CARDIOVASCULAR AND RESPIRATORY CHANGES DURING HEAT STRESS IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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INTRODUCTION

Numerous studies on the effect of temperature change on fish have been made (see bibliography by Raney & Menzel, 1969). Most of these have been concerned with behaviour, metabolism, reproduction, and lethal limits and have usually involved long-term effects where temperature acclimation becomes an important factor. Relatively little has been done on the physiological effects of rapid changes in temperature. Recently techniques involving measurements from indwelling catheters in the blood vessels and respiratory cavities have been applied to physiological studies in fish (e.g. Holeton & Randall, 1967; Hughes & Roberts, 1970). The latter measured changes in ventilatory and cardiac frequencies in lightly anaesthetized trout during rapid warming. Apparent uncoupling of the buccal and opercular pumps and brady-cardia occurred at high temperatures, findings not unlike those noted for trout sub-jected to hypoxia (Marvin & Heath, 1968; Hughes & Saunders, 1970). Cocking (1959) suggested that high-temperature death in fish might be due to insufficient oxygen being supplied to the tissues. In the present study blood pressure, blood oxygen con-tent, oxygen consumption and respiratory pressures were measured in unanaesthetized trout during warming. The physiological changes taking place shortly before death from heat stress were also examined since the causes of thermal death in fish are still poorly understood (Fry, 1967).

MATERIALS AND METHODS

Fifteen of the experimental runs were performed at Bristol using rainbow trout (*Salmo gairdneri*) from a hatchery at Nailsworth, Gloucestershire. Four additional experiments were performed at Blacksburg, Virginia, using rainbow trout from a hatchery at Marion, Virginia. The fish weighed 430–600 g and were acclimated to 15 °C for at least 1 week before experimentation. No fish was used on more than one occasion and no differences between the two populations were evident.

Each fish was anaesthetized with MS 222 (0·15 g/l) and placed ventral side upper-most on an operating table similar to that used by Smith & Bell (1964). The gills were irrigated with water containing MS 222 (0·10 g/l) while the cannulations were per-formed. The buccal and opercular cavities were cannulated with polyethylene tubing (Saunders, 1961) and the dorsal and ventral aortae were cannulated with 21 or 23 gauge

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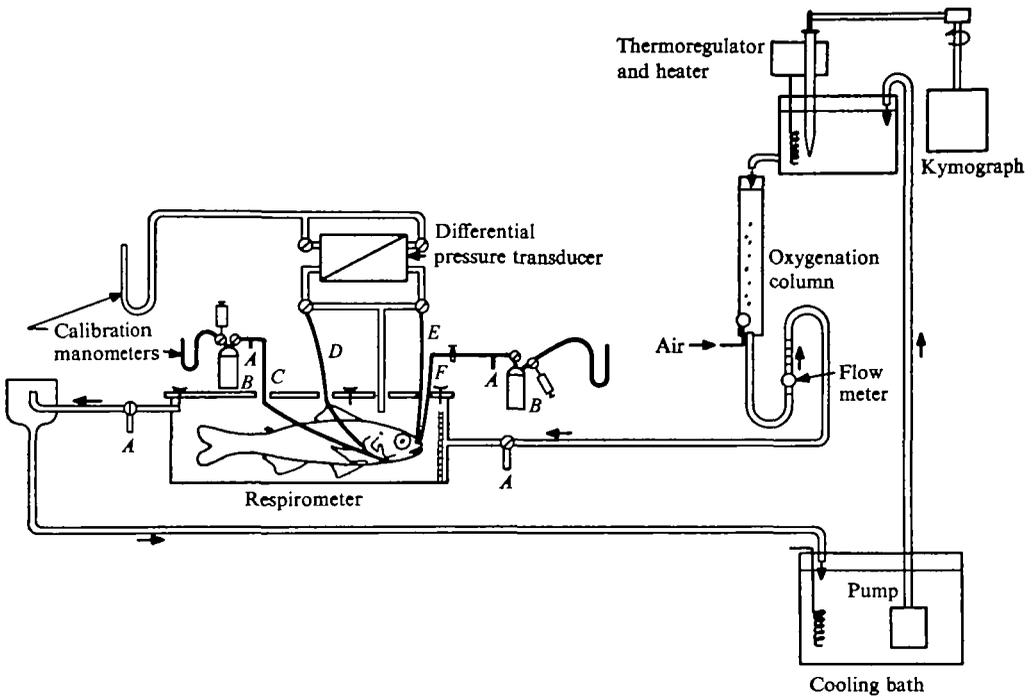
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Fig. 1. Diagram of experimental apparatus for the simultaneous recording of respiratory and cardiovascular parameters during warming. (*A*) Outlets for blood and water samples; (*B*) blood-pressure transducers; (*C*) ventral aortic catheter; (*D*) opercular catheter; (*E*) buccal catheter; (*F*) dorsal aortic catheter.

hypodermic needles attached to polyethylene tubing by the method of Holeton & Randall (1967). The polyethylene tubes from the needle cannulae were filled with heparinized Cortland saline and plugged with steel pins. The fish was then revived on the operating table by pumping fresh water through the gills and finally placed in a Perspex box which served as the fish chamber in the experimental apparatus (Fig. 1). Water flow through the chamber was maintained at 500–1000 ml/min. To avoid any accumulation of metabolic waste products a slow flow of charcoal-filtered tap water was maintained into the refrigeration bath. From 15 to 24 h were allowed for recovery from the operation before the experiments were begun.

The cyclic changes in the respiratory cavities produced by the ventilatory movements were recorded with a Sanborn 268 B differential pressure transducer which gave the differential pressure across the gills. It was also possible to record pressure changes in the opercular and buccal cavities individually by connecting one of the two sides of the transducer to the water in the fish chamber, which also served as the zero reference pressure (Hughes & Roberts, 1970). Respiratory frequencies were easily taken from the pressure records.

Blood pressures were measured with either Bell and Howell or Statham pressure transducers. The outputs from all pressure transducers were monitored with a Tektronix 502 A oscilloscope and recorded on a polygraph.

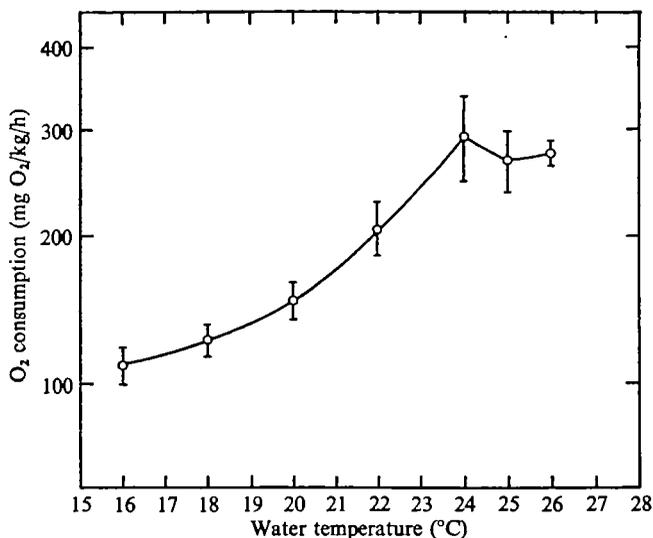Blood samples for oxygen analysis were taken from 'T' connexions in the catheter

Fig. 2. Effect of warming (1·5 °C/h) on oxygen consumption of seven fish. Vertical bars above and below the mean values represent ± 2 standard errors of the mean.

tube. Blood oxygen content was determined immediately on 10 μl samples by the method of Tucker (1967).

Oxygen consumption by the fish was determined by measuring the rate of water flow through the respirometer and the P_{O_2} in samples of the inflow and outflow water.

All experimental runs consisted in raising the temperature at a steady rate of approximately 1·5 °C/h from the acclimation temperature until death occurred while the above-mentioned parameters were measured at regular intervals. The temperature of the water in the respirometer was monitored continuously with a YSI Telethermometer.

In order to give a smooth rise in temperature a kymograph was attached to the knob of the thermoregulator by an endless belt. As the kymograph slowly turned, the thermoregulator was thus gradually adjusted to a higher and higher temperature.

Not all of the parameters were measured in every experiment. For example, some difficulties were encountered with the needle cannulae shifting in the blood vessels which interfered with blood-pressure and blood-oxygen measurements.

RESULTS

A. *Oxygen consumption* ($\dot{V}_{O_2}$)

The oxygen consumption at 16 °C averaged 110 mg*/kg/h which corresponds well with the standard oxygen consumption of brook trout at that temperature reported by Beamish (1964) and the routine oxygen consumption of rainbow trout reported by Skidmore (1970). Since no quantitative measure of activity was made, this value should be considered as routine $\dot{V}_{O_2}$.

When the temperature was raised most fish remained quiescent, except for an

* 1 mg O_2/l = 1 ppm = 0·7 ml O_2/l.
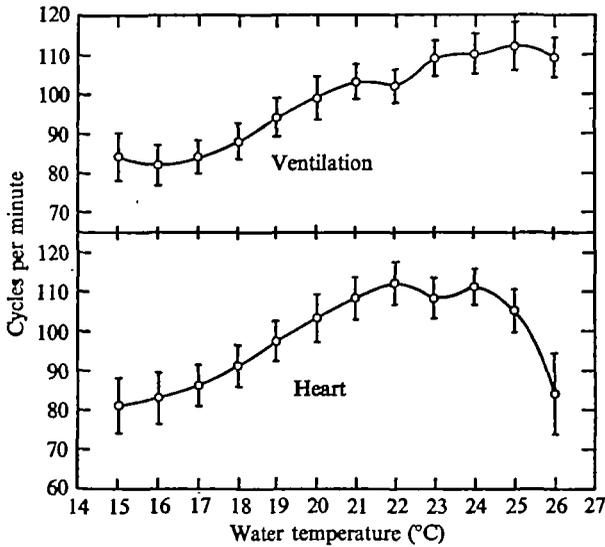
Fig. 3 Effect of warming (1·5 °C/h) on ventilation frequency of 14 fish and heart rate of 10 fish. Vertical bars above and below the mean values represent ±2 standard errors of the means.

occasional attempt at turning around in the respirometer. As the temperature of death (i.e. the lethal temperature) was approached, considerable struggling occurred in about half the preparations, but in the others death took place rather abruptly with no noticeable physical activity.

Fig. 2 shows the changes in average $\dot{V}_{O_2}$ as the water temperature was raised from 16 °C at a rate of 1·5 °C per hour. The Q_{10} between 16 and 20 °C was 2·35 and between 20 and 24 °C was 4·96, suggesting a strong reaction to the thermal stress above 20 °C. In most preparations, there was either a levelling off or a moderate decline in $\dot{V}_{O_2}$ during the hour preceding death while the temperature was still rising. In no case did $\dot{V}_{O_2}$ precipitously decline until respiratory and cardiac activity had nearly ceased.

B. *Effect on respiratory pumping*

Ventilatory frequency

Ventilatory frequency at 15–16 °C was variable, but in all preparations frequency rose with increasing temperature until a levelling off occurred at 23 °C (Fig. 3). The rate of increase with temperature was fairly constant with a Q_{10} of 1·38 between 15 and 23 °C. The maximum percentage increase was variable between different specimens, but tended to be less for those with the highest initial frequency (Table 1). Frequencies in excess of 150 cyc/min per minute were never recorded.

The temperature at which the maximum respiratory frequency was observed varied in different preparations from 20 to 26 °C with most occurring at 24 °C and above (Table 1). At the highest temperature ventilatory frequencies remained elevated with little change until death which resulted in an abrupt cessation of all measured parameters.

Table 1. *Minimum and maximum ventilatory frequencies observed during warming.*

(Minimum frequencies were measured at 15–16 °C. Temperatures at which the
maximum frequencies were observed are indicated in the table.)

Fish	Minimum	Maximum	Temperature	Difference	%
E	63/min	115/min	24 °C	52/min	83
F	90	120	29	30	33
G	84	115	22·5	31	37
H	78	124	25·5	46	59
I	78	127	25·5	49	63
J	73	102	25·8	29	40
K	64	102	24·0	38	60
L	75	90	20·0	15	20
M	99	145	20·0	46	46
N	72	102	24·0	30	42
O	58	100	26·0	42	73
P	90	150	25·0	60	67
Q	102	138	22·0	36	35
R	96	135	25·0	39	41

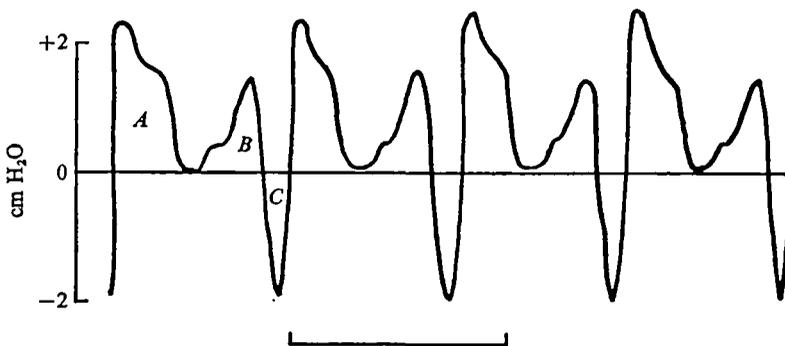Mean percentage change, 50%.

Fig. 4. Tracing of differential pressure recording to show the portions of the respiratory cycle used in the analyses. (*A*) Opercular suction phase; (*B*) buccal pump phase; (*C*) reversal phase. Time base is 1 sec.

Pressure changes in the respiratory cavities

The cyclic changes in pressure within the respiratory cavities of trout during breathing were described by Hughes & Shelton (1958) and Hughes & Roberts (1970) and a similar terminology has been adopted here. Fig. 4 illustrates a typical recording of the differential pressure across the gills in a quiescent trout. The relative contribution of the buccal and opercular pumps to the water flow over the gills may be estimated by either the area under the appropriate component of the differential curve or the amplitude of the buccal and opercular pressures. The relative contribution of the opercular and buccal pumps varied between different preparations and at different times in the same preparations. Similar variability has also been noted by Hughes & Roberts (1970) and Hughes & Saunders (1970).

During warming of the fish the amplitude of all components of the differential pressure increased, often several fold, indicating a considerable increase in stroke volume (Fig. 5). Some idea of the capacity for adjustment in the respiratory pumping
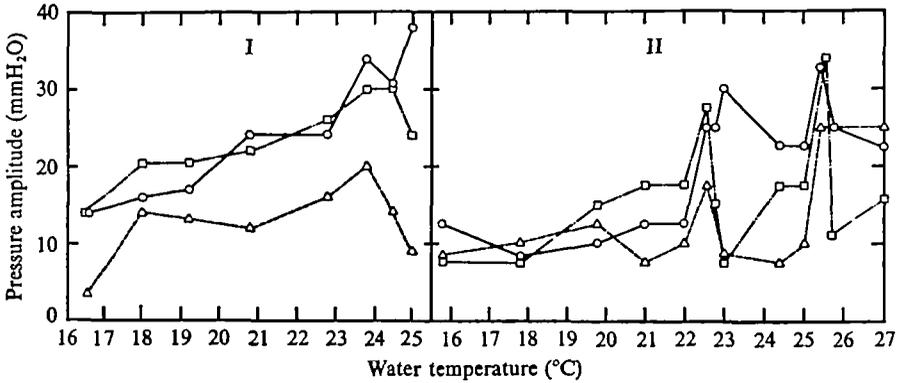
Fig. 5. Effect of warming (1·5 °C/h) on the amplitude of the components of the differential pressure wave. The two fish illustrated (designated as I and II respectively) were chosen to show some of the diversity of responses observed in different preparations. $A = \square$; $B = \bigcirc$; $C = \triangle$.

Table 2. *Minimum and maximum pressure amplitudes of the components of the respiratory differential pressure wave measured during warming of the water*

(A = opercular suction-pump phase; B = buccal pump phase; C = reversal phase (see Fig. 4).)

Fish	Component	Minimum	Maximum	Temperature range	Q_{10}
F	A	14 mmH₂O	30 mmH₂O		2·76
	B	14	34	16–24 °C	3·26
	C	4	20		8·55
G	A	10	42		6·01
	B	16	46	16–24	3·75
	C	0	29		14·60
H	A	15	48		4·29
	B	14	30	16–24	2·59
	C	6	19		4·23
J	A	8	38		5·17
	B	13	33	16–25·5	2·82
	C	8	38		5·17
L	A	14	32		3·27
	B	22	40	16–23	2·62
	C	4	14		5·97

may be gained by comparing the maximum amount of change during warming. Table 2 shows minimum and maximum pressure amplitudes measured from five preparations with Q_{10} calculated in each case. From these data there appears to be some tendency for the opercular pump to increase in stroke more than the buccal pump. Both yield Q_{10} values far in excess of the 1·4 found for ventilatory frequency. Additionally, the 'C' wave, which may indicate a reversal of flow, became very large at high temperatures.

The relative contribution of the two pumps to the gill water flow often changed. Occasionally this was quite marked, as seen in Fig. 5 II at 23 °C. At the higher temperatures double pressure reversals were sometimes observed in the differential pressure wave form (the 'C' wave, and a deflexion below the zero line between 'A' and 'B'). Such double reversals were also observed by Hughes & Roberts (1970) who attributed
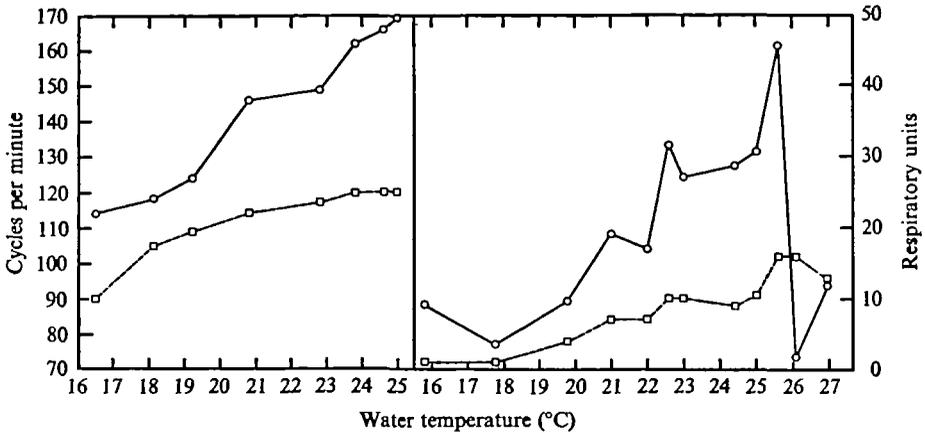
Fig. 6. Effect of warming on relative minute volume (respiratory units) and ventilation frequency in the same two fish shown in Fig. 5. O, Relative minute volume; □, ventilation frequency.

them to uncoupling between the buccal and opercular pumps. In most preparations a rather abrupt drop in the amplitude of all components of the differential pressure wave form preceded death.

Minute volume

The method that has generally been used to calculate minute volume involves measurement of the P_{O_2} of water taken from buccal and opercular cannulae and the data so obtained used in the Fick equation (e.g. Saunders, 1961; Holeton & Randall, 1967; Hughes & Saunders, 1970). This indirect method has been criticized (Hughes & Knights, 1968; Davis & Watters, 1970) because of the variability of the expired water samples and a tendency to yield high minute-volume values.

The area under the differential pressure curve $(A + B - C)$ times the respiratory frequency was used to indicate changes in minute volume. Since this is an indirect method that does not give absolute values for water flow, the figures obtained will be referred to as 'relative minute volume' (RMV) which is closely related to the mean differential pressure.

During the experimental runs, as the temperature rose, the relative minute volume rose in a more or less regular manner (Fig. 6). The increase in RMV was generally much greater than the increase in ventilation frequency. In some preparations there were abrupt rises or falls in volume with little change in frequency (e.g. Fig. 6II at 26 °C). While this was most common just before death, it sometimes occurred even at intermediate temperatures.

Relationship of relative minute volume (RMV) to oxygen consumption

Since the metabolic demand increases with an increasing temperature, there must be increased respiratory water flow to provide the necessary oxygen. Fig. 7 shows a fairly close relationship between RMV and $\dot{V}_{O_2}$ except at high temperatures when volume generally declines. The temperature at which this occurred varied from 23 to 25 °C in different preparations.
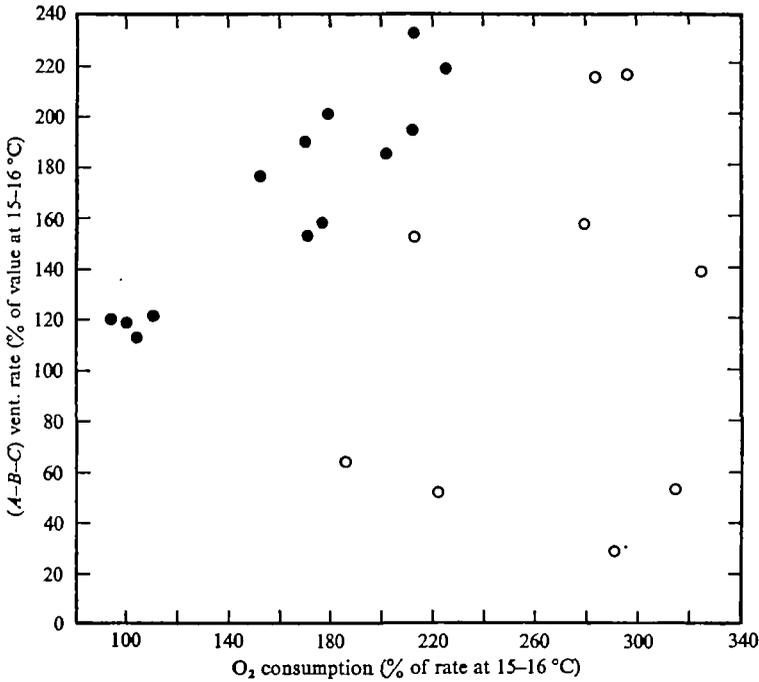
Fig. 7. Relationship of relative minute volume (area under the differential pressure curve times the ventilation frequency) to oxygen consumption for four individuals. Open circles refer to values taken at the high temperatures where respiratory failure was occurring.

Table 3. *Temperature at which bradycardia was first observed and temperature of death*

Fish	Temperature of bradycardia (°C)	Temperature of death (°C)
E	23·0	recovered
H	25·7	26·4
J	26·5	27·3
K	24·8	25·6
M	22·7	23·5
N	24·2	25·8
O	26·2	26·8
P	23·5	25·5
Q	21·0	23·0
R	25·0	27·0

C. *Effect on cardiovascular function*

Cardiac frequency

The cardiac frequency was determined by counting the dorsal or ventral aortic pressure pulses. Heart beat showed a steady rise in frequency as the temperature rose until about 24–25 °C when a bradycardia became evident (Fig. 2). The average Q_{10} for cardiac frequency between 15 and 23 °C was 1·6 which is close to that for ventilatory frequency (1·4). Over the temperature range of 15–23 °C, the heart and ventilation frequencies were often quite similar, as is indicated by the averages (Fig. 2).
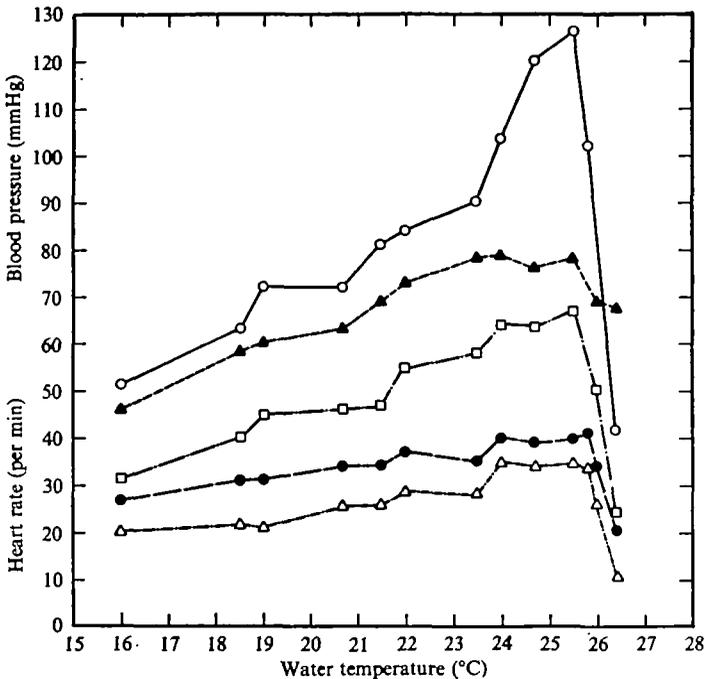
Fig. 8. Effect of warming on blood pressure in the dorsal and ventral aortae of one preparation to show the typical response.

	Systolic	Diastolic	
Dorsal aorta	●	△	Heart rate O
Ventral aorta	▲	□	

Synchrony (i.e. heart beat occurring regularly at one particular phase of the ventilatory cycle) was noted at all temperatures in one preparation and in half of the other preparations only at the higher temperatures (23–25 °C). In some fish the frequencies of heart and ventilation were similar but the heart beat would slowly shift out of phase with the respiration. These were not counted as being synchronous.

Bradycardia was noted at differing temperatures in the various preparations but usually occurred at a temperature 0·5–2·0 °C below the lethal temperature (Table 3). Because of this, bradycardia was a good indication that the lethal temperature was being approached even though this temperature varied between 23 and 27 °C in different preparations.

Blood pressure

As the temperature rose there was a more or less steady increase in blood pressure in both dorsal and ventral aortae (Fig. 8). The maximum pressure increase averaged 88 % in the ventral aorta and 48 % in the dorsal aorta. Thus the differential pressure across the gills increased with temperature. There was usually a levelling off of blood pressure at the higher temperatures which, however, was not necessarily associated with a bradycardia. In some preparations the heart rate continued to increase even with a levelling off of the blood pressure.
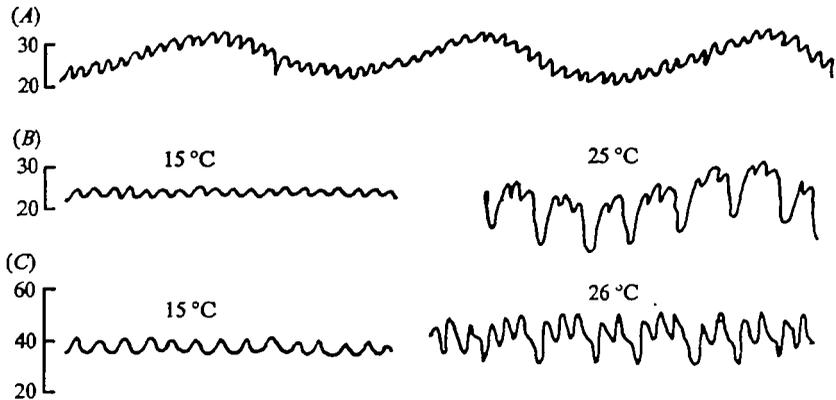
Fig. 9. (*A*) Tracing of dorsal aortic blood-pressure record taken at 20 °C to show the oscillations in the mean blood pressure. These were commonly seen but not always of this great an amplitude. (*B*) Tracing of dorsal aortic blood-pressure records at 15 and 25 °C showing the aberrations frequently observed in the cardiac cycle at the higher temperatures. (*C*) Tracing of ventral aortic blood pressure record at 15 and 26 °C showing the aberrations frequently observed at the high temperatures.

The high-temperature bradycardia was associated with an immediate decline in ventral aortic mean blood pressure, but the dorsal aortic pressure did not drop appreciably until the bradycardia became severe.

In many of the preparations there was a tendency for the mean blood pressure to fluctuate in a more or less regular manner (Fig. 9*A*). Little or no change in heart rate or pulse pressure was associated with the oscillations and they were not in any way related to the respiratory movements. The amplitude of the oscillations usually became greater at the higher temperatures.

Alterations in the cardiac cycle

Part of the purpose of this study was to examine the physiological changes that occur just before death from heat stress. By measuring the blood pressure using indwelling catheters, changes in the cardiac cycle associated with heat stress could be observed.

As the lethal temperature was approached the cardiac cycle began to show irregularities in both the dorsal and ventral aortic pressure waves (Fig. 9*B*, *C*). As the temperature rose the frequency of aberrant cycles increased so that every third or fourth beat was missing, giving a pressure record with an abrupt fall in diastolic pressure during the period of the missing heartbeat (Fig. 9). Since the heart beat was counted from the complete pressure waves, this was also interpreted as a decreased heart rate. As the lethal temperature was approached the heart cycles usually became more aberrant, but occasionally there would occur transient recoveries of short duration.

Blood oxygen content

The blood oxygen-content data obtained from analysis at random times during the experimental runs are shown in Fig. 10. The dorsal aortic blood oxygen-content data were variable but showed a tendency for a slight lowering in oxygen content at the
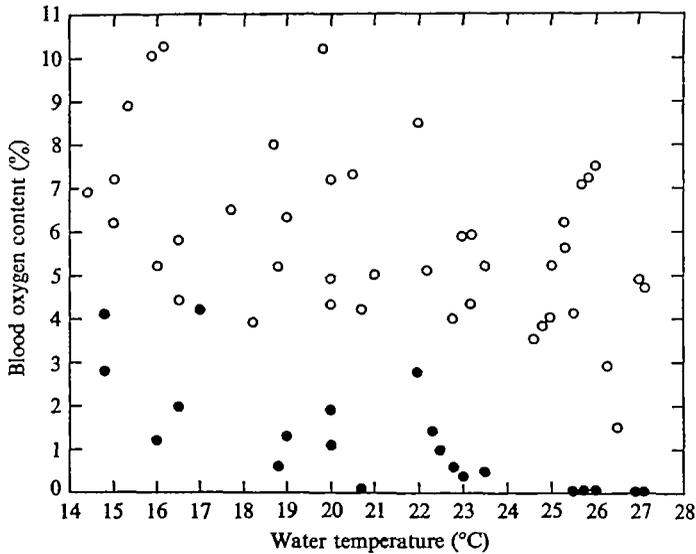
Fig. 10. Blood oxygen-content measurements from dorsal (O) and ventral (●) aortae of trout exposed to warming. The dorsal aortic data are from 10 fish, the ventral aortic data from six fish.
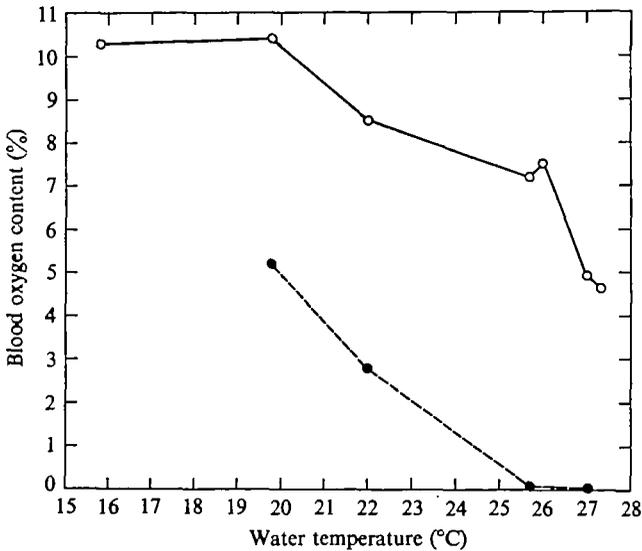

Fig. 11. Blood oxygen-content data from a typical fish showing the changes occurring during warming of the water. O, dorsal aorta; ●, ventral aorta.

higher temperatures. Within any given preparation, when the temperature rose above 20 °C, the dorsal aortic oxygen generally began to fall and just before death dropped more markedly (Fig. 11).

In order to determine whether the reduction in arterial oxygen content above 20 °C was due to a temperature effect on capacity, blood samples from four of the preparations were equilibrated with water-saturated air ($P_{O_2} = 151$ mmHg) in a tonometer
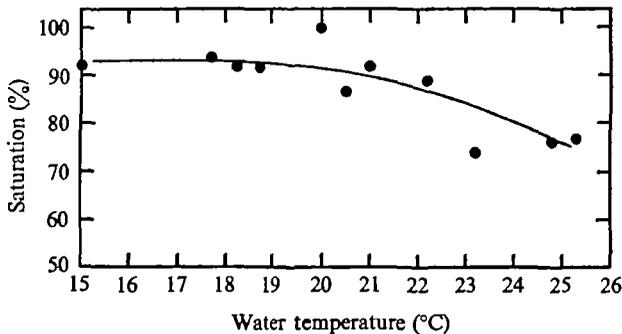
Fig. 12. Effect of warming on percentage saturation for oxygen of the dorsal aortic (arterial) blood of four preparations. The line was fit to the points by eye. The three points on the right of the graph are from three fish and the samples were taken before the final abrupt drop in oxygen content that occurs just before death.

at the same temperature as the fish and analysed for oxygen content. The values obtained were then used to compute percentage saturation of the dorsal aortic blood at that temperature. While these data, which are shown in Fig. 12, are somewhat limited, they indicate a reduced arterial oxygen saturation at the higher temperatures.

The ventral aortic oxygen content showed some reduction at temperatures between 15 and 20 °C, and always equalled zero as the lethal temperature was approached (Figs. 10, 11). This reduction in venous oxygen was more rapid with the temperature rise than was the reduction in arterial oxygen; thus the arterial-venous difference increased with temperature.

DISCUSSION

The usual rate of thermal acclimation in teleost fish is of the order of a degree or two change per day (Fry, 1967). Even assuming a faster rate it is doubtful whether any significant amount of thermal acclimation occurred during these experiments in which temperature rose at a rate of 1·5 °C per hour. The usual observation of a decreasing Q_{10} for oxygen consumption with higher acclimation temperature (Fry, 1957; Beamish, 1964) was not found in this study (Fig. 2). Furthermore, the overall Q_{10} in this study was over twice that usually found (Fry, 1957) which suggests that factors, in addition to the effect of temperature on cellular energy metabolism, must be contributing to the metabolic demand. Other than hormonal effects, two factors that are known to increase metabolic rate in fish are environmental hypoxia and bodily activity.

Metabolic rate rises during hypoxia due to increased activity of the ventilatory muscles (Hughes & Saunders, 1970). The ambient oxygen was maintained at near saturation at all times in the present study, although the decreased solubility of oxygen led to a decline in oxygen content of the water from about 10 mg/l at 15 °C to 8·2 mg/l at 26 °C. However, the partial pressure remained above 150 mmHg at 26 °C.

When the temperature of an ectothermic animal is changed there is frequently seen an overshoot in the oxygen consumption (Grainger, 1958). Increased activity or muscular tone of the organism may account for part or all of this effect which was undoudtedly present in this study. It would appear that the stress from the temperature

rise becomes progressively greater as the temperature approaches the lethal level so that the metabolic demand becomes considerable even in a seemingly quiescent fish.

The responses of the respiratory pumps of trout to thermal stress appear to be variable, but some trends are clearly evident. The frequency and amplitude of the ventilation movements both increase with a rise in temperature. Factors such as the rate of temperature change and use of sedation may affect the response, however. For example, Hughes & Roberts (1970) used a temperature rise of 1 °C/3 min. and recorded average ventilation frequencies above 200/min. at a brain temperature of 25 °C, while in the present work ventilation frequencies averaged 112/min. at a bath temperature of 25 °C (presumed equal to brain temperature). This difference is probably due to more marked stress produced by a more rapid rise in temperature in the Hughes and Roberts work. On the other hand the effect of temperature on the depth of breathing seemed to be more marked with the slower rate of temperature rise. While Hughes & Roberts observed increases in pressure amplitudes at temperatures between 15 and 21 °C, in most cases these declined at higher temperatures. During the present series of experiments the amplitude of the *A* and *B* phase of the differential pressure wave usually continued to increase often almost to the lethal temperature.

Trout subjected to gradually induced environmental hypoxia respond with increased stroke volume almost exclusively until the level of hypoxia becomes severe, then frequency increases (Hughes & Saunders, 1970). It thus seems that adjustments of ventilation volume in unanaesthetized trout, whether caused by external influences such as temperature or hypoxia or by increased muscular activity, are accomplished by alterations in stroke volume preferentially to ventilation frequency.

At the higher temperatures some uncoupling of the respiratory pumps in trout often occurs (this study, and Hughes & Roberts, 1970). It might, therefore, be predicted that oxygenation of the arterial blood would be affected. This indeed seems to be true in that the arterial blood oxygen saturation declines slightly at the highest temperatures. Roberts & Hughes (1967) observed decreased brain P_{O_2} levels at high temperatures in trout and attributed this to decreased respiratory flow because the effect was partially offset by directing a stream of water into the mouth.

There seems to be relatively little data on the effects of temperature change on cardiovascular parameters in fish. Randall (1968) observed a rise in heart rate with temperature (Q_{10} approximately 2) in lingcod (*Ophiodon elongatus*) but stroke volume remained unchanged. Using lightly anaesthetized rainbow trout Hughes & Roberts (1970) showed an increase from an average of 62 beats/min at 15 °C to approximately 115 beats/min at a body temperature of 24 °C. The 15 °C value is somewhat lower than that observed in this study, but the rates at the high temperature are almost the same. Spitzer, Marvin & Heath (1969) observed a threefold difference in heart rate of bluegill sunfish acclimated to 13 and 25 °C.

The changes in heart rate have been attributed to the direct effect of temperature on the pacemaker cells of the heart (Randall, 1970); however, there is some evidence that isolated fish hearts are more sensitive to temperature change than when *in situ* (Wilbur, 1961). Thus extrinsic factors such as vagal inhibition and hormonal levels must also be important. The effect of epinephrine on the trout heart is temperature-dependent. It causes decreased heart rate and increased stroke volume at higher

temperatures (15 °C) but the reverse situation prevails at lower temperatures (6 °C) (Randall, 1970). The oxygen uptake data in this study suggest some degree of stress at the higher temperatures. If it is assumed that the epinephrine level also increased, then stroke volume was probably positively effected while the heart rate was inhibited, the latter effect resulting in a Q_{10} of less than 2 for heart rate. Peripheral resistance probably decreases at higher temperatures (Davis, (1968) as cited by Randall (1970)) which would also help to maintain stroke volume in spite of an apparent negative inotropic effect of temperature. The increased pulse pressure seen in this study during the temperature rise supports the idea of increased stroke volume.

Bradycardia in fish at high temperatures has been noted by Roberts (1968, 1973) and Hughes & Roberts (1970). It is apparently the result of vagal stimulation since pretreatment with atropine partially abolishes it (Roberts, 1973). Fish subjected to hypoxia also show a bradycardia (Randall & Shelton, 1963; Marvin & Heath, 1968) so its presence in fish under severe thermal stress has been interpreted as a response to either internal or external hypoxia. Hughes & Roberts (1970) suggest the possibility that input from thermoreceptors may modulate vagomotor centres. In a study that supports this idea Spitzer et al. (1969) measured heart rates of bluegill sunfish subjected to environmental hypoxia at various temperatures. With this species bradycardia became more severe for a given level of oxygen at the higher temperatures. In the present study bradycardia was associated with a large increase in pulse pressure which may help to maintain cardiac output (Holeton & Randall, 1967).

In this study the cardiac cycle (as interpreted from the pressure pulse wave) tended to become progressively more irregular at the high temperatures. High temperatures might cause breakdown of heart tissue but this seems unlikely at 25 °C. Sporadic discharges from the cardiac control centre down the vagus nerve may be the cause since the skipped beats are frequently (but not always) associated with bradycardia. A third possibility would be the presence of abnormally high levels of potassium in the blood caused by the release of potassium from anoxic tissues. Potassium has the well-known effect on vertebrate hearts of favouring diastole, or stopping of the heart in the relaxed condition (Prosser & Brown, 1961).

In spite of a nearly threefold increase in $\dot{V}_{O_2}$ during the temperature rise, the average heart rate increased only 38 %. There was also some increase in stroke volume but this would probably not be sufficient for the increased oxygen uptake. Instead, the oxygen had to be carried by an increased amount being picked up by the blood in its passage through the gills. Blood at normal P_{O_2} holds only a finite amount of oxygen so the adjustment must take place by a greater removal of oxygen from the blood in its passage through the tissues, thus increasing the arterial-venous difference. When the oxygen content of the venous blood reaches zero, the limit is thus put on this adaptation. In these fish this limit appears to be around 24 °C where the ventral aortic oxygen reached zero and the $\dot{V}_{O_2}$ levelled off (Figs. 2 and 10). With a venous oxygen content of zero the P_{O_2} in many tissues must be below the level necessary to sustain aerobic respiration. This would be particularly important for nervous tissue and may account for the loss of equilibrium seen in fish subjected to heat stress (Cocking, 1958). In addition, it could cause discoordination of the respiratory movements which would further aggravate the situation of inadequate oxygen supply to the tissues by reducing the saturation of arterial blood (Fig. 11).

Salmonid fishes can normally increase their oxygen uptake during swimming by an amount greater than was observed in the temperature-stress experiments (Brett, 1964). This is not accompanied by any changes in venous oxygen (Stevens & Randall, 1967) so increased cardiac output must be sufficient to meet the oxygen transport needs. In lieu of any direct measurements of cardiac output under either condition it is difficult to make comparisons; one could make the tentative suggestion that the trout has the cardiovascular capability to transport oxygen to satisfy a quite high demand, but changes in oxygen demand induced by temperature alone do not produce an adequate stimulus to the cardiovascular control centre, and so the responses become progressively more inadequate the higher the temperature.

SUMMARY

1. Trout were subjected to a steady increase in water temperature ($1\cdot5$ °C/h) from 15 °C until death occurred, while several respiratory and cardiovascular parameters were monitored.

2. Oxygen consumption increased during the warming ($Q_{10} = 2\cdot35$ between 16 and 20 °C). At the higher temperatures the increase was more marked ($Q_{10} = 4\cdot96$ between 20 and 26 °C).

3. Ventilatory frequency increased during the rising temperature with a general levelling off observed above 23 °C. The amplitude of the pressure changes in the buccal and opercular cavities increased more than did the ventilatory frequency. Further analysis of the differential pressure across the gills suggests that the adjustment of respiratory pumping to the increased oxygen demand is predominantly in the volume pumped per stroke (cycle).

4. Heart rate rose steadily with the increasing temperature until about 24–25 °C, when a bradycardia usually became evident. Synchrony between the heart beat and the respiratory pumps was observed in some preparations at the higher temperatures.

5. Blood pressure increases during the warming were more marked in the ventral aorta than in the dorsal aorta. At the highest temperatures, abnormal cardiac cycles were frequently observed.

6. Arterial oxygen content declined slightly during warming and venous oxygen content dropped to zero above 23 °C.

7. It is suggested that cardiovascular adjustments may be a limiting factor in this type of stress.

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