resistance to flow through the systemic vascular bed decreased at all three temperatures (Fig. 6). There were two variables which showed little or no change during hypoxia at any temperature. Respiratory frequency increased significantly above the normoxic value at a P_{I,O_2} of 80 mmHg at 12 °C, but remained unaltered at 7 and 17 °C (Fig. 7). Neither pH_a or $pH_{\bar{v}}$ underwent any significant change during hypoxia at any of the three temperatures (Tables 1 and 2).

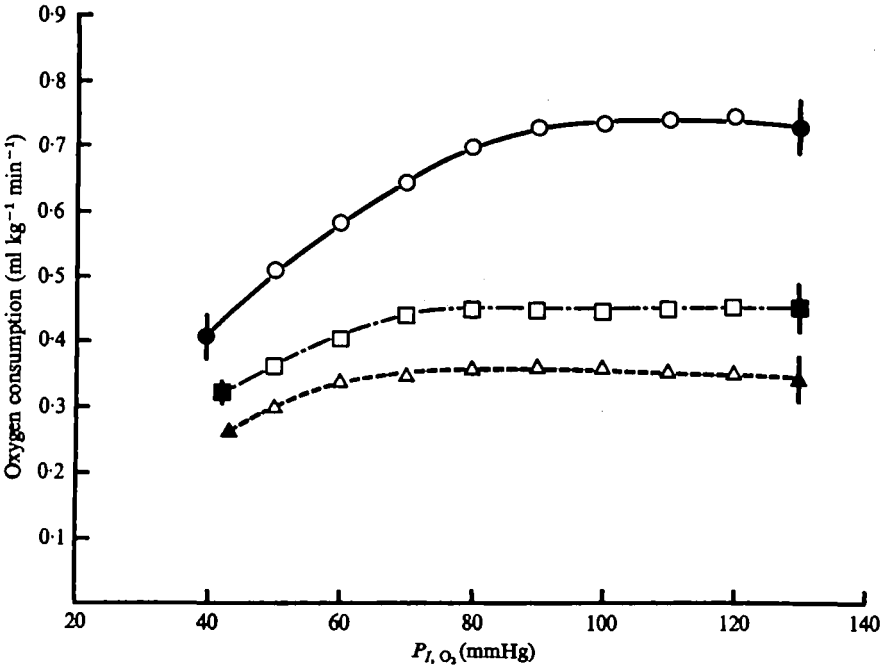

Fig. 4. Mean changes in oxygen uptake in dogfish during progressive hypoxia at 7 °C (△), 12 °C (□), and 17 °C (○). For further details see Fig. 1.

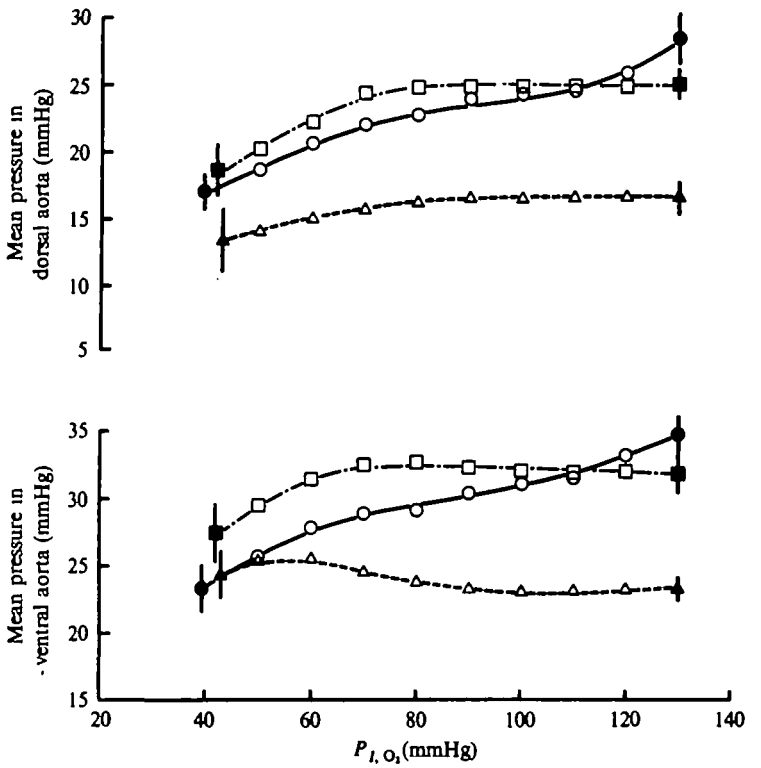

Fig. 5. Changes in mean pressures from the dorsal and ventral aortae in dogfish during progressive hypoxia at 7 °C (△), 12 °C (□) and 17 °C (○). For further details see Fig. 1.

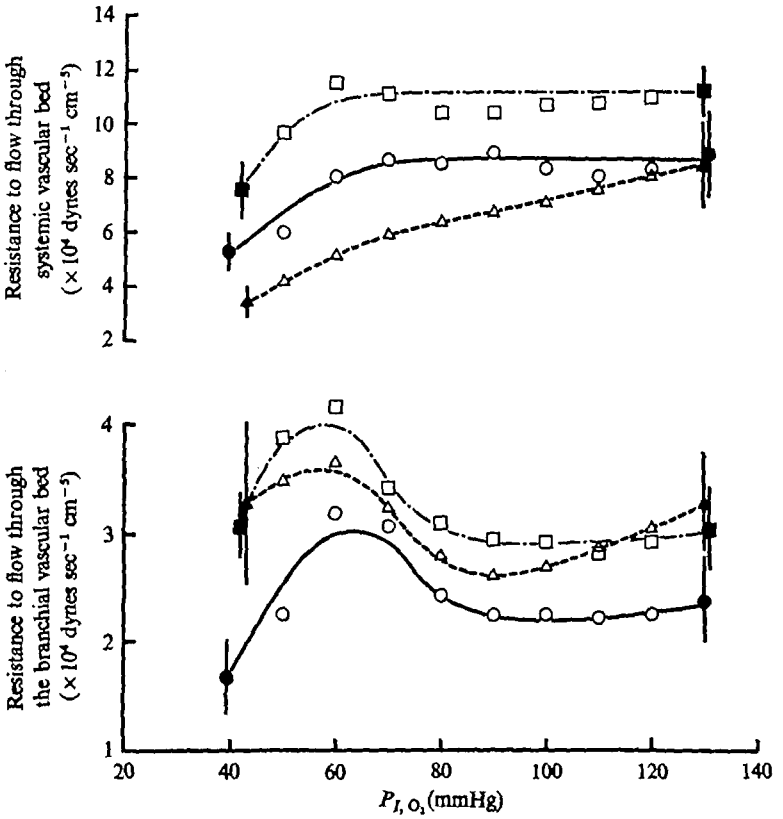

Fig. 6. Changes in resistance in the branchial and systemic vascular beds in dogfish during progressive hypoxia at 7 °C (△), 12 °C (□) and 17 °C (○). For further details see Fig. 1.

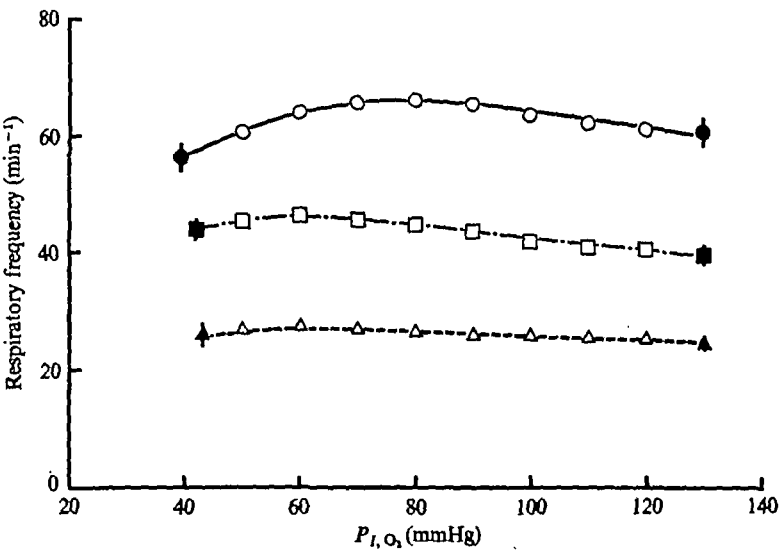

Fig. 7. Changes in respiratory frequency in dogfish during progressive hypoxia at 7 °C (△), 12 °C (□) and 17 °C (○). For further details see Fig. 1.

DISCUSSION

The present results are in general agreement with those that have already been published on the effects of temperature and hypoxia on respiration and circulation in fish (see Introduction for references). As in teleosts, a rise in temperature stimulates respiratory frequency, heart rate and oxygen uptake. There was, however, no indication of a reduction in peripheral resistance as temperature increased, as had been supposed by Stevens *et al.* (1972) for the lingcod, neither did the $a-\bar{v}_{O_2}$ content difference increase at higher temperatures as Watters & Smith (1973) found to occur in the flounder. Progressive hypoxia caused bradycardia, a rise in stroke volume, a reduction in oxygen uptake, and eventually a decline in the $a-\bar{v}_{O_2}$ content difference. As has already been reported (Hughes & Umezawa, 1968*a*; Piiper *et al.* 1970; Butler & Taylor, 1971) dogfish, unlike teleosts, show little or no change in respiratory frequency during hypoxia.

Although some of the results in this and similar investigations are qualitatively alike, quantitative differences are often apparent, and it has been pointed out that such variations in results may reflect either species differences or differences in techniques (Randall, 1970). One obvious difference in technique as far as fish and all poikilotherms are concerned, is the temperature at which the animals are held in captivity and subsequently experimented upon. Piiper *et al.* (1970) suggest that the dogfish *S. stellaris*, acclimated to 17 °C, should be classified as an oxygen conformer (Prosser, 1955), but Spitzer *et al.* (1969) have shown that a fish can be an oxygen conformer or a regulator, depending upon the temperature to which it has been acclimated. Certainly, *S. canicula* shows complete respiratory independence at a temperature of 7 °C, but if the trend seen in the present experiments continues, then it could well be classified as a total conformer at some temperature above 17 °C. Piiper *et al.* (1970) also reported a 32% reduction in heart rate at a P_{I,O_2} of 54 mmHg in their dogfish, whereas in an earlier paper on *S. canicula* acclimated to 12 °C, Butler & Taylor (1971) found a 16% reduction in heart rate at a similar P_{O_2}. In the present series of experiments, at a P_{I,O_2} of 55 mmHg, heart rate showed a 17% reduction at 12 °C and a 35% reduction at 17 °C. So in both cases cited above, it is clear that variations in acclimation and subsequent experimental temperature have given rise to differences in response to hypoxia which might otherwise have been interpreted as specific differences. The influence of acclimation temperature on the response to hypoxia is not confined to fish. It has, for example, been shown to occur in decapod Crustacea (Taylor, Butler & Sherlock 1973; Taylor & Butler, 1973), and may well be a universal influence amongst poikilotherms.

The cardiac chronotropic response to hypoxia in fish has often been used as an indicator of chemoreceptor stimulation (Satchell, 1961; Randall & Smith, 1967; Butler & Taylor, 1971), although the location of these receptors is still uncertain (Randall & Smith, 1967; Taylor, Houston & Horgan, 1968; Hughes & Umezawa 1968*b*; Saunders & Sutterlin, 1971; Laurent & Rouzeau, 1972; Randall & Jones, 1973; Bamford, 1974). One of the arguments used when favouring a central position of the chemoreceptors, is that the P_{I,O_2} causing bradycardia during hypoxia would be the same at all temperatures if receptors were placed peripherally (Spitzer *et al.* 1969). This assumes that the receptors respond to a fixed threshold P_{O_2}. A similar

argument was used by Hughes & Roberts (1970) to explain the bradycardia resulting from increased environmental temperature, even though P_{I,O_2} remained unaltered. The present investigation has shown, however, that bradycardia occurred at progressively higher values of P_{I,O_2}, P_{a,O_2} and $P_{\bar{v},O_2}$ with increasing temperature. This indicates that whatever the location(s) of the chemoreceptors may be, they do not have the same sensitivity at different environmental temperatures. From our knowledge of the arterial chemoreceptors in mammals, this apparent shift in sensitivity with temperature is not surprising. The stimulus for the chemoreceptors is a fall in oxygen supply below the level necessary to meet the extremely high metabolic requirements of these cells (Biscoe, 1971; Paintal, 1973). We know that in fish, metabolism and critical oxygen tension rise with temperature. As the chemoreceptors are likely to experience similar changes in oxygen demand and supply, it is equally likely that they will show a greater sensitivity to any reduction in P_{O_2} at higher temperatures, whatever their location. Direct recording from sensory nerve fibres is the only likely way of resolving this, and in fact Paintal (1971) has demonstrated that the aortic body chemoreceptors in cats do have their sensitivity reduced at low temperatures. Attempts to record nerve impulses related to chemoreceptor activity in fish might be more profitable therefore, if the experiments are conducted at temperatures at the upper end of the animals' physiological range. If successful, such studies would indicate whether the effect of temperature on the cardiac response to hypoxia is mediated entirely at the sense organs or whether the C.N.S. is involved.

The bradycardia during hypoxia is accompanied by a rise in stroke volume such that cardiac output is maintained. Even at low acclimation temperatures where there is no bradycardia during hypoxia, stroke volume still increases. The bradycardia is known to be the result of a rise in vagal tone in both teleosts (Randall & Shelton, 1963; Randall, 1966; Randall & Smith, 1967) and in elasmobranchs (Satchell, 1961; Butler & Taylor, 1971). The positive inotropic response could be the result of increased filling as the heart slows (Starling's Law), or it could be the result of adrenergic stimulation. Preliminary experiments (Butler & Taylor, unpublished observations) have shown that in dogfish the increase in cardiac stroke volume during hypoxia is abolished after injection of an adrenergic β-receptor blocking agent (Propranolol). This indicates that cardiac activity is augmented during hypoxia by catecholamines. There is, however, no morphological (Young, 1933) or physiological (S. Short, personal communication) evidence of sympathetic innervation of the heart in elasmobranchs. Gannon, Campbell and Satchell (1972) have recently demonstrated that adrenergic control of the elasmobranch heart could occur by way of catecholamines released from chromaffin tissues into the blood in the posterior cardinal sinus which would then aspirate directly into the heart. This mechanism could provide rapid and relatively specific cardiac control. A similar function has been speculatively attributed to the pericardial glands in decapod crustaceans (Taylor, 1970).

Dogfish can be added to the growing list of poikilotherms that show a reduction in blood pH with a rise in temperature, and lend further support to the hypothesis that, whatever the temperature, the relative alkalinity of the blood with respect to neutrality of pure water is maintained approximately constant (Rahn, 1966; Howell *et al.* 1970; Howell *et al.* 1973). During hypoxia, however, neither pH_a nor $pH_{\bar{v}}$ changed at any acclimation temperature, and these observations are consistent with the findings

of Holeton & Randall (1967*b*) in trout, and Eddy (1974) in tench. These authors also report an increase in blood lactate during hypoxia, and it is likely that this lactate counteracts any tendency towards alkalosis resulting from a rise in $\dot{V}_g$ (Dejours, 1973).

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