SOME ASPECTS OF EXERCISE PHYSIOLOGY

IN FISH

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ABSTRACT

The limiting factors of swimming performance were studied in fish exercising in a water tunnel. The relationship between (10 min) critical velocity and body length determined in 10 species of freshwater teleosts is discussed with respect to the ratio of lateral red body musculature to total body weight. Electromyographic recording from red and white portions of the body musculature in four species of fish showed that red muscle fibers alone are active during steady swimming at sustained speeds. White muscle fibers are active only during bursts of violent swimming, such as during rapid acceleration and for a brief period preceding fatigue. Thus red muscle fibers, generally accepted as having an aerobic metabolism, appear to be the major determinant of sustained swimming speed.

To establish the time course of cardiovascular and respiratory changes during swimming; heart rate, ventilation rate, dorsal aortic, ventral aortic, and right common cardinal blood pressures were monitored during steady swimming following abrupt changes in water velocity. Under these circumstances most of the heart rate increase occurred in the first thirty seconds and heart rate did not change further after 3-15 minutes at a given swimming speed. Ventilation rate tended to increase initially and then decline, reaching a constant value after 15-30 minutes at a given swimming speed. Dorsal and ventral aortic blood pressure increased more slowly than heart rate, peaking after six minutes then declining to constant values after about 30 minutes. Blood pressure in the common cardinal vein was constant during exercise. The animals were considered to be in a steady state with regards to these circulatory and respiratory variables after about 30 minutes.

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Oxygen consumption increased from a mean of 0.58 ml kg⁻¹ min⁻¹ at rest to a mean maximum of 4.34 ml kg⁻¹ min⁻¹. Under the same circumstances cardiac output increased from a mean of 17.6 ml kg⁻¹ min⁻¹ at rest to a mean maximum of 52.6 ml kg⁻¹ min⁻¹. The corresponding stroke volume was 0.46 ml kg⁻¹ stroke⁻¹ at rest and 1.03 ml kg⁻¹ stroke⁻¹. Arterio-venous oxygen difference at rest was 3.29 volumes % and increased to 8.3 volumes % as a result of a decrease in venous saturation (to lower than 10% in some cases) during exercise.

Heart rate at rest was 31.75 min^{-1} and increased during exercise by a mean of 1.33 times. Ventral aortic blood pressure rose from 38.8 Torr at rest to 61.7 Torr. The corresponding ventral aortic pulse pressure rose from 11.6 Torr at rest to 26 Torr. Dorsal aortic mean pressure at rest was 31 Torr and increased to 37 Torr with exercise, accompanied by an increase in pulse pressure from 5.8 Torr at rest to 10 Torr. Ventilatory volume at rest was 211.4 ml kg⁻¹ min⁻¹ and increased to about 1700 ml kg⁻¹ min⁻¹ at maximal sustained swimming speed. The capacity rate ratio of oxygen exchange between water and blood increased from 0.6 at rest to 1.8 during exercise. Arterial blood of resting trout was 97% saturated with oxygen and % saturation did not change with exercise. Blood lactate at rest and at swimming speeds as high as 93% of critical velocity was 0.5 µM/ml. One minute after fatigue the blood lactate level had increased about five fold and continued to increase, reaching a maximum value (6-10 µM/ml) 2 to 2.5 hours after fatigue.

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INTRODUCTION

The term exercise, in the physiological sense, is used to refer to activity of skeletal muscle. Most vertebrates must use muscular activity in their daily lives, to find and consume food, escape predators, tend their offspring, and carry out other bodily functions. In addition muscles must be exercised periodically or muscle fibers will degenerate and become nonfunctional (Falls, 1968). Skeletal muscle is composed of basically two different types of muscle fibers; red type muscle fibers which contract slowly and white type fibers which have a fast or "twitch" contraction (Hess, 1970). The slow muscle fibers derive their energy from aerobic metabolism of fat and carbohydrate, whereas "twitch" fibers metabolize glycogen anaerobically (George and Naik, 1958; Love, 1970).

During exercise the contraction of muscle results in an increase in the animal's oxygen consumption. Oxygen to be consumed by an animal must be exchanged between the animal and its environment, transported to the tissues by the blood, and transferred from the blood into the tissue where it becomes the end acceptor of hydrogen in the aerobic metabolic process. In such a sequential process any one of the steps could limit the whole sequence. The possible limiting factors of maximal oxygen consumption then are:

(1) The rate of oxygen exchange between the animal and its environment

(2) Rate of transport of oxygen by the blood

(3) Rate of diffusion of oxygen into the tissue (muscle)

(4) A limitation in the ability of muscle to further increase its metabolism

(5) Other factors such as inability to remove waste products (e.g. CO₂, lactate, heat).

Increases in the amount of oxygen exchanged at the gas exchange organ (lung or gill in most vertebrates) can occur by increasing the amount of oxygen removed from each unit of medium, or by increasing the amount of medium breathed, or both. Transport of oxygen by the blood can be increased by increasing the amount of blood pumped (cardiac output), by increasing the amount of unloading of oxygen from the blood (increasing the arterio-venous oxygen difference), or both. Most vertebrate blood contains hemoglobin in cells or corpuscles. Hemoglobin not only increases the oxygen carrying capacity of blood but also because of its sigmoid dissociation curve and modulation by other molecules (CO $_{2}$ and H⁺) allows the unloading of a large proportion of the oxygen from the blood without a large change in oxygen tension. After unloading from the blood the diffusion of oxygen into the tissue is facilitated by the presence, in red muscle fibers, of myoglobin, a compound similar to hemoglobin but having a higher affinity for oxygen than hemoglobin (Wittenberg, 1970). A muscle contraction is the result of the contraction of many individual muscle fibers. The intensity of contraction of a muscle (and therefore its metabolic rate) can be increased by recruitment of more muscle fibers to contract, or, since contraction of red fibers is graded, by increasing the contraction of individual fibers (Hess, 1970). It is therefore evident that when both recruitment and contraction of all muscle fibers is maximal the oxygen consumption of the muscle will be maximal.

In order to determine which of these factors may limit an animal's maximal aerobic exercise capacity changes in variables related to the possible limiting factors must be studied in relation to exercise. In mammals, particularly man, many studies have been conducted on the effect of exercise on

cardiovascular, respiratory, and metabolic variables (Bevegardand Sheperd, 1967; Ekelund, 1967; Rowell, 1974). At the beginning of an exercise period oxygen consumption increases rapidly then plateaus after about 1 minute, at which time the animal is said to be in the "steady state" of exercise (Asmussen, 1965). In mammals a short exercise period (5 min) of increasing intensity results in increases in oxygen consumption up to a point beyond which oxygen consumption does not increase when intensity of exercise is further increased. However, when long test periods are used (1 hour) no plateau is observed in oxygen consumption versus intensity of exercise. Under these circumstances maximal oxygen consumption corresponds to the highest exercise rate (Taylor, 1970, 1974).

During exercise ventilation volume increases in proportion to oxygen consumption at low exercise levels but intense exercise causes ventilation to increase more rapidly than oxygen consumption (Asmussen, 1967). Cardiac output rises with rising oxygen consumption, this is accomplished by progressive increases in stroke volume, heart rate, or both. In man stroke volume reaches the highest value at about 40 per cent of maximal oxygen consumption, the remaining increase in cardiac output being the result of an increase in heart rate. Arterio-venous oxygen difference increases with increasing oxygen consumption; at maximal oxygen consumption 80-85% of all available oxygen in the blood is extracted by the tissues. This high extraction is the result of two adjustments; firstly, oxygen extraction by working muscles is increased, secondly, blood flow to areas which normally have high blood flow rates but low oxygen extraction is reduced. In man blood flow is reduced to splanchnic tissue, kidney, and nonexercising muscle. Dogs, on the other hand, show only a slight change in splanchnic blood flow even at maximal exercise levels

(Herrick et al., 1940; Rushmer et al., 1961; Lacroix and Leusen, 1966; Van Citters and Franklin, 1969). However when cardiac output is reduced in dogs by heart block, blood redistribution similar to that in humans is reported (Holtman, 1967; Vatner et al., 1971; Millard et al., 1972). The difference in blood redistribution during exercise in man and dogs may be the result of the difference in posture between the two animals but this is not clear on the basis of existing evidence (Rowell, 1974). Oxygen removal from blood increases with exercise (one of the factors accounting for the increase in arteriovenous oxygen difference) (Rowell, 1974). Since both oxygen consumption and oxygen extraction (A-VO₂ diff) are larger in physically trained than in untrained individuals, oxygen extraction from the blood (oxygen supply to tissue) is not likely to be a limiting factor in setting maximal oxygen consumption in mammals (Rowell, 1974).

If muscle is not limited in its supply of oxygen the overall oxygen consumption may still be limited by the ability of the muscle to increase its metabolism. One of the ways that the metabolic rate of muscle could be limited is by the amount of oxidative enzymes present in the muscle fibers. Holloszy (1967), on the basis of his findings that mitochondrial oxidative enzymes increase in rats physically trained at near maximal exercise levels, considers training to be a process of increasing muscle aerobic metabolic capacity and suggests that maximal oxygen consumption is limited by the tissue. Another possibility (which is not exclusive of the first) is that the maximal oxygen consumption is determined by the amount of contractile protein in red muscle. That is to say that, at maximal oxygen consumption, all of the contractile proteins of red muscle are adequately supplied with ATP and are working to their maximal capacity.

The energy charge of blood perfused, maximally exercised, red muscle is not known in any animal. In mammals both the musculature and mode of locomotion are very complex; the proportion of red and white muscle fibers in different muscle masses is not constant (some are pure red; others are mixed in various proportions) and the number of muscles involved in locomotion is large. In fact it is not even clear as to how many muscles are involved in various kinds of terrestrial locomotion (Taylor, personal communication).

Cardiovascular and respiratory phenomena have also been investigated in birds during flight (Hart and Roy, 1967; Tucker, 1968; Berger <u>et al.</u>, 1970; Butler <u>et al.</u>, 1975). Birds are capable of somewhat higher maximal oxygen consumption rates than mammals (Lasiewski, 1960). Since the cost of flying (per unit distance) is less than the cost of running (Tucker, 1970) oxygen consumption during level flight is probably comparable to that of running mammals. The cardiovascular and respiratory responses to flight in pigeons are similar to those to exercise in mammals (Butler et al., 1975).

In birds the power for flight is provided by basically two muscle³, the pectoralis and the supracoracoideus. The proportion of red type muscle fibers in the flight muscles of many species of birds has been investigated (George and Berger, 1966). The domestic chicken, which does not fly, has a pectoralis muscle composed predominantly of white type muscle fibers, whereas very strong flyers (house sparrow) and very active birds (hummingbirds) have pectoralis muscles composed predominantly (house sparrow) or exclusively (hummingbird) of red fibers. Those birds which soar have a predominance of intermediate type fibers (George and Berger, 1966). The physiological significance of the proportions of different fiber types has not been investigated and it can only be speculated that this morphological distribution is related to aerobic metabolic

capacity. It is known that flying hummingbirds have a higher oxygen consumption per unit weight than has been recorded for any other vertebrate (Pearson, 1950; Lasiewski, 1960). No data are available on the lactate accumulation during flight in birds such as hummingbirds; an accumulation would signify an insufficiency of oxygen supply at the tissue.

The limitation of maximal aerobic exercise in mammals and birds is not known; it may be one of the factors discussed above or it could be some other factor such as accumulation of heat. In some species of homeotherms body temperature, which increases as a result of exercise, has been shown to correlate favourably with the inability of the animals to continue exercise. In the cheetah and rhea body temperature during exercise increases until the animal appears exhausted and refuses to run further. The body temperature of a cheetah during a run to exhaustion increases by 1.0 to 1.5°C (Taylor, 1974). Gazelles can run faster than the cheetah for a longer period of time. During exercise of this intensity a gazelle's body temperature increases by as much as 6°C. Under these conditions the brain is maintained at a lower temperature than the rest of the body by a heat exchanger system located at the base of the skull of the gazelle (Taylor, 1974). Thermoregulation during flight may be a limiting factor in birds flying at high ambient temperatures, under which circumstances the birds must rely on evaporative cooling. Pigeons flying at an ambient temperature of 26°C increase their body temperature (at exhaustion) by about 2°C (Butler et al., 1975). At lower temperatures cooling by convection and radiation is probably adequate and temperature regulation is probably not a limiting factor (Dawson and Hudson, 1970). Likewise heat accumulation is not a problem in small mammals (rats, mice) or in long distance runners (African hunting dog, domestic dog) at submaximal exercise levels; at maximal

exercise levels however this could be a limiting factor but has not been investigated (Taylor, 1974).

The only other class of vertebrates in which exercise studies have been conducted is the teleost fishes. Fish as animals for the study of exercise have certain advantages. Because fish live in a medium with a large heat capacity and a low oxygen content, the gills (during gas exchange) act as heat sinks, resulting in blood being cooled to environmental temperatures. For this reason fish (except for a few which have heat exchangers to conserve heat in parts of the body) have no problem with an accumulation of heat in their bodies during exercise. Another advantage in studying fish is that they have a single circuit circulatory system in which it should be easier to determine the relationships of variables and their control than it is in mammals and birds.

Extensive studies have been carried out on the oxygen consumption of various species of fish in relation to swimming speed (Brett, 1964; Brett and Sutherland, 1965; Rao, 1968; Kausch, 1968; Farmer and Beamish, 1969; Beamish, 1970). Oxygen consumption increases exponentially with increases in swimming speed and a logarithmic transformation of oxygen consumption results in a straight line relationship of oxygen consumption to swimming speed (Brett, 1964). Sockeye salmon show a decrement in swimming speed and maximal oxygen consumption at temperatures in excess of 15°C. This is attributed to a decrease in the oxygen content of water at higher temperatures (Brett, 1964). Jones (1971) hypothesized that the energy demand of the branchial pump will limit the amount of oxygen available to the tissue at high temperatures.

The cardiovascular and respiratory responses of fish to exercise have been the subject of only a few studies. Smith, Brett and Davis (1967) described the

heart rate, ventilatory rate and dorsal aortic blood pressure responses in relation to swimming speed in sockeye salmon. Stevens (1968) examined the responses of the cardiovascular and respiratory systems of trout to exercise of short duration (5 min). Sutterlin (1969) examined the effect of exercise on cardiac and ventilatory frequency in pumpkinseed (Lepomis gibbosus), bullheads (Ictalurus nebulosus) and brown trout (Salmo trutta). Priede (1974) studied the effects of exercise (up to maximal 30 min sustained) on heart rate and attempted to determine the role of the vagus in the control of heart rate in rainbow trout. No studies have been conducted on the cardiovascular and respiratory responses of fish to prolonged exercise.

In those fish which swim with the tail (anguiliform mode) only the myotomal muscles are used for swimming. The myotomal musculature in teleosts consists of a lateral muscle mass composed entirely of red muscle fibers and a much larger white or mosaic muscle mass composed of white fibers (white mass) or of white and red or possibly intermediate type fibers (mosaic mass) (Boddeke <u>et al.</u>, 1958). It is known that the mosaic muscle mass of trout is active at low swimming speeds (Hudson, 1973) but the relative contributions of the two muscle masses at other submaximal and maximal sustained speeds is not known. However this morphological separation of red and white muscle fibers (at least in some fish) provides a convenient means for a comparative physiologist to study the recruitment of the different muscle fiber types during exercise in relation to maximal sustained exercise.

The first part of this study was devoted to examining the interspecific variability in swimming speeds of fish in relation to the proportions of red and white muscle in the myotome and the recruitment of the two muscle types during swimming. The second section deals with the cardiovascular, respiratory

and metabolic changes which occur in trout in relation to their maximal exercise capability.

SECTION I

Recruitment of Red and White Myotomal Muscle During Swimming and its Relationship to Critical Velocity

INTRODUCTION

Since the attempt by Regnard (1893) to determine the maximum swimming speed of fish in the laboratory, a large number of papers have presented data from both laboratory and field observations which allow assessment of this variable. Brett (1964) established a fatigue curve for yearling sockeye salmon (Oncorhynchus nerka), recognising two definite transition points; one at 12-24 sec and another at 300 min. The transition point at 12-24 sec represents the termination of burst swimming and 300 min marks the end of steady performance. After this latter transition the fish enters a period of sustained performance which can, theoretically, be maintained indefinitely. During burst swimming, speeds up to 3-4 times the sustained performance can be achieved. Data obtained by Blaxter and Dickson (1959) and Blaxter (personal communication in Bainbridge, 1960) and Bainbridge (1960, 1962) confirms the early transition from burst to steady performance for a variety of fishes. Gray (1953) reported that trout from a standing start can accelerate to full speed in 50 msec. Consequently there seems adequate experimental evidence that fish can reach extremely high forward velocities very quickly for periods of several seconds and that some fish can swim for very long periods of time. The reasons for large interspecific variations in sustained swimming speed have not be investigated. One of the possible explanations for these differences may be found in the characteristics of the muscles generating the power for swimming.

In most fish the myotomal musculature is composed of two main types of muscle fibers. The two fiber types are usually distinguished by their colour,

myoglobin and mitochondrial content, vascular supply and enzymatic properties, diameter, and, to some extent, innervation (Boddeke et al., 1959; Bone, 1966; Webb, 1969). White fibers have been found to propagate muscle action potentials, and are fast "twitch" fibers, in contrast to the "tonic" red fibers which do not propagate action potentials (Barets, 1961; Jansen et al., 1963; Stanfield, 1972). Red fibers are well supplied with blood capillaries, have large numbers of mitochondria, usually contain fat droplets, and are generally accepted as having an aerobically based metabolism (Boddeke et al., 1959; Bone, 1966; Webb, 1969; Love, 1970). White fibers are larger than the red ones, are more uniform in size, are poorly supplied with blood capillaries, have few mitochondria, and no fat reserves. On the basis of poor blood supply, glycogen depletion and lactate accumulation during their action, white fibers are considered to metabolize glycogen anaerobically (Boddeke et al., 1959; Bone, 1966; Webb, 1969; Hess, 1970; Love, 1970; Pritchard, 1971). Red muscle fibers are always innervated by small motor nerves ending in fine filaments with minute expansions on the ends (multiple innervation), whereas white type fibers are innervated by larger nerve fibers ending in either a single end plate or multiple endings (Hess, 1970).

In fish the fiber types are generally organized into a lateral superficial red muscle mass situated immediately below the skin at the lateral line. This muscle mass is composed entirely of red type fibers (Boddeke et al., 1959). The deeper myotomal musculature (often referred to as the white muscle mass) is composed mostly or entirely of white muscle fibers. In many species (such as salmonids and carp) the "white muscle mass" also contains some intermediate type muscle fibers. These are intermediate in size between red and white but contain fat and are thought to be a slow fiber type (Boddeke et al., 1959).

The contractile and electrophysiological nature of these fibers has not been investigated. A muscle mass composed of red or intermediate type fibers and white fibers is referred to as a mosaic muscle.

Direct myographic recordings from electrodes inserted into red and white muscle masses in sharks have shown that at low swimming speeds only the red fibers are active. Electrical activity from the white fibers is observed only during bursts of swimming (Bone, 1966; Roymer and Kennan, 1967). Boddeke et al. (1959) correlated the arrangement of the myotome in a number of freshwater fish species to their feeding habits and related this to the type of swimming capability the various fish might be expected to have.

The present experiments were designed to:

- measure the maximum steady swimming performance which could be maintained for 10 minutes by a variety of teleost fishes;
- (2) examine the electrical activity of the red and white portions of the myotome, and the proportion of red musculature in some species, which differed widely in swimming performance (in terms of maximum sustained velocity), in order to determine to what extent the action of the two muscle types can be related to the maximal sustained swimming speed.

MATERIALS AND METHODS

- (1) The Water Tunnels
- (a) The laboratory tunnel

At U. B. C. fish were exercised in a tunnel similar to that described by Brett (1964). Basically the respirometer was a recirculating water tunnel incorporating a cylindrical Plexiglass fish chamber (126.5 cm² in crosssectioned area) connected to a pump through expansion and contraction cones. The maximum output of the pump was about 300 gal/min against a developed head of 40 ft, providing a maximum velocity of 103 cm/sec.through the fish chamber. The total volume of the respirometer was 35 L. The expansion cone and three turbulence screens leading to the fish chamber were designed so that a relatively consistent, flat velocity profile occurred over the range of velocities at which fish were forced to swim (10 cm/sec - 103 cm/sec). That the flow profile was a streamlined flow of minutely turbulent water was checked by the use of dyes injected upstream of the pump but downstream of the fish tunnel. This procedure ensured adequate mixing of dye before it reached the fish chamber. The water velocity in the tunnel was monitored continuously using a counter which monitored the revolutions of the pump shaft (Rev-counter) or by means of the setting on the pump's variable speed hydraulic gear (Setting). The Revcounter and pump setting were calibrated in terms of water velocity using an Ott meter. At the end of this series of experiments the tunnel was recalibrated using the Ott meter and it was found that, except at the position where the Rev-counter was superseded by the pump setting, values assessed from measurements made by these systems did not vary by more than 3% from those obtained using the Ott meter (Table 1). Replicate determinations made in the same position of the tunnel over a series of several days agreed within $\pm 2\%$ of one

TABLE 1. Comparison between water velocities in the fish tunnel assumed from the Rev-counter or pump setting and actual measurements made with the Ott meter.

A - Rev-counter/Setting (cm/sec)	B - Measured velocity (Ott meter) (cm/sec)	% Error between A and B
24.37	24.73	-1%
51.77	50.45	+3%
*77.7	73	+7%
102.8	100	+3%

*Rev-counter superseded by pump setting at this velocity.

another. Determinations of water velocity at the centre, top, bottom and sides of the tunnel, with the Ott meter, gave values for water velocity which were no more than 4% different between any two positions, thereby confirming the results of the dye injections in that the velocity profile was relatively flat. The tunnel was dismantled and the turbulence screens cleaned periodically to prevent scales and other detritus from blocking the grids and thereby reducing the maximum water velocity in the tunnel. As recommended by Brett (1964) a covered area at the front of the chamber was provided for the fish. Temperature was controlled within $\pm 0.5^{\circ}$ C by a flow of a refrigerant through a heat exchanger, counterbalanced by a 500-watt heater and relay. Fresh aerated water was fed

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continuously into the system from a reservoir located above the respirometer. The water was heated to a temperature slightly above that in the water tunnel and aerated to avoid problems of oxygen supersaturation of the introduced water. In the majority of experiments the water was renewed at a rate of 2 1/min. The oxygen tension of the water flushed out of the respirometer was monitored using a Radiometer or Beckman oxygen electrode and remained constant during any one experiment.

(b) The field tunnel

The exercise apparatus in the field was considerably simpler in design than that used in the laboratory. As there was an abundant water supply available a one-pass flow system was designed. A gasoline powered trash pump was used to draw water from the river and this was expelled through a 24 cm internal diameter Plexiglass fish tunnel (Fig. 3a). As in the laboratory, the fish chamber had a forward covered area (Fig. 1). The pump, powered by a Ford engine, was rated at 40,000 gals/hr at zero head. In the 24 cm tube this gave a maximum water velocity of about 100 cm/sec. The suction hose and hoses connected to the fish tunnel were of 12.5 cm diameter, mating between them being achieved by use of an expansion cone. Three screens of 1 cm mesh were placed at the upstream end of the fish tunnel. An electrified grid was placed at the downstream end of the fish tunnel and a further contraction cone was used to mate the tunnel to the discharge hose (12.5 cm diameter). The grid was electrified at 5 v A.C. by using an inverter which was powered from the battery on the pump engine.

The turbulence screens were of considerably larger mesh size than is desirable in this kind of tunnel; however, by suitable orientation of the meshes in each grid, relative to one another, it was possible to achieve a fairly flat, albeit skewed, flow profile across the tube over the range of

Figure 1

Photograph of the 25 cm diameter water tunnel used in field experiments.



velocities used (10 cm/sec to 100 cm/sec). Large mesh sizes for the screens were chosen because it was felt that this might eliminate the need for continual dismantling of the tunnel to allow the turbulence screens to be cleaned.

A T-piece and screw-valve was sited between the pump and the fish chamber. With the motor running at idling speed low flows in the fish chamber could only be achieved by opening the valve and exhausting some of the pump's output to the river. Above water velocities of 40 cm/sec the by-pass valve was shut and water velocity in the fish chamber controlled by the throttle on the pump engine. In order to keep the fish chamber filled with water at low velocities it was necessary to raise the level of the discharge pipe above that of the river. As the water velocity was increased the discharge pipe was lowered into the river.

Water velocities in the tunnel were monitored using an Aqua-log boat speedometer, suitably modified to cover the range of water velocities encountered during these experiments. Calibrations of the Aqua-log in the field with the 0tt meter (sited in the mid-position of the tunnel) revealed considerable discrepancies at low speeds but above 40 cm/sec the difference between the two readings was slight (\pm 3%) (Table 2). Measurements made with the 0tt meter on two different days showed that by reading the Aqua-log alone the water velocity in the tunnel could be adjusted to \pm 1% of the required value. Since the water tunnel was floating on a dock surges of flow occurred when the level of the discharge hose changed; however, even violent fluctuations in level (produced by jumping up and down on one end of the raft) only caused fluctuations in water velocity of \pm 2% when velocities were measured over a period of 100 sec with the 0tt meter. In these tests no attempts were made to correct for these flow fluctuations by altering the throttle control on the pump motor.

TABLE 2. Comparison between water velocities in the fish tunnel measured by the Aqua-log and the Ott meter.

A - Aqua-log (cm/sec)	B - Ott meter (cm/sec)	% Error between A and B
10	11.46	-14%
20	26.5	-32%
30	33.4	-11%
40	41.27	-3%
50	50,26	0
60	58.9	+2%
70	71.35	2%
80	82.36	-3%
90	93	-3%
100	102.4	-2%

Dye injections, made just downstream of the pump, revealed that the flow profile was markedly different from the laboratory tunnel. The flow profile was skewed in such a way that the fastest flow velocity occurred at the top and one side of the tunnel and the slowest velocities at the bottom and opposite side (Fig. 2a). It was apparent that the sharp bend in the tubing between the pump and the fish chamber was responsible for the shape of the flow profile but the necessity for even weight distribution on the floating dock prohibited any attempt at correcting this design fault. The nature of the flow profile was confirmed by measuring water velocities at the sides, top and bottom of the tunnel with the Ott meter. Taking the value in the centre of the tunnel as

Figure 2

- (a) Diagrammatic representation in side view of the flow profile in the field water tunnel (25 cm diameter) as determined from photographs of dye injections made over a range of water velocities.
- (b) Water velocities at the periphery of the field tunnel expressed as a % of water velocity in the centre. All determinations (average of 3 at each position) made with an Ott meter over 100 sec time periods.



100% it was found that velocities at the top and left side (looking into the flow) of the tunnel were some 118-120% of this value whereas velocities at the bottom and right hand side were some 91-93% of water velocity in the centre of the fish chamber (Fig...2b). In practice it was noted that fish seldom swam at the top or left hand side of the tunnel; the majority of animals, after the acclimation period, frequented the centre, bottomoor right hand side of the tube. Consequently values for critical velocity determined in the field may be overestimated by, at most, some 9%.

Due to the fact that the spectacularly muddy Liard River enters the Mackenzie River just upstream and on the same side as the island on which Fort Simpson is sited, it was impossible to locate the water tunnel in the immediate vicinity of Fort Simpson. This seemed a wise choice in that few fish are caught in the Liard River water and it therefore seemed unreasonable to expect fish to swim well in such a silt load. Furthermore since the Mackenzie River drops considerably in level over the summer months it was not feasible to site the apparatus on the bank opposite Fort Simpson. As a compromise the whole apparatus was assembled on a 12 ft by 10 ft raft and towed across the Mackenzie River and moored at the mouth of the Harris River (Fig...:3). Due to this procedure a relatively clear water supply was ensured throughout the period of the study.

(2) The Fish

Except for trout (<u>Salmo gairdneri</u>, Richardson), carp (<u>Cyprinus carpio</u>, L.), and herring (<u>Clupea harengus</u>, L.) the fish species used in this study were supplied by the Fisheries Service from Fort Simpson, Norman Wells, Arctic Red River and Aklavik. In the laboratory experiments individuals from all 4 field stations were used whereas the field tunnel only utilised fish caught in the

Figure 3

Photograph of the field apparatus moored in position on the river.



vicinity of Fort Simpson. The fish were seined, gill-netted or caught on hook and line. At the field operation the fish were placed in holding pens sited alongside the floating dock and were experimented upon 24 hours after capture.

For the laboratory operation the fish were shipped to Vancouver by air freight after being held by the Fisheries Service in pens for up to 3 days after capture. The fish were not fed before shipment. For shipping, the fish were placed in clean double plastic bags, a layer of newspaper being placed between the bags if the fish had spiny rays. This prevented puncture of the outside bag and subsequent loss of water. Water was placed in the inner bag, sufficient just to cover the fish, and the air was squeezed out completely and a length of hose, connected to an oxygen cylinder, was used to inflate the inner bag to about 2-3 times the volume of the enclosed water. The bag was then twisted tight and taped. The outer bag was then tied in the same manner. In virtually all shipments the fish were packed individually to avoid contamination of specimens should one of a group die in transit. The bags were then placed in styrofoam boxes which contained one or two freezer packs wrapped in The boxes were sealed with tape. As a general rule 1-2 large or newspaper. 3-4 small fish could be adequately contained in a single styrofoam box.

Fish caught by stations at Aklavik and Arctic Red River were shipped via Northward Aviation to Inuvik, International Jet Air to Whitehorse, and C. P. Air to Vancouver. The total time spent in transit by this routing was less than 12 hours. Unfortunately after only 3 shipments a labour dispute at C. P. Air forced use of P. W. A. from Inuvik to Edmonton and thence to Vancouver. Under favourable circumstances (no delays) transit time via this routing was 15 to 18 hours. In the later part of the study an attempt was made to further reduce the transit time on this route by shipping the fish to Inuvik by air charter.
From Norman Wells and Fort Simpson fish were shipped by P. W. A. to Vancouver, via Edmonton, the transit time being of the order of 18 to 24 hours. Under favourable circumstances (no delays and care taken in packing) mortality was low, being less than 10% of any shipment. It was established that about 30% mortality occurred when transit time exceeded 30 hours and 100% if transit: time exceeded 36-40 hours.

On arrival at U. B. C. the fish were introduced to 2,000 1 circular tanks in which water circulation was maintained by means of pumps. The water velocity varied in the tanks from zero at the centre to 35-40 cm/sec at the edge of the tank. Temperature was maintained at 12-13°C by means of thermostatically controlled heaters working against the input water flow. The input water was at 9°C. After introduction to the tanks - this procedure taking about one hour to allow fish to adapt to the different water hardness between the Mackenzie River water and water at U. B. C. - pumps were placed in the "mixing" mode and the fish treated with "nitrofurazone" (10 g/2,000 1). After 24 hours in the tanks fish were offered trout pellets, artemia and minnows and the pumps were placed in the circulation mode. Fish were fed every other day and after 3-5 days were used in experiments, if the water temperature in the field was close to that in the holding tanks. However, on some occasions, fish were left in the holding tanks for longer periods (5-10 days) to allow for thermal acclimation to the range of 12-13°C since they were taken in the field at temperatures from 17-19°C. Fish were not fed for 24 hours before any series of experiments.

Some fish were also acclimated, in October, to a temperature of $7^{\circ}C$ in 1,000 l refrigerated tanks. These fish were taken from the Mackenzie River at Fort Simpson, which was at a temperature of 8-9°C at that time. Water circula-

tion was maintained in these tanks by means of submersible pumps. The fish were allowed, on average, about 15 days for the period of thermal acclimation before the start of the experiments. These fish were offered trout pellets and minnows every other day before the start of the experiments.

Juvenile herring (Clupea harengus) were caught by seine netting in the Georgia Straits and after treatment with nitrofurazone (10 g/2,000 1) for 2 days they were held in tanks through which aerated seawater $(6-8^{\circ}C)$ was circulated until they were used for experiments (one week). During the holding period they were not fed. Female trout (Salmo gairdneri) were purchased from a commercial supplier (Trout Lodge, Ephrato, Washington, U. S. A.) and transported to U. B. C. by tank truck, where they were held in large cylindrical tanks (8000 1). Constant inflow of fresh dechlorinated water (9-10°C) was maintained at all times. Carp (Cyprinus carpio) were caught by seine netting in the Fraser Valley east of Vancouver, and held under the same conditions as the trout. Trout and carp used for experiments were physically trained for a minimum of two weeks. Training was accomplished by holding the fish in 2000 1 circular tanks in which water was kept in motion by water jets driven by pumps. The water velocity varied from nearly 0 at the centre of the tank to 30-40 cm/sec at the circumference. The fish tended to swim constantly in the high water velocity zone. Trout and carp were fed Clarke's Trout Pellets six times weekly throughout the training period.

(3) Experimental Procedures

At the field station only increasing velocity tests, to allow determination of critical velocity, were carried out; whereas in the laboratory fatigue trials were performed in addition to the increasing velocity tests. The experiments in the field were performed from mid-July to the end of September

whereas the laboratory experiments ran from mid-May to mid-October. The effects of temperature on swimming performance were investigated both in the In the field all experiments were performed at ambient laboratory and field. temperature (the tunnel offering no opportunity for temperature regulation) and the field experiments were carried out at temperatures between 12-13°C and 18-20°C. Since the fish were caught and held in the same body of water as was passed through the tunnel it was assumed that the fish were acclimated to the respective temperature regimes. The laboratory data gave critical velocity determinations at 7-10°C and 12-13°C whereas the field data gave values for fish run at temperatures of 12-13°C and 18-20°C. Therefore comparison for any differences between laboratory and field data, for most species, could be made at 12-13°C whereas the effect on swimming performance of acclimation to higher and lower temperatures could be assessed by comparing the critical velocities obtained with either the laboratory or field data at 12-13°C. In the field, following fatigue of the fish, it was removed from the tunnel, weighed, measured (fork length and cross-sectional area) and tagged. The tagged individuals were returned to the tanks and after several days were used in fatigue or temperature shock experiments. Following these experiments the fish were killed and opened in the ventral midline to allow determination of the state of maturity or sex of the individual.

(a) Increasing velocity tests

Fish were introduced into the respirometer by means of the access port and the downstream grid electrified with voltages varying from 3-10 v A.C. depending on the sensitivity of the fish. Great care was taken when introducing fish to the tunnel to avoid undue excitement of the animal and to this end the transfer was usually achieved using a series of nets and water filled

buckets. The fish was allowed at least one hour's acclimation to the tunnel with the water velocity at its lowest speed (9.5 cm/sec in the laboratory tunnel and 11 cm/sec in the field tunnel). It was confirmed by visual observation that fish expended relatively little effort in maintaining their position in the tunnel against this water velocity. Following this introductory period the fish were subjected to sequential water velocity increments every 10 min. In the laboratory the water velocity was increased in steps of not more than 1/2 L/sec (L = fork length, cm) for small fish and 1/3 L/sec for large fish (>25 cm fork length). However, in the field, the velocity increments were of the order of 10 cm/sec regardless of the length of the fish. In order to introduce some standardization to the results, data from fish which failed to complete 3 velocity increments in the laboratory or 2 increments in the field, was not used. As a rule only large fish were rejected on this score and, in many cases, their poor performance could often be related to prominent fungus growth or gill-net marks. A fish was deemed to have become fatigued when it could not remove itself from the electrified grid even when a voltage 3-4 times greater than that established in the acclimation period was applied. Further confirmation of fatigue was obtained by trying to force the fish to remove itself from the grid in response to strong mechanical stimuli. The fatigue velocity (Vf) and time taken for complete fatigue (t) at that velocity was noted, along with the water velocity applied before the fatigue velocity was reached (Vp = penultimate velocity). In the laboratory, it proved possible to measure fork length and cross-sectional area, weigh and tag the exhausted fish without the use of anaesthetics. Three hundred and sixty critical velocity tests were made in the field and three hundred and one in the laboratory.

Since fish almost always fatigued during the last velocity increment in a time interval less than the desired time period (10 min) a measure of critical swimming speed was obtained empirically as has been described by Brett (1964), viz:

$$Cv = Vp + ([Vf - Vp] \times \underline{t})$$

where

Cv = critical velocity (cm/sec)
Vp = penultimate velocity (cm/sec)
Vf = final velocity (cm/sec)
t = time to fatigue (min)

Because of the closed nature of the water tunnel, swimming fish experience a drag higher than that expected at any given free-stream velocity. For fish, corrections must be made for the extra drag arising from horizontal buoyancy and solid-blocking effects (Pope and Harper, 1966) as well as a "propeller correction" (Webb, 1970). The horizontal buoyancy and propeller corrections are small and opposite in effect to each other so they tend to cancel one another (Webb, 1971). However, the solid-blocking effect, which results from the decrease in effective cross-sectional area of the tunnel through which water can flow due to the presence of the fish, will obviously vary depending upon the respective cross-sectional areas of the fish and tunnel. The increase in water velocity around the fish can be calculated from the following general formula - and must represent the actual velocity at which the fish is swimming in the tube, viz:

$$Cv_2 = [1 + f(\frac{Af}{At - Af})] \times Cv_1$$

where

 Cv_1 = uncorrected critical velocity (cm/sec) Af = maximum cross-sectional area of the fish (cm²)

At = cross-sectional area of the fish chamber (cm^2)

f = factor

Cv₂ = corrected critical velocity (cm/sec)

Paulik and Delacy (1957) took f = 1 in their calculations but Webb (1970) suggested that this gave erroneously high results unless the maximum crosssectional area of the fish was a large proportion of the cross-sectional area of the tunnel. In the present experiments, to calculate the actual critical velocity of the fish, f was taken as 0.8 and corrections were only made when the cross-sectional area of the fish was greater than 10% of the cross-sectional area of the tunnel. Apart from 7 or 8 exceptions the maximum crosssectional area of the fish was less than 30% of that of the laboratory tunnel meaning that even if f = 1 the error between that value for critical velocity and the one calculated, assuming f = 0.8, would be only 5%. For the field tunnel (24 cm internal diameter) only 3 fish had maximum cross-sectional areas greater than 10% of the cross-sectional area of the tunnel and, for these animals, the error between assuming f = 0.8 and not 1.0 would be about 2.5%.

Due to the large diameter of the field tunnel several fish were run in each trial but, in the laboratory, only when very small animals were tested (10 cm fork length) was it possible to run more than one fish at a time. As noted previously (Jones, 1971) interaction between individuals did not appear to affect swimming performance. Temperature was recorded throughout each experiment in the field and laboratory and, in addition, the oxygen tension of the water flowing from the laboratory respirometer was continuously monitored using a Radiometer or Beckman oxygen electrode. By appropriate adjustment of the water inflow it was possible to keep the oxygen tension of the water constant during each test.

(b) Fixed velocity tests

One hundred and thirty-two attempts were made in the laboratory to establish fatigue curves for various species using a somewhat modified procedure from that outlined by Brett (1967). All fish used in these experiments had had their critical velocities determined 5-10 days prior to this test. After removal of the tag and introduction of the fish to the water tunnel, as outlined in section (a), the speed was increased by small steps, following the one hour acclimation period, until a velocity of 60-90% of the fish's previously determined critical velocity was obtained. Usually 5 steps were used to reach the test velocity and a period of 3 min was allowed between each velocity increment. When the desired test velocity was reached the time to fatigue was measured using 100 min maximum. If the fish did not fatigue in 100 min the experiment was terminated. Fatigue, when it occurred, was judged as outlined in section (a). The temperature and oxygen tension of exhalent water from the respirometer was measured continuously. No marked changes in either variable occurred during any one experiment. All experiments were conducted at the temperature to which the fish were acclimated (12-13°C). After fatigue, or at the termination of the experiment, the fish were removed from the respirometer, weighed, measured (fork length and maximum cross-sectional area) and re-tagged. (c) Electromyography

To prepare fish for electromyography they were anaesthetized with MS222 (1:15000) and electrodes were inserted into the lateral red muscle, and the epaxial portion of the white muscle mass. Electrodes consisting of a pair of 40 swg insulated copper wires glued together with epoxy for a distance of 5 mm from the tip, bent into a hook shape and bared at the end, were used in the red

muscle of all species studied and for white muscle in herring. In the other species the individual electrodes of each pair used for recording from white muscle were separated by a distance of 2-3 mm.

After instrumentation the fish were allowed to recover from the anaesthetic. Herring were allowed to recover in a bucket of seawater for 20-30 min; then if the electrodes were functioning the fish were transferred to the water tunnel. Herring were found to be so delicate that it proved impractical to allow a long recovery period after anaesthesia. The freshwater fish were allowed to recover for 18-20 hours in the water tunnel before electromyographic recording was undertaken.

Electromyograms were recorded from the fish at increasing swimming speeds until the maximum sustained speed of the individual was reached. In recording from the freshwater fish (trout, carp, pike) signal to noise ratio was increased by addition of small amounts of seawater to the water in the water tunnel for the duration of the most critical recording periods. The water temperature in the water tunnel was held at the same temperature as the water in which the fish had been held, $\pm 0.5^{\circ}$ C. The potentials from the muscle were amplified by a Tektronix 122 preamplifier and recorded on a Brush 220 oscillographic pen recorder. After the experiments, some fish were killed, cooked in boiling water, and the proportion of lateral red muscle to total body weight determined. This was done first weighing the cooked fish, then removing and weighing the lateral red muscle.

(4) <u>Analysis of Data</u>

(a) Increasing velocity tests

The aim of the analysis was to provide information for the following formula for each species:

 $V = K L^{e}$

 $(\log V = \log K + e \log L)$

where

V = critical velocity (cm/sec)

K = constant (y intercept)

L = body length (cm)

e = exponent (slope of the line)

Obviously not all species were examined in sufficient numbers to give a regression equation in which any confidence could be placed and, in these cases, only mean values for various size classes is presented. For reasons given previously it was also necessary to define the effects of temperature of acclimation, and state or maturity, on swimming performance. Consequently a series of statistical tests was performed which ultimately led (or not, as the case may have been) to the final regression analysis. In all statistical procedures 5% was regarded as the fiducial limit of significance.

Analysis of co-variance was used to test for critical velocity differences between immature, male and female individuals and individuals at different temperatures for each species with length as the co-variate. No significant differences were found between the different groups in any species. The data for each species were therefore pooled and a regression equation was calculated. Pearson product moment correlations were performed on log critical velocity versus log length to assess the correlation of the two variables.

(b) Fixed velocity tests

The data are presented graphically as plots of time to fatigue (min) against swimming speed expressed as a per cent of the animal's previously determined critical velocity. Since the latter represented the maximum speed that the animal could swim at for 10 min, a fixed point regression was perform-

ed on the data with 10 min representing the fixed point of 100%. As the maximum time period of the experiment was 100 min, if an unreasonably low speed was chosen for the fish to swim at, it would be possible with this type of analysis to obtain unreasonably low values, as a proportion of their previously determined critical velocity, for sustained performance. Consequently, it was arbitrarily decided to eliminate all those 100 min points which were more than 10% below the mean of the two lowest percentages of critical velocities at which any two fish had fatigued within the 100 min time period.

RESULTS

(1) Preliminary Experiments

(a) Increasing velocity tests

Increasing velocity tests were structured to give the maximum steady performance for 10 min but it has been claimed that longer periods between velocity increments should be used in trials of this type (e.g., 20-60 min) so, in a preliminary series of experiments, 5 longnose suckers (<u>Catostomus</u> <u>catostomus</u>) and 3 arctic grayling (<u>Thymallus arcticus</u>) were examined with both 10 and 20 min periods between velocity increments. Although there was a slight reduction in critical velocities achieved, by the same fish, with the longer time period, the mean values for both species were not significantly different. Also, it has been suggested that the period of acclimation to the respirometer before the start of the test may affect critical velocity determined in this manner (Brett, 1967), although in a series of experiments with grayling and longnose suckers there was no significant difference in the critical velocities achieved after 16, 12, 2 or 1 hour of acclimation to the water tunnel.

(b) Fixed velocity tests

Brett (1967) suggested that the minimum time period to termination of a fixed velocity test should be 200 min, but in the present series tests were terminated at 100 min as this represented 10 times the time period used to delimit the maximum steady performance. Consequently in some fixed velocity trials the ultimate time period was extended to 300 min, which represents the transition point to sustained performance in sockeye (<u>Oncorhynchus nerka</u>) (Brett, 1964). The extended time period was imposed on 4 longnose suckers,

1 grayling and 1 burbot (Lota lota, L.). The suckers were swimming at 68.5 to 89% of their previously determined critical velocities, the arctic grayling at 89%, and burbot at 96.6%. All animals except one longnose sucker, which was swimming at 83.5% of its previously determined critical velocity, continued to swim throughout the extended time period.

(2) Increasing Velocity Tests

Increasing velocity tests were performed on 179 longnose suckers (<u>Catosto-</u> <u>mus catostomus</u>, Forster), 20 white suckers (<u>Catostomus commersoni</u>, Lacépède), 169 humpback whitefish (<u>Coregonus clupeaformis</u>, Mitchill), 24 broad whitefish (<u>Coregonus nasus</u>, Pallas), 105 arctic grayling (<u>Thymallus arcticus</u>, Pallas), 192 pike (<u>Esox lucius</u>, L.), 54 yellow walleye (<u>Stizostedion vitreum vitreum</u>, Mitchill), 53 burbot (<u>L. lota</u>), 34 carp (<u>C. carpio</u>), 25 trout (<u>S. gairdneri</u>), and 11 arctic char (<u>Salvelinus alpinus</u>, L.). The tests on burbot, broad whitefish, trout, and carp were performed exclusively in the laboratory; all other species were tested both in the laboratory and in the field. Of the 11 species studied only 10 were represented by a sufficiently wide size class to obtain a regression equation ($V = KL^e$). Table 3 shows the values for K and e of the 10 species along with a probability value for the correlation of critical velocity on length for each of the species.

(3) Fixed Velocity Tests

Fixed velocity tests were conducted on 5 species in the laboratory at 12-13°C. All animals were acclimated to this temperature. The data are illustrated, for each species, in Figure 4. It is of interest that the fixed point regression lines are of the same slope for pike, longnose sucker and burbot, showing that, on the average, these fish can maintain 60% of their 10 min maximum performance for 100 min (Fig...4). For both char and grayling

Species	К	e	Probability of V not being correlated to L	Conditions
pike	4.9	.55	.001	* 1 . 12–13 [°] C
yellow walleye	13.07	.51	.1	*F 18-20 ⁰ C
arctic grayling	36.2	.193	.02	F 12 1 12 F 20 ⁰ C
longnose sucker	11.03	.529	.0001	1 7 F 18-20 ⁰ C
white sucker	10.3	.552	.02	F 12, 19 ⁰ C
burbot	30.6	.07	.1	1 7, 12 ⁰ C
humpback whitefish	18.2	. 35	.0001	1 7 F 12, 19 ⁰ C
broad whitefish	9.7	.45	.003	1 12 [°] C
trout	12.4	.55	.0001	1 10 [°] C
carp	7.52	.65	.0001	1 10 ⁰ C

TABLE 3. Tabulation of the K and e values for the equation $V = KL^{e}$ for 10 species of freshwater fish.

*1. - Laboratory

F - Field

Figure 4

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Fatigue curves for 5 species of fish determined in fixed velocity tests. The tests were terminated at 100 min if the fish failed to fatigue.



the slopes of the regression lines are twice as steep as those for the above 3 species. Char and grayling can therefore maintain about 80% of their 10 min maximum performance for 100 min (Fig. 4). It must also be pointed out that some of the individuals represented by these figures could maintain their 10 min critical velocity for a period of 100 min or (as is suggested by the one burbot swimming at 96.6% of critical velocity for 300 min) much longer. In all of the species subjected to incremental increases in water velocity it was observed (in about 80% of the cases) that fish tend to fatigue within the first 3 min of a new increment (regardless of the length of the test period).

(4) Electromyography

(a) Herring

In herring, swimming was of one of two distinct patterns: either, the fish swam smoothly maintaining its position in the water tunnel or, it could not maintain its position while swimming smoothly but rather drifted backwards then accelerated up the tube with bursts of violent tail beats. During smooth swimming red muscle fibers alone were active (Fig. 5a, b & c). When the water velocity was increased to the point at which the fish was unable to maintain position by swimming smoothly large potentials were recorded corresponding in time to bursts of violent swimming (Fig. 5d). These large potentials are 4-5 times as large as the ones associated with smooth swimming and are thought to be white fiber action potentials. A period of such violent swimming always resulted in fatigue in 1-2 min. The red portion of the myotomal musculature was 5.6% of the body weight in herring.

(b) Carp

Electromyographs were successfully recorded from the red and white portions of the myotome. Electrical activity was detected from the mosaic portion of

Figure 5

Electromyographs recorded from herring with electrodes placed within the red myotomal muscle mass. Lines a-c are records at increasing swimming speeds. d is an electromyograph obtained from fish swimming with violent tailbeats.



the myotome even at the lowest swimming speed (20 cm/sec) (Fig. 6a). At increased swimming speeds, the potentials from both portions of the myotome increased in amplitude while decreasing in duration. This increase in amplitude suggests recruitment of more fibers at the higher swimming speed. Occasional rapid large potentials were observed (Fig. 6b) at intermediate swimming speeds. These were faster events than those which composed the remainder of the electrical activity, and are presumably muscle action potentials of white muscle fibers. At the maximum speed (10 min critical velocity) rapid potentials (Fig. 6c) formed the major part of the activity from the white muscle and were picked up by the electrodes located in the red muscle More information was obtained by alternately increasing and decreasing mass. the speed slightly while the fish was swimming at near to its critical velocity. Under these conditions the fish could be induced to swim as did the herring, interspersing periods of steady swimming with a few violent tail beats while accelerating. During this type of swimming (Fig. 6d) two types of muscle discharges were recorded. Violent tail beats (indicated by asterisks in Fig. 6d) resulted in large potentials being recorded from the white muscle mass. These are presumably extracellularly recorded white fiber action potentials. In the period between violent tail beats electrical activity from the white muscle mass consisted of small potentials resembling those recorded from the red fibers. Histologically two types of muscle fibers were identified within the white muscle mass: a broad fiber and a narrow fiber. Lateral red musculature in carp is 2.6% of the body weight.

(c) Pike

In pike there was no evidence of electrical activity in the white muscle mass at speeds of less than the individual's critical velocity (Fig. 7a & b).

Figure 6

Electromyographs from carp myotomal musculature. M denotes record from mosaic muscle mass, and R denotes that from red muscle mass. a-c indicate records at increasing swimming speed from minimal to 10 min critical. d indicates record obtained when fish was swimming at near critical speed with interspersed violent tailbeats (denoted by * on record).



Figure 7

Electromyographs from pike red (R) and white (W) myotomal muscle masses. a-c indicate records at minimal to 10 min critical velocity.



At critical velocity or in excess of critical velocity large fast potentials of the action potential type were recorded from the white muscle mass (Fig. 7c). The lateral red muscle in pike was found to be only about 1% of the weight of the whole fish.

(d) Trout

The lateral red muscle mass in trout forms 2.5% of the body weight. Red muscle was active at all swimming speeds but no electrical activity was detectable in the mosaic muscle at low speeds except for a few discharges at the beginning of some speed increments when the fish was swimming unsteadily (Fig. 8a & b). During steady swimming at speeds up to 99% of the individual's critical velocity no electrical activity was observed in the mosaic muscle. Water velocity in excess of critical velocity resulted in the fish using violent bursts of swimming during which large action potential type discharges were recorded from the mosaic musculature indicating that white muscle fibers were being used. This period of white muscle activity at high swimming speeds was observed in all species studied and was followed by fatigue.

Figure 8

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Electromyographs from trout red (R) and mosaic (M) myotomal musculature. a-c are records at minimal swimming speed to 99% of 60 min critical velocity. d is a record at a velocity in excess of 60 min critical velocity.

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DISCUSSION

There can be no doubt, for those species studied by both fixed and increasing velocity tests, that in the 10 min increasing velocity tests the animals were in the zone of steady performance. It would also appear reasonable to assume that this was true of all other species studied in increasing velocity tests alone. Bainbridge (1962) argued that there are major specific or familial differences in the relation of speed to size and Fry and Cox (1970) urged that this should be tested in a thorough comparative study. For most salmonids the sustained speeds vary essentially as L^{0.5} (Bainbridge, 1960, 1962; Blaxter and Dickson, 1959; Brett, 1964, 1965; Fry and Cox, 1970), while for other species the exponents range from 1 for burst speeds of herring (Blaxter and Dickson, 1959) and dace (Bainbridge, 1960) to 0.31 for sustained speeds of pumpkinseed (Lepomis gibbosus) (Brett and Sutherland, 1965). In the present experiments for species showing a good length to swimming speed correlation, the range in exponents was from 0.19 for grayling to 0.65 for carp. Figure 9 is a graphical representation of the relationship of 10 min critical velocity to length in the different species studied, along with the proportion of lateral red muscle in some of the species. Swimming speed is more size dependent in some species than others. This may be related to the feeding and migratory activity of the different species in that small grayling (<20 cm), which feed in the same riffle areas as large grayling, have a higher critical velocity than individuals of the same length $\hat{a}S$ the other freshwater species studied. In general the larger proportion of red muscle in the body musculature (Fig. 9) the greater the critical velocity.

The present observations of electromyograms of red and white myotomal

Figure 9

The relationship of length to critical velocity (10 min). The points are for fish which were not represented by a sufficiently large size class to derive a length velocity regression equation. Values in parentheses represent the weight of lateral red myotomal musculature as a per cent of total body weight.



muscle masses of several fish species have shown that:

- 1. At low swimming velocities red muscle fibers alone are active, not the white ones, in all the species studied.
- 2. At speeds high enough to produce fatigue during the test period white muscle as well as the red is active in all of the species studied. In those fish which have only white fibers in the white portion of the myotome (herring, pike) there is no electrical activity in the white muscle in the absence of violent tail beats. Mosaic muscle in the physically trained trout used in this study did not produce the small slow potentials typical of red muscle, which were shown in mosaic muscle of untrained trout by Hudson (1973). This difference between the two groups of trout (Hudson's and mine) may be the result of physical training somehow changing the pattern of recruitment of the red type fibers in the mosaic muscle or it may be a difference between the two stocks of fish. The stock of fish used by Hudson (1973) have previously been shown to have a very low critical velocity (about half of that of fish in this study) (Webb, 1971). Carp in this study showed recruitment of the red fibers in the white (mosaic) myotomal muscle mass at submaximal swimming speeds as was observed by Hudson (1973) in trout. The narrow muscle fibers in the mosaic muscle of trout contain myoglobin and fat and would therefore be expected to have an aerobically based metabolism.

In examining the available evidence it does not seem probable that white muscle operating anaerobically can or does contribute to the power output of a fish for periods longer than a few minutes without precipitating fatigue. It would also seem that the more red muscle a fish has in the muscles which power swimming, the faster it can swim on a sustained basis.

SECTION II

Changes in Cardiovascular, Respiratory and Metabolic Variables Accompanying Prolonged Exercise in Fish

INTRODUCTION

In Part I of this study it was demonstrated that during sustained swimming in several teleosts only the red muscle fibers are involved and that the proportion of the lateral red muscle of the myotome is related to the maximal sustained swimming speed. The oxygen consumption rate of fish during prolonged swimming periods has been extensively investigated and found to increase exponentially with swimming speed until the fish fatigues (Brett, 1964). Changes in cardiovascular and respiratory variables during short duration (5 min) exercise in fish have been studied (Stevens, 1968). In mammals the cardiovascular compensations to exercise change when exercise is prolonged beyond 10-15 min. Under these conditions heart rate tends to rise and stroke volume decreases (both relative to the value at 10-15 min of exercise) (Cobb and Johnson, 1963; Ekelund and Holmgren, 1964; Saltin and Stenberg, 1964; Ekelund, 1967). This is thought to be due to an increase in blood flow to the skin for thermoregulatory purposes. If this is the case an animal which has no thermal load, such as fish, would not be expected to show such changes during prolonged exercise. The effect of prolonged exercise on cardiovascular and respiratory variables and their relationship to metabolic variables has not been investigated in fish.

The experiments in this section were designed to seek answers to the following questions:

(a) What is steady state with respect to cardiovascular and respiratory aspects of exercise in fish?

(b) How do the cardiovascular and respiratory systems in fish adjust to steady state exercise?

(c) What determines, and causes termination of, maximal exercise (i.e., what is the limiting factor, and what is fatigue)?

MATERIALS AND METHODS II

(1) Animals

Trout (40-53 cm, 0.9-1.5 kg) were purchased from either the Trout Lodge, Ephrato, Washington, U. S. A. or Colebrook Trout Farm, Surrey, B. C. and transported to U. B. C. by tank truck where they were held as described in Section I. All fish were barren females and came from the same stock (i.e., Colebrook Trout Farm bought fish from Trout Lodge). The experiments were carried out at water temperatures of 9-10.5°C on fish which had been trained for a minimum of two weeks (see Section I for training procedure).

(2) <u>Surgical Procedures</u>

The fish was anaesthetized in a bucket of MS222 Sandoz (1/15,000), weighed, measured, and placed on an operating table similar to that of Smith the Bell (1964). Flow of water or anaesthetic (MS222, 1/20,000) was maintained over the gills during all surgical procedures. Cannulae for blood sampling were placed in the dorsal aorta, ventral aorta and common cardinal vein. The dorsal aorta was cannulated as described by Smith and Bell (1964) using a 45 cm length of PE 60 tubing terminated with a 1 cm section of Huber point 21 G needle. Ventral aortic cannulation was accomplished using a cannula similar to the one for the dorsal aorta except that the needle end was 2 cm long and bent at a 60° angle 6 mm from the tip. This cannula was inserted into the ventral aorta through the tongue at the level of the third gill arch. The ventral aortic cannula was firmly sutured to the tongue and led straight out of the mouth.

The right common cardinal vein cannula consisted of a 3 cm section of Huber pointed 18 G needle bent at 90[°] 18 mm from the tip and attached to a 45 cm piece of PE 160 tubing (Clay Adams). This cannula was inserted perpen-

dicular to the right side of the fish at a point about 3 mm posterior to the cliethrum and 3 mm ventral to the lateral line. The cannula was then oriented so that the open end in the vein was directed ventrally before being sutured in place. All blood vessel cannulae were filled with heparinized (10 μ /ml) Courtland saline (Wolf, 1963) and plugged with pieces of wire of the appropriate diameter.

Wires for ECG recording were inserted, one immediately posterior to the middle of the pectoral girdle and one medio-dorsally posterior to the operculae. These wires were sutured to the skin at several locations along the right side of the fish up to the anterior edge of the dorsal fin. At this point the wires together with a protective wire braid were firmly tied to the fin. Any existing dorsal fin damage was repaired by suturing to help prevent snagging of cannulae and wires.

To measure ventilation volume a skirt was made of the wrist portion of a disposable surgical glove and placed over the head of the fish to lie in contact with the body. After proper orientation the skirt was sutured in place around the lower jaw and up over the operculae in such a manner that the anterior of the skirt formed a close fit to the head of the fish, whereas the posterior part was loose to allow free movement of the operculae. The excess membrane was then trimmed off, leaving an area of membrane extending about 2 cm posterior to the operculum. A water sampling cannula (PE 60) 60 cm long was placed with its open end ventro-medially anterior to the pectoral girdle (under the skirt), and sutured in place, and then led up posteriorly to the left pectoral fin and sutured in place dorsally. In order to measure respiration rate a buccal cannula (45 cm PE 60) was placed as described by Saunders (1961).

After completion of surgical procedures the fish were placed in the water

tunnel and allowed 18-24 hours to recover from the anaesthetic and surgery. During this time water velocity in the water tunnel was at the lowest setting, about 10 cm/sec.

(3) Experimental Techniques and Equipment Used

(a) Pressure measurement

Samborn 267B tranducers were used for dorsal and ventral aortic blood pressure measurements and a Statham P23v transducer was used to measure common cardinal blood pressure. Buccal pressures were measured with a Samborn 267B transducer. During the cardiovascular transients experiments it was desirable to have an indicator of water velocity (in the water tunnel) recorded along with the other variables. One way that the velocity of a fluid flowing in a pipe may be measured is by detecting the pressure change as the fluid passes around an elbow in the pipe or through a constriction. Since the water tunnel has, incorporated in its design, two contraction cones (effective constrictions) the pressure change across one of these was measured using a Samborn 268B differential pressure tranducer and the output recorded on the strip chart along with the other variables.

To ensure that the amplitudes of the recorded blood pressure waves were accurately recorded the transducers and cannulae used for blood pressure recording were tested by the free vibration method described by Macdonald (1974). By this method the natural frequency of the Sanborn 267B transducers and cannula was found to be 15 Hz with a damping 35% of critical. The Statham transducer and cannula used for common cardinal pressure measurement had a natural frequency of 10 Hz and damping was 36% of critical. Pressure transducers used for dorsal and ventral aortic pressures were calibrated against a pressure head of 40 cm of saline (zero being the level. of the water tunnel). The Statham P23v transducer used for common cardinal blood pressures was calibrated against a head of 15 cm of saline, as was the transducer used for buccal pressure recording. All calibrations were checked frequently.

(b) Signal conditioning and recording

An EKG analog ratemeter was used (triggered by the QRS wave of the ECG) to obtain beat to beat heart rate in the study of heart rate transients. Recording of all electronic signals was done on either a Brush model 220 two channel pen recorder or a Techni-rite 8 channel recorder, model number TR8-88; both recorders writing on rectilinear co-ordinates.

(c) The water tunnel

The water tunnel used was basically the same design described by Brett (1964). For a complete description of this equipment refer to Section I.

(d) Blood sampling procedures

Blood samples were taken from the cannulae after first bleeding the saline out of the cannula. A sample of blood for oxygen content determination was then taken from the cannula by inserting the needle of a needle-tipped micro buret (previously heparinized and dried) into the end of the cannula and allowing the buret to fill the required amount by blood pressure (dorsal and ventral aortic samples) or by siphoning (common cardinal vein). A blood sample for pH and PO_2 was taken from the cannula with a 1 ml heparinized syringe and a three-way valve arrangement. The valve and syringe were rinsed with a small amount of blood (rinse blood saved), the sample (0.5 ml) taken, and immediately injected into the previously calibrated pH and PO_2 equipment. A sample (0.3-1 ml) of blood was taken in a similar manner on occasions when lactate was to be determined and immediately diluted 1.0:3.5 v/v in cold 8% HClO₄.
(e) Oxygen tension

Oxygen tensions were measured with a Radiometer type E5046 electrode in a type D616 thermostated cell. The zero setting was established, using a $0.01 \text{ M Na}_2\text{BO}_7$ solution with 5 mg/20 ml Na_2SO_3 added, twice daily. The span was set before each sample or group of samples in the case of duplicates using air equilibrated water at the ambient water temperature. Both calibrations were reproducible to 0.5 Torr over the period of the measurements.

(f) Measurement of pH

Measurement of pH was done with a Radiometer type G297/G2 blood pH electrode calibrated using precision buffers (type S1500 and S1510). One or both calibrations were done before each blood sample depending on the stability of the electrode. Readout from the oxygen and pH electrodes was on a Radiometer Acid-Base Analyzer.

(g) Determination of oxygen consumption

Before each oxygen consumption determination the water tunnel was checked for trapped air bubbles. Any air bubbles that were found were removed. Oxygen consumption was determined by closing the system (shutting off the input of fresh water) and measuring the rate of change of the oxygen tension of the water circulating in the water tunnel. This was done by taking a water sample with a 5 ml syringe from the water tunnel at the same time that the inflowing water was shut off and another sample at the end of a suitable time period. The length of time (5-45 min) between the samples was determined by the size and intensity of swimming of the fish. During this time the oxygen tension of the water dropped about 10-15 Torr. The oxygen tension of the water samples was measured in duplicate immediately after each was taken. Oxygen consumption was then calculated using the formula:

$$VO_2 = \Delta PO_2 \cdot V \cdot \alpha$$

 \dot{VO}_2 - oxygen consumption (ml O₂ ATPS/min) ΔPO_2 - change in oxygen tension (Torr) V - volume (34.51) α - solubility coefficient of O₂ in H₂O (ml O₂/1 H₂O·Torr) t - time (min)

(h) Blood oxygen content

Oxygen content of arterial and venous blood was determined by the method of Tucker (1967). The chamber used was larger (3 ml) than that described by Tucker and was used in conjunction with a Radiometer type E5046 oxygen electrode. The volume of the chamber varied depending on how deep the electrode was inserted, and was measured daily. All oxygen content determinations were done at 32° C as described by Tucker except that the blood volumes were larger (50 µl for venous and 25 µl for arterial blood) because the chamber was larger. The blood samples were pipetted into the chamber within 30 sec of sampling. Arterial and venous samples were done serially 5-10 min apart.

(i) Hematocrit

The blood remaining in the micro buret after the oxygen content determination was transferred into three 20 μ l micro pipettes sealed with Seal-ease (Clay Adams). The excess length of pipette was then cut off, the samples were spun in a commercial micro hematocrit centrifuge and measured. After hematocrit determination the samples were labelled and frozen for blood iron determination.

(j) Lactate determination

Lactate determinations were done by a co-worker (W. Driedzic) in the following manner: the sample (see under blood sampling) was centrifuged to remove protein and the supernatant was neutralized with 3 M K_2CO_3 containing 0.5 M triethanolamine. KClO₄ was removed by centrifugation and an aliquot of the supernatant was analyzed for lactate enzymatically (Sigma bulletin #826). Assays were carried out on a Unicam SP 1800 dual beam spectrophotometer connected to a strip chart recorder.

(k) Blood iron concentration

Micropipettes containing the 20 μ l blood samples saved (frozen) from hematocrit determinations were rinsed on the outside and the pipettes broken up inside clean borosilicate glass scintillation vials. After an overnight drying period at 80°C the vials and contents were placed in a muffle furnace and ashed at 680°C for a minimum of 8 hours or until only a white powder remained in the pipette sections in the vials. Upon cooling to room temperature the vials were re-labelled and the contents dissolved in 0.2 N HC1. Recovery of sample was checked by an identical treatment of standard solutions in pipettes, and found to be 100%. The samples were analyzed on a Tectron model AA120 atomic absorption spectrophotometer using a wavelength of 248.3 mm from a Varian FeCo hollow cathode lamp against standards ranging from 0 to 10 mg Fe/1. The standard was made by dissolving 0.1 g of Iron Powder (99+ Fisher chemicals) in a minimum quantity of Analar HC1 and diluting in 0.2 N HC1 to the appropriate concentrations.

(1) Ventilation volume

Ventilation volume can be determined by measuring the oxygen tension of the inhalent and exhalent water and the oxygen consumption of a fish. From the

above information and the solubility coefficient of oxygen in water the ventilation volume can be calculated by the Fick equation:

$$V_g = VO_2 / (P_1O_2 - P_EO_2)\alpha$$

where

 \dot{v}_2 = oxygen consumption (ml $0_2/min$)

 $\dot{V}g$ = ventilation volume (ml H₂0/min)

 P_1O_2 = oxygen tension of inhalent water (Torr)

 $P_E O_2$ = oxygen tension of exhalent water (Torr)

 α = solubility coefficient of 0₂ in water (ml 0₂/ml H₂0 Torr)

One way to measure the oxygen tension of exhaled water is by sampling water from within the opercular cavity by means of opercular or cliethral cannulae. Both of these methods have been found to have low reliability (Davis and Watters, 1970).

The observation of Ballintijn and Hughes (1965) that the pressure in the opercular cavity is negative until the end of the opercular stroke, together with visual observations of the movement of the opercular valve, indicated that there is a backflow of water from outside into the opercular cavity when the operculum opens fully. Water sampled by an opercular or cliethral cannula would therefore be of higher oxygen tension than the water which has just come across the gills. A method was devised of attaching a rubber skirt to loosely cover the area at the opening of the operculae (described under surgical procedures), thus providing a buffer zone of exhaled water between the opercular cavity and the outside water. This is essentially a modification of the method of Davis and Cameron (1970) for use on free-swimming fish.

Davis and Watters theorized that the reason for the large variability in the oxygen tensions of water samples taken from cannulae in different locations in the opercular cavity was variations in oxygen extraction in differ-

ent regions of the gills. If such is the case the large volume (relative to opercular volume) under the skirt should act as a mixing chamber as well as a buffer for any backflow which occurs during the respiratory cycle. To determine whether water samples taken from different locations under the skirt and the same PO2, and whether the skirt imposed a load on the opercular musculature, the following procedure was used: The resting \dot{VO}_2 and \dot{VO}_2 max (\dot{VO}_2 at critical velocity) of a trout were measured before any surgical procedures were carried out (a normal trout). A skirt was then attached to the trout (as described under surgical procedures) with cannulae attached at the lateral and latero-ventral positions as well as in the ventral position. After 18-24 hours recovery the \dot{vo}_2 and oxygen tensions of exhaled water collected from the three sampling cannulae were measured at rest and at various swimming speeds up to critical velocity. The measurement of water samples taken from the cannula in different positions in two fish at rest and during swimming at various speeds showed no detectable difference (42 samples). Since there was no difference in the oxygen tension of the water samples from different positions on the first two fish a ventral cannula alone was used on the two remain-Resting VO2 of four fish before and after attaching the skirts were ing fish. not significantly different. The VO₂ max of each of these individuals was the same before and after attaching the skirt. Critical velocity (60 min) was decreased by an average of 15 cm/sec over the values obtained for the same individuals previous to installation or after removal of the skirt. This effect on critical velocity was also obtained by attaching dummy wires and cannulae (of the type used for blood sampling and ECG recording) and is presumably the effect of increased drag on the fish.

(m) Experimental procedure and data analysis

Fish were instrumented as described in Surgical Procedures and allowed to recover in the water tunnel for 18-24 hours. Figure 10 is an illustration of a fully instrumented trout. Such a fish did not exist; for instance, determinations of ventilation volume were not done on the same individuals as measurement of cardiovascular variables (except heart rate in two cases). Venous cannulation was either of the ventral aorta or the common cardinal vein, not both. Basically three types of experiments were performed:

- (1) Transients experiments
- (2) Blood and cardiovascular experiments
- (3) Ventilation experiments.

In the transients experiments only heart rate, ventilation rate and blood pressure in the dorsal aorta, ventral aorta, and common cardinal vein were monitored, along with the velocity of the water in the water tunnel. After recording the different variables at rest the water velocity was increased abruptly by about 1/6 of critical velocity and maintained at that velocity for 60 min. The variables were recorded after 0.5, 1, 3, 6, 10, 15, 30, 45, and 60 min, at which time the velocity was increased again and the measurements repeated.

The variables were measured at each test time in each trial. Results were analyzed in the following way: Heart rate for each time in each trial was expressed as the heart rate at that time divided by the heart rate immediately before the beginning of the velocity increment. In the case of blood pressure the differences between the pressure at each time and the pressure before the start of the increment were computed. Respiratory rate was treated as a rate per min for each time. Means and standard errors were then calculated for the normalized variables.

Schematic diagram of the various instrumentation carried by the experimental fish.



In the experiments investigating the blood and cardiovascular variables and venous arterial and measurements of heart rate, arterial oxygen content, venous oxygen tension, venous and arterial hematocrit, venous and arterial CO_2 contents, blood pressure, arterial and venous pH, lactate, oxygen tension of inhaled water and oxygen consumption, were attempted on each individual. In this set of experiments 89 trout were cannulated. During recovery several fish died due to internal bleeding as a result of cannulation; in some one or both cannulae became non-functional, and in others the hematocrit was too low as a result of internal bleeding. On the remaining fish as much information as possible was obtained. Oxygen consumption was determined on 25 individuals, blood pressure in 10, lactate in 7, ventilation volume in 5 and heart rate in 30. The samples of blood were taken from the resting fish (sitting quietly on the bottom of the water tunnel at a water velocity of about 10 cm/sec) and analyzed as previously described.

When all other variables had been measured the $\dot{v}0_2$ of the fish was determined. After $\dot{v}0_2$ determination at rest the water velocity in the water tunnel was increased by about 1/4 to 1/3 of the estimated critical velocity of the particular individual. The fish was checked periodically or continuously to make certain that it was swimming constantly and not tangling on the cannulae and wires throughout the test period. After swimming for 50 min of the test increment blood sampling commenced and all the variables were measured before the oxygen consumption was determined and swimming speed again increased. At maximal swimming speed when it was suspected that the fish was about to fatigue the sampling procedure was sometimes carried out before the 50 min time.

The data from these experiments were analyzed relative to critical velocity or to \dot{vo}_2 max of each individual. This treatment was necessary since

swimming speed is affected by drag, which is determined by the amount and type of instrumentation attached to the fish, whereas the critical velocity and $\dot{v}0_2$ max of an individual represent that individual's maximal aerobic capacity.

Ventilation experiments were carried out using the same test period (50 min) as the blood and cardiovascular system experiments. Exhaled water samples were taken throughout the exercise periods as were samples of inhaled water. Towards the end of each exercise period oxygen consumption was determined. Per cent utilization ($%U = P_I O_2 - P_E O_2 / P_I O_2$) and ventilation volume $(\dot{v}g = \dot{v}O_2 / (P_I O_2 - P_E O_2)\alpha)$ were calculated.

RESULTS

(1) <u>Changes in Cardiovascular and Respiratory Variables in Response</u> to <u>Incremental Speed Increase</u> (Transient Responses)

In order to determine the length of time to reach steady state during prolonged exercise in trout five variables (heart rate, ventilation rate, and blood pressure in the dorsal aorta, ventral aorta, and common cardinal vein) were monitored in 5 fish exposed to increases in swimming speed. Heart rate was found to be steady after about 15 minutes. Ventilation rate was extremely variable even at rest and the variability increased during exercise. The ventilation rate tended to increase at the beginning of an exercise period and to decline by about 15-30 minutes. Dorsal aortic and ventral aortic blood pressures were steady after about 30 min (see Fig. 11) while common cardinal blood pressure showed no change at any time. On the basis of the transient response of these variables the animals are in steady state after about 30 minutes.

Apart from defining the length of time needed to reach steady state the heart rate and blood pressure transients are worthy of detailed description. During the time that swimming speed was being increased (10 sec) bradycardia was observed, followed by tachycardia. Often several cycles of bradycardia and tachycardia were observed in the first 30 seconds of a new velocity increment. This apparent hunting disappeared after about 30 sec while the heart rate continued to increase at a somewhat slower rate than at the beginning of the increment. Heart rate reached a maximum value for that particular swimming speed at 3-15 minutes. The heart rate response described above was typical of all incremental velocity increases up to about 90% of critical

Change in dorsal aortic and ventral aortic blood pressures following an incremental increase in swimming speed. The pressure at any given time minus the pressure before the increase in swimming speed was calculated for each time; the means (points) and standard errors (horizontal bars) were then calculated for each pressure at each time. Numbers above the records represent the number of determinations.



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TIME (MIN)

velocity, above which heart rate was maximal and did not change with further increases in swimming speed. The cardiac interval at steady state was constant.

Dorsal aortic blood pressure (both systolic and diastolic) showed a transient increase (Fig. 11) following an incremental increase in swimming speed. This increase was present immediately (5 sec) after the water velocity was increased and peaked at about 6 min, then diminished. Pulse pressure in the dorsal aorta did not change significantly. Ventral aortic systolic and diastolic pressure showed the same transient response as did the dorsal pressures (Fig. 11). Common cardinal blood pressure did not change during or following increases in swimming speed.

(2) <u>Cardiovascular</u>, <u>Respiratory and Metabolic Responses to Swimming</u> <u>at Steady State</u>

Table 4 and Figures 12-14 contain the results of detailed measurements (on 6 individual trout) of cardiovascular parameters at steady state. Resting heart rate in cannulated animals tended to be higher (37.8 ±1.505 min⁻¹, n=9) than the mean for all trout studied (31.75 ±0.98 min⁻¹, n=32) (Fig. 15). Heart rate in all cases increased with swimming until a maximum heart rate was reached. Mean maximum heart rate for cannulated animals was 51.4 ±2.478 (n=4) with a mean maximum increase of 1.33X resting. CaO_2 at rest was 10.4 ±0.544 v% (n=6) and changed little during increased swimming. Mean arterial oxygen saturation was 97.0 ±1.29% (n=8) at rest and did not change with intensity of exercise (Table 4). Venous oxygen content decreased with increasing swimming activity, resulting in increased A-VO₂ differences with exercise. A-VO₂ difference at rest was 3.29 ±0.266 v% (n=8) and increased to a mean maximum of 8.30 ±0.507 v% (n=4) (an increase of 2.84X) (Fig. 13).

Speed	H.R.	Ca02	^{Cv0} 2	Pa02	Pv02	Hcta	Hctv	рНа	pHv	A-VO2	vo2	ġ	. S.V.	P102	Sa02
%Cv	min ⁻¹	v%	v%	Torr	Torr	%	%			v %	ml kg ⁻¹ min ⁻¹	ml kg ⁻¹ min ⁻¹	m1 kg1 ST1	Torr	%
rest	37.8± 1.505 n=9	10.4± 0.544 n=9	7.1± 0.715 n=9	137± 4.23 n=8	33.2± 3.056 n=8	22.6± 1.02 n=9	24.2± 1.809 n=8	7.932 +7.991 -7.879 n=5	7.959 +8.025 -7.902 n=4	3.29± 0.266 n=9	0.56± 0.025 n=9	17.6± 1.095 n=9	0.46± 0.021 n=9	152.9± 1.959 n=8	97.0 ±1.291 n=9
41- 63% Cv	42.7± 3.18 n=3	9.8± 0.737 n=3	4.4± 0.833 n=3	123.5± 7.5 n=2		22.7± 1.386 n=3	24.45± 1.05 n=2		· ·	5.4± 0.1 n=3	1.52± 0.245 n=3	28.4± 4.996 n=3	0.62± 0.079 n=3	152.0± 2.309 n=3	96.0 ±5.00 n=3
70- 78% Cv	49.0± 1.00 n=5	9.02± 0.497 n=5	3.4± 0.391 n=5	123.0± 4.203 n=4	23.5± 2.062 n=4	20.34± 1.391 n=5	21.85± 2.447 n=4	7.924 +8.046 -7.829 n=3	7.988 +8.081 -7.911 n=3	5.6± 0.585 n=5	1.9± 0.276 n=5	34.8± 4.809 n=5	0.7± 0.091 n=5	155.75± 0.947 n=4	98.75 ±1.03 n=5
81- 91% Cv	51.3± 4.667 n=3	10.2± 1.31 n=3	2.9± 1.477 n=3	128.0± 5.033 n=3	29.3± 6.173 n=3	22.5± 1.348 n=3	25.8± 0.939 n=3	7.859 +7.970 -7.770 n=2	7.883 +7.950 -7.825 n=2	7.3± 0.492 n=3	3.12± 0.379 n=3	42.9± 5.446 n=3	0.86± 0.157 n=3	146.7± 0.666 n=3	99.7 ±0.667 n=3
max. (92%- Cv)	51.4± 2.478 n=4	9.7± 0.732 n=4	1.35± 0.413 n=4	126.0± 5.431 n=4	16.0± 2.121 n=4	25.7± 0.882 n=4	27.4± 1.181 n=4	7.610 +7.620 -7.600 n=2	7.548 +7.630 -7.480 n=2	8,3± 0,5068 n=4	4.34± 0.1687 n=4	52,6± 2,160 n=4	1.03± 0.074 n=4	151.8± 2.658 n=4	98.5 ±0.866 n=4

TABLE 4. Cardiovascular and respiratory variables at rest and during exercise in trout.

Cardiac output of trout during rest and exercise. The values are single determinations normalized for weight of the fish and plotted against \dot{VO}_2 . Thirty-one determinations were done on 6 trout.



Arterio-venous oxygen differences in trout at rest and during exercise plotted against VO_2 . Thirty-one determinations were done on 6 fish.



Stroke volume of trout heart at rest and during exercise normalized for weight of the fish and plotted against VO_2 . Thirty-one determinations were done on 6 fish.



Heart rate of trout at rest and during exercise plotted against water velocity as a per cent of each individual's critical velocity. Point at less than 25% CV is for animals which were resting. The points are means of individual determinations, the horizontal bars denote one standard error, and the numbers above the points indicate the numbers of determinations on 29 animals.



 \dot{Q} (cardiac output) at rest was 17.6 ±1.095 ml kg⁻¹ min⁻¹ (n=9) and increased with swimming by 2.86X to a mean maximum of 52.6 ±2.160 ml kg⁻¹ min⁻¹ (n=4) (Fig. 12). Concomitant with the rise in \dot{Q} , stroke volume (SV) increased from a resting value of 0.46 ±0.021 ml kg⁻¹ stroke⁻¹ (n=9) by 2.12X to 1.03 ±0.074 ml kg⁻¹ stroke⁻¹ (n=4) (Fig. 14).

Oxygen consumption (\dot{VO}_2) increased exponentially with swimming speed (Fig. 16). Mean resting \dot{VO}_2 for the cannulated animals was 0.56 ±0.025 ml kg⁻¹ min⁻¹ (n=7) as compared to 0.580 ±0.010 ml kg⁻¹ min⁻¹ (n=31) for all resting \dot{VO}_2 determinations (Fig. 16). \dot{VO}_2 max for the cannulated trout was 4.34 ±0.168 (n=4) ml kg⁻¹ min⁻¹ as compared to 4.344 ±0.070 ml kg⁻¹ min⁻¹ (n=13) for all trout. The mean maximum \dot{VO}_2 increase for the cannulated trout (n=4) was 7.71X. \dot{VO}_2 max whenever determined was remarkably constant regardless of how the individual was instrumented (i.e., no instrumentation, ECG electrodes only, cannulae, opercular skirt) as can be seen from the small amount of scatter of the points in Figure 16 as 100% CV is approached. However when swimming speed rather than %CV is used as the x axis the scatter of points is very large.

Arterial oxygen tension in the six trout examined in detail was 137 \pm 4.23 (n=6) Torr at rest, whereas the mean for all PaO₂ determinations done on resting fish was 134.4 \pm 2.14 Torr (n=21). The arterial oxygen tensions did not change significantly with exercise. Venous and arterial oxygen tensions plotted against per cent saturation (as determined from oxygen content and blood iron concentrations) showed a sigmoidal <u>in vivo</u> oxygen dissociation curve (Fig. 17).

Neither arterial nor venous hematocrit showed a statistically significant change during swimming but showed a trend towards an increase in individuals

Oxygen consumption of trout expressed per kilogram animal plotted against water velocity as a per cent of each individual's critical velocity. Points at velocities of less than 25% CV are for animals at rest. One hundred and one determinations are presented on 25 individual trout.



In vivo blood oxygen dissociation curve. Per cent saturation was derived from the measured iron concentration and measured blood oxygen content. The 61 determinations were performed on 20 fish and a curve fitted by eye.



at maximal exercise levels (see Table 5 in Appendix). Arterial and venous pH tended to decrease with exercise.

Blood pressure in the ventral aorta increased with increasing intensity of exercise (Fig. 18). Mean pressure in the ventral aorta at rest was 38.8 Torr and rose to a mean of 61.7 Torr at 80-100% of critical velocity. The corresponding pulse pressure was 11.5 Torr and 26 Torr. Blood pressure in the dorsal aorta (Fig. 18) showed a smaller increase over the same speed range. Pulse pressure in the dorsal aorta at rest was 5.8 Torr and increased to 10 Torr during exercise, while mean pressure increased from 31 Torr to 37 Torr. Blood pressure in the right common cardinal vein at rest was 1.4 \pm 0.281 Torr (n=4), and increased by an insignificant amount to 1.9 \pm 0.375 Torr (n=4) at critical velocity. No increases in venous pressure were observed at intermediate speeds.

Gill ventilation volume (Vg) increased with increased oxygen consumption during swimming (Fig. 19). The mean resting Vg was 211.4 $\pm 5.81 \text{ ml kg}^{-1} \text{ min}^{-1}$ (n=5) and increased to about 1700 ml kg⁻¹ min⁻¹ at maximal VO₂. Per cent utilization remained constant at a mean value of 33.0 $\pm 0.43\%$ (n=74) during rest and swimming at speeds up to 99% of critical.

Blood lactate in trout remained about 0.5 umoles/ml during rest and swimming up to 93% of critical velocity. One minute after fatigue blood lactate had increased to 5X the resting level (Fig. 20).

(3) Recovery from Fatigue

Following fatigue blood pressure remained constant for about 2 min, then decreased gradually, reaching a resting value after about 120 min. Heart rate (Fig. 21) remained at the maximal value for 10-30 min then declined slowly to

Diastolic and systolic blood pressure in both the dorsal (DA) and ventral (VA) aortae during exercise. Horizontal bars indicate one standard error of the mean and the number above each point indicates the sample size. Horizontal axis is the swimming speed expressed as a per cent of the individual's critical velocity.



2% 2%

93

Figure 19

The relationship of ventilation volume to oxygen consumption at rest and during exercise of incrementally increased intensity up to and including maximal 60 min sustained exercise. Twentyone determinations were done on 5 trout.



Blood lactate levels of individual swimming trout at specified swimming speed and following fatigue. Multiple points at a given percentage critical velocity are representative of repeat runs on different days. All blood samples taken from dorsal aorta except one represented by 0 which is from ventral aorta. The curve is a regression line drawn through all points prior to fatigue Thirty determina-

tions were done on four fish.

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Blood Lactate (µmoles/ml)



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Heart rate during recovery from fatigue. Curve fitted by eye.


resting level over a period of 12-18 hours. Blood lactate levels increased after fatigue, reached a maximum of 6-10 µmoles/ml at 2-2:1/2 hours and declined thereafter to resting levels after 11-17 hours. Seventeen to 18 hours after fatigue heart rate, arterial and venous pH, A-VO₂ difference, cardiac output, and stroke volume had returned to pre-exercise levels.

DISCUSSION

The response of heart rate to changes in swimming speed in trout was rapid, as reported by Priede (1973) and Sutterlin (1969). There was considerable oscillation in heart rate before a new plateau was reached after an increment increase in swimming speed. This oscillation may be due to hunting in the control system or it may be due to variations in the intensity of swimming of the individuals at the beginning of an incremental increase in water velocity. Unlike the case in mammals, where heart rate rises continuously (Cobb and Johnson, 1963; Eklund, 1967; Eklund and Holmgren, 1964; Smith <u>et al.</u>, 1952) during periods of prolonged exercise (more than 10-15 min), trout reach a steady state with regards to heart rate after some 15-30 minutes.

Blood pressure did not show any evidence of hunting. Both ventral and dorsal aortic blood pressures increased smoothly, peaked, and then decreased gradually to reach a steady state after about 45 min. In mammals prolonged exercise causes a reduction in systemic blood pressure (Eklund, 1967; Erikson <u>et al.</u>, 1971; Smith <u>et al.</u>, 1952). Eklund <u>et al.</u> (1964) suggest that this change is due to increased peripheral vasodilation which results in a reduced cardiac filling pressure and resultant decrease in stroke volume. The peripheral vasodilation may be partly that of the cutaneous circulation for purposes of thermoregulation (Christensen <u>et al.</u>, 1942; Zitink and Lorenz, 1969). In fish there is no requirement for cutaneous thermoregulation by vasodilation, therefore the observed blood pressure transient must be due to other factors. The initial increase in cardiac output at the onset of an increase in exercise intensity may be in excess of the required cardiac output and a slow compensatory decrease may follow to optimize the system, or an

increase in cardiac output which is faster than decreases in peripheral resistance (in muscle) may be the cause of the pressure transient. The relationship of the variables which govern oxygen transport by the blood to oxygen consumption (\dot{VO}_2) is stated by the Fick equation:

 $\dot{VO}_2 = \dot{Q}(A-VO_2)$ and $\dot{Q} = HR \times s.v.$

As oxygen consumption increases during increased activity cardiac output can be varied by increasing either heart rate or stroke volume or both. In the present experiments heart rate reaches a maximum at about 90% of critical velocity but oxygen consumption continues to rise with increasing swimming speed until critical velocity is reached. At speeds in excess of 90% of critical velocity the increases in oxygen transport must be by increases in stroke volume and A-VO2 difference. Mean stroke volume at VO2 max was 1.03 ml kg^{-1} stroke⁻¹ and mean venous saturation was 16.5%. It is unlikely however that either of these variables is limiting VO2 max since stroke volumes as high as 1.23 ml kg⁻¹ stroke⁻¹ and venous saturations lower than 10% were observed (not in the same animal). Individuals which were not cannulated had the same \dot{v}_2 max as the cannulated individuals in spite of the fact that the former individuals had hematocrit values of 30% or greater as compared to 25% or less for cannulated individuals. This together with the high arterial oxygen saturation values observed is further evidence that \dot{v}_2 max is not set by the exchange rate or transport of oxygen to the tissue.

Studies of blood lactate levels during swimming at increasing speeds showed that blood lactate levels do not increase appreciably at swimming speeds up to 93% of critical. This is not surprising since electromyography (see Section I) shows that white muscle is not involved to any extent in swimming at speeds of less than critical velocity in this group of animals. The large

increase in blood lactate following fatigue suggests either a large washout of accumulated lactate from the white muscle following fatigue or a very rapid production of lactate immediately preceding fatigue. Since white muscle activity was always observed immediately before fatigue (Section I) and blood pressure was maintained following fatigue, it is unlikely that the sudden appearance of large quantities of lactate in the blood immediately after fatigue is the result of a large washout of lactate accumulated over a long period of time but therefore due to a large production precipitating fatigue.

During swimming at speeds less than 91% of critical velocity arterial and venous pH dropped slightly. Swimming at speeds in excess of 91% of critical velocity caused a large decrease in both arterial and venous pH. This suggests either an accumulation of CO_2 or some other acid in the blood. Measurement of whole blood and plasma CO_2 content was attempted by Cameron's method (1971) but in our hands this method did not work very well on blood sampled from fish. From those results which were obtained it does not seem likely that a net accumulation of CO_2 is the cause of the change in blood pH. Whatever the cause of the low pH it did not affect the loading of hemoglobin with oxygen since arterial blood remained 98.5% saturated at the maximal exercise level.

Since percent utilization remained constant during exercise, ventilation volume increased in proportion to oxygen consumption. Ventilation perfusion ratio $(V\dot{g}/\dot{Q})$ was 12:1 at rest and increased to 32:1 at \dot{VO}_2 max. The capacity rate ratio given by the formula $\dot{VgaWO}_2/\ll BO_2\dot{Q}$ (Hughes and Shelton, 1962), where \approx is the solubility coeff cient for O_2 in water (W) and blood (B), was 0.6 at rest and increased to 1.8 at maximal exercise. The mean gradient (ΔPG) for diffusion from one side to the other in a counter-

current exchanger such as the gills is given by the formula $\Delta PG = 1/2(P_1O_2 + P_EO_2)-1/2(P_aO_2 + P_vO_2)$ (Jones <u>et al.</u>, 1970). During exercise the mean gradient for diffusion of oxygen increased from 120 Torr at rest to 136 Torr at $\dot{v}O_2$ max. This increase in mean gradient is reflected in the change in transfer factor for oxygen: $TO_2 = \dot{v}O_2/\Delta PG$. The transfer factor for oxygen increased only 5.92X, from 0.013 at rest to 0.077 at $\dot{v}O_2$ max, while $\dot{v}O_2$ increased by 7.75X. Per cent utilization ($\%U = \frac{P_1O_2 - P_EO_2}{P_1O_2} \times 100$) remained constant (33%) of all

values of \dot{v}_2 during exercise, whereas effectiveness for oxygen removal from the water,

$$E = \frac{P_{I}O_{2} - P_{E}O_{2}}{P_{I}O_{2} - P_{V}O_{2}} \times 100$$

decreased from 42.2% at rest to 36.9% at vO_2 max as a result of a decrease in P_vO_2 . Effectiveness values of 11-30% have been obtained in other studies for oxygen removal from water by Randall <u>et al</u>. (1967), who thought that this low effectiveness was the result of the fish facing into a water stream. In view of the criticism by Davis and Watters (1970) of opercular cannulation as a method of sampling expired water and observations of backflow of water into the opercular cavity by myself, it seems more likely to attribute these low values to technical difficulties associated with water sampling. Per cent utilization as determined in my study (33%) is higher than the values obtained by opercular cannulation (10%) (Stevens, 1968), but lower than those reported for restrained fish (oral membrane method, 46%) (Cameron and Davis, 1970). The fish in the Cameron and Davis study had a low P_aO_2 (105 Torr) suggesting that they were not ventilating adequately, thus increasing the per cent utilization.

Using the data on cardiac output and mean blood pressures in the ventral

aorta, dorsal aorta, and common cardinal vein, the resistances to blood flow were calculated. The resistance to blood flow in the gills was 0.5 Torr min/ ml at rest and decreased to 0.46 Torr min/ml (0.92X) at maximal exercise. Resistance to blood flow in the systemic circulation decreased from a resting value of 1.68 Torr min/ml to 0.68 Torr min/ml (0.41X) when maximal exercise was imposed on the fish. Pulsatility, defined as pulse pressure/mean pressure, increases in both the ventral and dorsal aortae despite the increase in heart rate (1.33X) which, in the absence of other factors, should reduce diastolic run-off and hence reduce pulsatility. These anomalous increases in pulsatility cannot be explained on the basis of the observed changes in gill and systemic resistances and are most easily described by appealing to an electrical model (Fig. 22). In this model the heart is represented by an electrical generator, vascular compliances by their electrical analogue, capacitors, and the gill and systemic resistances by resistors. As frequency (heart rate) increases the impedance of the dorsal aortic capacitance (Cda) drops (since impedance varies as the inverse of frequency for a capacitor). At the same time peripheral resistance (Rs) decreases resulting in a further decrease in the impedance across which ventral aortic capacitance (Cva) discharges (parallel combination of Cda and Rs) allowing the ventral aortic capacitance to discharge more each Thus the effect of increases in heart rate on capacitive impedance diastole. overcompensates for the reduced duration of the cardiac cycle and ventral aortic pressure and flow pulsatility increase. In the dorsal aorta there is an increased inflow pulsatility resulting from the increased pulsatility in the ventral aorta. Consequently the increased pulsatility in the circulatory system during exercise is related to the effects of elevated heart rate on the fluid impedance of the ventral and dorsal aortic compliances.

Figure 22

Electrical model of fish circulatory system (Satchell, 1971).



GENERAL DISCUSSION

Steady state aerobic exercise is a state of dynamic equilibrium. Oxygen is being consumed by red muscle at a rate dependent on the amount of red muscle which is being used, which in turn is dependent on the amount of power required for that particular intensity of exercise. During exercise the respiratory and cardiovascular systems increase the uptake and transport of oxygen to the tissue to meet the increased demand. The amount of oxygen removed from the water per unit volume remains the same as ventilation volume is increased. Cardiac output increases as a result of increases in both heart rate and stroke volume and in addition, venous saturation is decreased. Aerobic exercise capacity (VO_2 max) does not appear to be limited by the ability of the respiratory and cardiovascular systems to supply oxygen at 9°C.

Different species of fish differ greatly in their sustained swimming ability. The proportion of lateral red musculature to total body weight is related to swimming ability in the species studied. Electromyographic studies on several species of teleosts showed that white muscle is not continuously active during sustained swimming, but is active during violent swimming such as that observed before the fish fatigues. After fatigue fish can still swim at a reduced speed but under no circumstances were fatigued fish observed to struggle. It therefore seems that fatigue is a result of a loss of function of the white muscle system rather than the red. Since blood supply is sparse and the white muscle fibers are large (Boddeke et al., 1959) it is unlikely that white muscle can depend on the circulatory system for supply of nutrients during periods of contraction. This is supported by the finding of Stevens and Black (1966) (in trout) that mosaic muscle glycogen is depleted during intense swimming, that

lactate accumulates at the same rate as glycogen is depleted, and that replenishing of glycogen after severe exercise is very slow. In mackerel which has a white rather than mosaic muscle mass Pritchard (1971) found a near total depletion of white muscle glycogen at fatigue. It therefore seems that fatigue of the white muscle is related to a depletion of its glycogen reserve.

The rate of appearance of lactate in the blood following fatigue may be explained on the basis of a poor blood supply to the mosaic muscle mass. A rapid initial washout of lactate as observed may be from the white muscle fibers closest to the blood capillaries followed by a slower diffusion of lactate from deeper areas.

A number of problems have come to mind during this study, one of which has been the subject of much work in mammalian exercise physiology, namely, what is the nature of the stimulus which initiates the cardiovascular and respiratory responses characteristic of muscular exercise. Asmussen and Nielsen (1964) found that bicycle ergometer exercise in humans during occlusion of muscle blood flow produced respiratory and cardiac responses equal to or greater than observed without occlusion even though there was no change in oxygen consump-They attributed their findings to a chemoreceptor located in the muscle. tion. Asmussen (1967) proposed that the initiating "work factor" in regulation of respiration was of peripheral nervous origin closely correlated with aerobic metabolism and somehow dependent on the mechanical condition of the muscle. Coote et al. (1971), working on anaesthetized and decerebrate cats in which the hindlimbs were induced to exercise (by electrical stimulation of the ventral roots L6-S1), obtained increases in systemic blood pressure and heart rate. The responses increased with increasing tension in the muscle. Abolition of muscle contraction by gallamine or section of dorsal roots abolished the

response, confirming the reflex nature of the initiator stimulus. Occlusion of the leg circulation potentiated the responses. The authors concluded that the stimulus was chemical rather than mechanical and that the receptors were the free endings of group III and IV sensory nerve fibers around the blood vessels in the muscle.

McCloskey and Mitchell (1972) found that cessation of isometric exercise during blood occlusion resulted in a small decrease in the cardiovascular and respiratory responses followed by a much larger decrease at the release of occlusion. They concluded that mechano- as well as chemoreceptors were involved. Comroe and Schmidt (1943) found that passive movement of the knee joint in anaesthetized dogs even when all the muscles were cut produces hyperpnoea. The response was totally abolished when procaine was injected into the joint. Similar observations were made by Barron and Coote (1973) but the contribution of the articular receptors to the reflex is small (1/4-1/2) compared to the response produced by electrical stimulation of the motor nerves to the muscles (as in Coote et al., 1971). Kao et al. (1967) using anaesthetized cross perfused dogs demonstrated both a neural stimulus for initiation of increases in cardiac output and ventilation volume, and a humoral one. The authors suggest the humoral factor to be CO_2 , since the effect of hypercapnia on an anaesthetized dog is similar to that of exercise of the companion dog. Neural and humoral factors were additive.

Freyschuss (1970), by paralysing arm muscles of human subjects and recording cardiovascular response during attempted handgrip exercise, found that paralysis reduced the response slightly but did not abolish it. Mediation of the cardiovascular response by the central nervous system with possible feedback from the periphery (muscles) is suggested.

Since fish have a simpler circulatory system the controls of cardiovascular responses to exercise may be more easily understood. Sutterlin (1969) observed that the initiation of tachycardia at the onset of exercise was not due to the stimulus of water flow past the fish since water flow past nonswimming fish produced an initial bradycardia. Increases in heart rate and stroke volume due to increased venous return (by muscle pumping) are ruled out on the basis of the response being too slow, the pressures required are too high (13. Torr (Sutterlin, 1969) as compared with 2 Torr observed in this study), and there is no increase in venous pressure during exercise in trout.

The possibility of receptors in the muscle initiating the cardiac and ventilatory responses to exercise has been investigated in trout. Passive movement of the body in trout, under MS222 or urethane anaesthesia produced acceleration of ventilatory rate but no change in heart rate (West, 1975, personal communication). The resting heart rates tended to be high (40-60 min) and may have masked any possible cardiac response. The ventilatory response was eventually found to be a generalized one to any disturbance, such as turning on the apparatus without any contact with the fish and still occurred after transection of the spinal cord and lateral line nerves. (West, 1975, personal communication). It is therefore doubtful whether the initiation of this response has anything to do with exercise. Another approach might be to examine changes in heart rate and blood pressure and flow during spontaneous swimming in a fish which tends to swim for only a brief period of time (such as sole and rockfish). The analysis of the time courses of the cardiovascular responses to short periods of activity (5-10 sec) may provide some information on the nature of the initiation of the exercise response.

Likewise the cause of the blood pressure transient observed at the beginning of exercise increments could be investigated by measuring blood flow in the ventral aorta as well as heart rate and blood pressures during long swimming periods. Blood pressures (dorsal aortic and venous) and blood flow could then be used to calculate peripheral resistance at different times to determine whether the observed blood pressure transient is the result of an overshoot in the increase of cardiac output followed by a slight decrease in cardiac output, or to an increase in cardiac output which leads the decrease in peripheral resistance

In summary (cardiovascular and respiratory variables in trout reach a constant level (steady state) about 30 min after an incremental increase in swimming speed. Heart rate, cardiac output, stroke volume, and A-VO₂ difference all increase with increased exercise intensity. Respiratory volume increases in proportion to oxygen consumption. The cardiovascular and respiratory systems do not appear toe limit maximal oxygen consumption, but rather the maximal oxygen consumption and maximal sustained swimming speed appear to be deterimined by the amount of red muscle in the body musculature of fish. Thus when all available red muscle is recruited during swimming no further increase in power output can be accomplished without recruitment of white muscle fibers. The use of white muscle fibers results in a depletion of their glycogen reserves and an accomulation of lactate, one or both of which result in fatigue of the white muscle.

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LITERATURE CITED

- Asmussen, E. 1965. Muscular exercise. In: Handbook of Physiology. Respiration. Washington, D. C. Am. Physiol. Soc., sect. 3, vol. II, chapt. 36, 939-978.
- Asmussen, E. 1967. Exercise and the regulation of ventilation. Suppl. I. Circ. Res. 20, 21: 1-132 - 1-145.
- Asmussen, E. & Nielsen, M. 1964. Experiments on nervous factors controlling respiration and circulation during exercise, employing blocking of the blood flow. Acta Physiol. Scand. 60: 103-111.
- Bainbridge, R. 1960. Speed and stamina in three fish. J. Exp. Biol. 37: 129-153.
- Bainbridge, R. 1962. Training, speed and stamina in trout. J. Exp. Biol. 39: 537-555.
- Ballintijn, C. M. & Hughes, G. M. 1965. Respiratory pumps in trout. J. Exp. Biol. 43: 349-362.
- Barets, A. 1961. Contribution a l'etude des systemes moteurs "lent" et "rapide" du muscle lateral des Teleosteens. Archs. Anat. microsc. Morph. exp. 51: 92-187.
- Barron, W. & Coote, J. H. 1973. The contribution of articular receptors to cardiovascular reflexes elicited by passive limb movement. J. Physiol. 235(2): 423-436.
- Beamish, F. W. H. 1970. Oxygen consumption of largemouth bass, <u>Micropterus</u> <u>salmoides</u>, in relation to swimming speed and temperature. Can. J. Zool. 48: 1221-1228.
- Bevegård, B. S. & Shepherd, J. T. 1967. Regulation of the circulation during exercise in man. Physiol. Rev. 47: 178-213.
- Berger, M., Hart, J. S. & Roy, O. Z. 1970. Respiration, oxygen consumption and heart rate in some birds during rest and flight. Z. vergl. Physiologie 66: 201-214.
- Blaxter, J. H. S. & Dickson, W. 1959. Observations on the swimming speeds of fish. J. Cons. Expl. Mer. 24: 472-479.
- Boddeke, R., Slijper, E. J. & Van der Stelt, A. 1959. Histological characteristics of the body musculature of fishes in connection with their mode of life. Proc. K. ned. Akad. Wet. Ser. C 62: 576-588.
- Bone, Q. 1966. On the function of the two types of myotomal muscle fibre in elasmobranch fish. J. mar. biol. Ass. U.K. 46: 321-349.

- Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can. 21(5): 1183-1226.
- Brett, J. R. & Sutherland, D. B. 1965. Respiratory metabolism of pumpkinseed (Lepomis gibbosus) in relation to swimming speed. J. Fish. Res. Bd. Can. 22: 405-409.
- Brett, J. R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (<u>Oncorhynchus nerka</u>). J. Fish. Res. Bd. Can. 22: 1491-1501.
- Brett, J. R. 1967. Swimming performance of sockeye salmon (<u>Oncorhynchus</u> <u>nerka</u>) in relation to fatigue time and temperature. J. Fish. Res. Bd. Can. 24: 1731-1741.
- Butler, P. J., Jones, D. R. & West, N. H. 1975. Cardiovascular and respiratory responses to exercise in pigeons. In preparation.
- Cameron, J. N. 1971. Oxygen dissociation characteristics of the blood of the rainbow trout, <u>Salmo gairdneri</u>. Comp. Biochem. Physiol. 38: 699-704.
- Cameron, J. N. & Davis, J. C. 1970. Gas exchange in rainbow trout (Salmo gairdneri) with varying blood oxygen capacity. J. Fish, Res. Bd. Can. 27: 1069-1085.
- Christensen, E. H., Nielsen, M. & Hannisdahl. 1942. Investigations of the circulation in the skin at the beginning of muscular work. Acta Physiol. Scand. 4: 162-170.
- Cobb, L. A. & Johnson, W. P. 1963. Hemodynamic relationships of anaerobic metabolism and plasma free fatty acids during prolonged, strenuous exercise in trained and untrained subjects. J. Clin. Invest. 42: 800-810.
- Comroe, J. H. & Schmidt, C. F. 1943. Reflexes from the limbs as a factor in the hyperphoea of muscular exercise. Am. J. Physiol. 138: 536-547.
- Coote, J. H., Hilton, S. M. & Perez-Gonzalez, J. F. 1971. The reflex nature of the pressor response to muscular exercise. J. Physiol. 215: 789-804.
- Davis, J. C. & Cameron, J. N. 1971. Water flow and gas exchange at the gills of rainbow trout, <u>Salmo gairdneri</u>. J. Exp. Biol. 54: 1-18.
- Davis, J. C. & Watters, K. 1970. Evaluation of opercular catheterization as a method for sampling water expired by fish. J. Fish. Res. Bd. Can. 27: 1627-1635.
- Dawson, W. R. & Hudson, J. W. 1970. Birds. In: Comparative Physiology of Thermoregulation, edited by G. C. Whittow. New York and London. Academic Press.

- Ekelund, L.-G. 1967. Circulatory and respiratory adaptation during prolonged exercise. Acta Physiol. Scand. 70, Suppl. 292.
- Ekelund, L.-G. & Holmgren, A. 1964. Circulatory and respiratory adaptation during long-term, non-steady state exercise, in the sitting position. Acta Physiol. Scand. 62: 240-255.
- Eriksson, B. O., Grimby, G. & Saltin, B. 1971. Cardiac output and arterial blood gases during exercise in pubertal boys. J. Appl. Physiol. 31: 348-352.
- Falls, H. B. 1968. Exercise physiology. Ed. H. B. Falls. Academic Press, New York.
- Farmer, G. J. & Beamish, F. W. H. 1969. Oxygen consumption of <u>Tilapia</u> <u>nilotica</u> in relation to swimming speed and salinity. J. Fish. Res. Bd. Can. 26: 2807-2821.
- Fry, F. E. J. & Cox, E. T. 1970. A relation of size to swimming speed in rainbow trout. J. Fish. Res. Bd. Can. 27: 976-978.
- George, J. C. & Berger, A. J. 1966. Avian Myology. Academic Press, New York and London.
- George, J. C. & Naik, R. M. 1958. Relative distribution and chemical nature of the fuel stores of two types of fibers in the pectoralis major muscle of the pigeon. Nature 181: 709.
- Gray, J. 1953. The locomotion of fishes. In: Essays in Marine Biology, 1-16. Edinburgh: Oliver and Boyd.
- Hart, J. S. & Roy, O. Z. 1966. Respiratory and cardiac responses to flight in pigeons. Physiol. Zool. 39: 291-306.
- Hess, A. 1970. Vertebrate slow muscle fibers. Physiol. Rev. 50: 40-62.
- Herrick, J. F., Grindlay, J. H., Baldes, E. J. & Mann, F. C. 1940. Effect of exercise on the blood flow in the superior mesenteric, renal and common iliac arteries. Am. J. Physiol. 128: 338-344.
- Holloszy, J. O. 1967. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. Biol. Chem. 242(9): 2278-2282.
- Hudson, R. C. L. 1969. Polyneuronal innervation of the fast muscles of the marine teleost <u>Cottus scorpius</u>, L. J. exp. Biol. 50: 47-67.
- Hudson, R. C. L. 1973. On the function of the white muscles in teleosts at intermediate swimming speeds. J. Exp. Biol. 58: 509-522.

- Hughes, G. M. & Shelton, G. 1962. Respiratory mechanisms and their nervous control in fish. Adv. comp. Physiol. Biochem. 1: 275-364.
- Hultman, E. 1967. Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. Scand. J. Clin. Lab. Invest. 19, Suppl. 94: 1-63.
- Jansen, J., Andersen, P. & Jansen, J. K. S. 1963. On the structure and innervation of the parietal muscle of the hagfish (Myxine glutinosa). Acta Morphol. Neerl. Scand. 5: 329-338.
- Jones, D. R. 1971. The effect of hypoxia and anaemia on the swimming performance of rainbow trout (Salmo gairdneri). J. Exp. Biol. 55: 541-551.
- Jones, D. R., Randall, D. J. & Jarman, G. M. 1970. A graphical analysis of oxygen transfer in fish. Resp. Physiol. 10: 285-298.
- Kao, F. F., Lahiri, S., Wong, C. & Mei, S. S. 1967. Ventilation and cardiac output in exercise: interaction of chemical and work stimuli. Suppl. I Circ. Res. 20, 21: 1-179 - 1-191.
- Kausch, H. 1968. Der Einfluss der Spontanaktivitat auf die Stoffechselrate junger Korpfen (Cyprinus carpio, L.) im Hangen and bei Fatterung. Arch. Hydrolbiol. Suppl. 33: 263-330.
- Lacroix, E. & Leusen, I. 1966. Splanchnic hemodynamics during induced muscular exercise in the anesthetized dog. Arch. Intern. Physiol. Biochem. 74: 235-250.
- Lasiewski, R. C. 1960. Oxygen consumption of torpid, resting, active, and flying hummingbirds. Physiol. Zool. 36: 122-140.
- Love, R. M. 1970. Chemical Biology of Fishes. Academic Press, New York.
- McCloskey, D. I. & Mitchell, J. H. 1972. Reflex cardiovascular and respiratory responses originating in exercising muscle. J. Physiol. 224: 173-186.
- McDonald, D. A. 1974. Blood Flow in Arteries. Williams & Wilkins, Baltimore.
- Millard, F. W., Higgens, C. B., Franklin, D. & Vatner, S. F. 1972. Regulation of the renal circulation during severe exercise in normal dogs and dogs with experimental heart failure. Circulation Res. 31: 881-888.

Pearson, O. P. 1950. The metabolism of hummingbirds. Condor 52(4): 145-152.

Paulik, G. J. & Delacy, A. C. 1957. Swimming abilities of upstream migrant silver salmon, sockeye salmon and steelhead at several water velocities. Univ. Wash. Sch. Fish. Tech. Rep. 44: 1-40.

Pearson, O. P. 1950. The metabolism of hummingbirds. Condor 52(4): 145-152. Pope, A. & Harper, J. J. 1966. Low-speed Wind Tunnel Testing. New York: Wiley.

- Priede, I. G. 1974. The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. J. Exp. Biol. 60: 305-319.
- Pritchard, A. W., Hunter, J. R. & Lasker, R. 1971. The relation between exercise and biochemical changes in red and white muscle and liver in jack mackerel, <u>Trachural symmetricus</u>. Fish. Bull. (Wash.) 69: 379-386.
- Randall, D. J., Holeton, G. F. & Stevens, E. D. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol. 6: 339-348.
- Rao, G. M. M. 1968. Oxygen consumption of rainbow trout (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool. 46: 781-786.
- Regnard, P. 1893. Sur un dispositif qui permet de mesurer la vitesse de translation d'un poisson se mouvant dans l'eau. C. R. Soc. Biol., Paris, Ser. 9, 5: 81-83.
- Rowell, L. B. 1974. Human cardiovascular adjustments to exercise and thermal stress. Physiol. Rev. 54: 75-159.
- Royner, M. D. & Keenan, M. J. 1967. Role of red and white muscles in the swimming of the skipjack tuna. Nature 214: 392-393.
- Rushmer, R. F., Franklin, D. L., Van Citters, R. L. & Smith, O. A. 1961. Changes in peripheral blood flow distribution in healthy dogs. Circulation Res. 9: 675-687.
- Saltin, B. & Stenberg, J. 1964. Circulatory response to prolonged severe exercise. J. Appl. Physiol. 19: 833-838.
- Satchell, G. H. 1971. Circulation in Fishes. Cambridge: Cambridge Univ. Press.
- Saunders, R. L. 1961. The irrigation of gills in fishes, I. Can. J. Zool. 39: 637-653.
- Smith, J. H., Robinson, S. & Pearcy, M. Renal responses to exercise, heat and dehydration. J. Appl. Physiol. 4: 659-665.
- Smith, L. S. & Bell, G. R. 1964. A technique for prolonged blood sampling in free-swimming salmon. J. Fish. Res. Bd. Can. 21: 711-717.
- Smith, L. S., Brett, J. R. & Davis, J. C. 1967. Cardiovascular dynamics in swimming adult sockeye salmon. J. Fish. Res. Bd. Can. 24: 1775-1790.
- Stanfield, P. R. 1972. Electrical properties of white and red muscle fibers of the elasmobranch fish, <u>Scyliorhinus caricula</u>. J. Physiol., Lond. 222: 161-186.
- Stevens, D. E. 1968. Cardiovascular dynamics during swimming in fish, particularly rainbow trout (Salmo gairdneri). Ph.D. Thesis, Univ. of British Columbia, Vancouver, B. C.

- Stevens, E. D. & Black, E. C. 1966. The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, <u>Salmo gairdneri</u>. J. Fish. Res. Bd. Can. 23: 471-485.
- Sutterlin, A. M. 1969. Effects of exercise on cardiac and ventilation frequency in three species of freshwater teleosts. Physiol. Zool. 42: 36-52.
- Taylor, C. R. 1974. Exercise and thermoregulation. In: Environmental Physiology. Vol. 7, edited by D. Robertshaw. University Park Press, Baltimore.
- Taylor, C. R., Schmidt-Nielsen, K. & Raab, J. L. 1970. Scaling of energetic cost of running to body size in mammals. Am. J. Physiol. 219: 1104-1107.
- Tucker, V. A. 1967. Method for oxygen content and dissociation curves on microliter blood samples. J. Appl. Physiol. 23: 410-414.
- Tucker, V. A. 1968. Respiratory exchange and evaporative water loss in the flying budgerigar. J. Exp. Biol. 48: 55-66.
- Tucker, V. A. 1970. Energetic cost of locomotion in animals. Comp. Biochem. Physiol. 34: 841-846.
- Van Citters, R. L. & Franklin, D. L. Cardiovascular performance of Alaska sled dogs during exercise. Circulation Res. 24: 33-42. 1969.
- Vatner, S. F., Higgins, C. B., White, S., Patrick, T. & Franklin, D. 1971. The peripheral vascular response to severe exercise in untethered dogs before and after complete heart block. J. Clin. Invest. 50: 1950-1960.
- Webb, P. W. 1970. Some aspects of the energetics of swimming fish with special reference to the cruising performance of rainbow trout. Ph.D. Thesis, University of Bristol.
- Webb, P. W. 1971. The swimming energetics of trout. I. Thrust and power output at cruising speeds. J. Exp. Biol. 55: 489-520.
- Wittenberg, J. B. 1970. Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle. Physiol. Rev. 50: 559-636.
- Wolf, K. 1963. Physiological salines for freshwater teleosts. Progve. Fish. Cult. 25: 135-140.
- Zitnik, R. S. & Lorenz, R. R. 1969. Sensitivity of methods for detection of active changes in venous wall tension. Am. J. Cardiol. 24: 220-223.

APPENDIX

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TABLE 5. Cardiovascular and respiratory variables at rest and during exercise and after eighteen hours recovery in individual trout.

Fish	Trial	Speed	H.R.	CaO_2	Cv02	A-VO ₂	vo2	ġ	s.v.	Hcta	Sv0 ₂	Pv0 ₂	Pa0 ₂	Sa0 ₂
		%Cv	min ⁻¹	v%	v%	٧%	ml kg ⁻¹ min ⁻¹	ml kg min ⁻¹	ml kg ⁻¹ ST ⁻¹	%	%	Torr	Torr	%
Т32	1	15	37	8.4	4.2*	4.2	.68	16.2	.44	18	⁵⁰ (1)	23	138	
		41	39	8.4	2.8*	5.6	1.10	19.6	.50	20	³³ (1)	22	137	
		70	48	8.35	2.55*	5.8	2.05	35.3	• • 80	19	³¹ (1)	17	136	
т70	1	18	44	9.95	6.76	3.19	.57	17.9	.41	22.5	68	35	144	99
		78	52	7.96	2.89	5.08	2.26	44.5	.86	18	36	24	114	99
		98	53	9.15	.20	8.95	4.30	48	.91	23.5	[.] 2	11	133	97

* venous blood from ventral aorta

(1) per cent saturation calculated assuming that ${\rm CaO}_2$ is 100% (no iron content done on blood of this individual).

				•		Tab1	.e 5 (con	t'd)						
Fish	Trial	Speed	H.R.	^{Ca0} 2	Cv02	A-VO ₂	vo2	ġ	S.V.	Hcta	Sv02	Pv02	Pa0 ₂	Sa0 ₂
		%Cv	min ⁻¹	v %	٧%	v%	ml kg ⁻¹ min ⁻¹	ml kg ⁻¹ min ⁻¹	ml kg ⁻¹ ST ⁻¹	%	%	Torr	Torr	%
T65	1	16	38	12.1	9.2	2.9	.51	17.6	•46	25	81	39	140	99
		16	38	12.3	10.2	2.1	.48	22.9	.60	23.7	81	43	138	99
		16	38	12.3	9.8	2.5	.50	20	.51	24	81	40	142	101
		61	40	10.9	5.6	5.3	1.95	36.9	.77	23.7	47	37	131	91
120		81	50	12.1	5.6	6.5	3,35	51.5	1.03	25	46	35	124	101
т65	2	16	38	8	5.8	2.2	.56	25.5	.67	18.5	64	37	120	89
		81	44	10.9	2.7	8.2	3.63	44.3	1.01	20,4	25	35	122	99
		100	44	10.7	1.9	8.8	4.74	53.9	1.23	26.5	18	20	123	97
		16	44	3.4	1.6	1.8	.60	33.3	.76	7.3	53	19	134	103
T71	1	17	35	9.9	6.8	3.1	.51	16.5	.47	25	68	20	139	99
		74	49	10.1	4.2	5.9	2.38	40.3	.82	24,3	42	18	134	101
		98	54	10.0	1.3	8.7	4.40	50,6	.93	25,2	13	14	136	101
		17	36	8.8	6.0	2,8	.49	17.5	.49	20.1	68	21	138	100

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Table 5 (cont'd)

Table 5 (cont'd)

Fish	Trial	Speed	H.R.	CaO_2	Cv02	A-VO ₂	vo2	ģ	S.V.	Hcta	Sv02	Pv0 ₂	$Pa0_2$	Sa0 ₂
		%Cv	-1 min	v %	v %	v %	ml kg ⁻¹ min ⁻¹	ml kg min ⁻¹	¹ ml kg ⁻¹ ST ⁻¹	%	%	Torr	Torr	%
т69	1	17	37	9.9	6	3.9	.63	16.2	.43	25.2	60	21	150	99
•		63	49	10.1	4.8	5,3	1.52	28.7	.59	24.5	48		116	101
		101	55	8.8	2.0	6.8	3,92	58	1.05	27.6	23	19	112	100
т69	2	17	55	8.75	3.58	5,17	.59	11.4	.21	17.1	41	21	140	99
		91	60	7.76	.50	7,26	2,38	32.8	,55	22	6	17	138	99
		17	55	5.57	1.99	3,58	•58	16.2	.29	10,6	. 36		131	100
т72	1	19	. 29	11.14	6.57	4.57	.51	11.2	. 39	23	59	32.5	110	93
		70	46	10.35	2.88	7.47	1.24	16.6	. 36	23	43	28	124	96
т72	2	19	44	7.96	4.77	3.19	.64	20.1	.46	17	57	36	133	95
		73	50	8.36	4.48	3.88	1.45	37.4	.75	17.4	54	24	120	99
		108	55	6.76				1990 1993 June 1996.		16.5	— —			
		19	34	7.40	1.89	5.51	.43	7.8	.23	15.0	26	22	107	100