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THE EFFECT OF EXERCISE ON THE CARDIAC OUTPUT AND BLOOD FLOW DISTRIBUTION OF THE LARGESCALE SUCKER *CATOSTOMUS MACROCHEILUS*

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Summary

Cardiac output ($\dot{Q}$) and blood flow distribution were measured in adult largescale suckers at rest and while swimming. Cardiac output was directly measured using an ultrasonic flowprobe in fish during the summer (16˚C), fall (10˚C) and winter (5˚C). Largescale suckers were adept at holding station against a current without swimming and, when engaged in this behavior, they did not significantly increase $\dot{Q}$ relative to that found in fish in still water. When fish began to swim, $\dot{Q}$ increased significantly. From 16 to 10˚C, the critical swimming speed (U_{crit}), maximum $\dot{Q}$ and scope for $\dot{Q}$ of the suckers did not change. However, from 10 to 5˚C all three traits were significantly reduced. Thus, these fish respond to variation in water temperature in two different ways. From 16 to 10˚C, the fish compensate perfectly for the change in temperature with respect to cardiac and swimming performance. From 10 to 5˚C, however, largescale suckers experience a dramatic decline in cardiac and swimming performance that may be associated with a quiescent overwintering strategy.

Blood flow distribution in the fish at rest and while swimming was measured at 16˚C using injection of colored microspheres. In the resting fish, over 10% of the microspheres were recovered from the kidney and over 43% were recovered from white muscle. When the fish were swimming, there was a 60-fold increase in blood flow to the red muscle while blood flow to all other tissues remained consistent with that at rest.

Introduction

Over the past 20 years, a relatively large number of studies have measured cardiac output ($\dot{Q}$) in teleost fishes (Farrell and Jones, 1992). However, most of these studies only determined $\dot{Q}$ on resting fish at one water temperature. Six studies have quantified the increase in $\dot{Q}$ with prolonged exercise (Stevens and Randall, 1967; Kiceniuk and Jones, 1977; Axelsson and Nilsson, 1986; Davie and Forster, 1980; Axelsson, 1988; Axelsson *et al.* 1989). The reported change in $\dot{Q}$ varies from a threefold increase in the rainbow trout *Oncorhynchus mykiss* (Kiceniuk and Jones, 1977) to a non-significant increase in

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the eel *Anguilla australis* (Davie and Forster, 1980). The effects of temperature on $\dot{Q}$ have been directly measured in two studies. The Q_{10} for resting $\dot{Q}$ was found to be 2.60 for rainbow trout (Barron *et al.* 1987) and 2.70 for the winter flounder *Pseudopleuronectes americanus* (Cech *et al.* 1976). Although the effects of water temperature and exercise on $\dot{Q}$ have been studied to some degree independently, little is known about how these factors interact. There is no information on maximum $\dot{Q}$ as a function of temperature in swimming fishes. Thus, one objective of our study was to determine the $\dot{Q}$ of largescale suckers (*Catostomus macrocheilus*) at rest and while swimming during the summer (16°C), fall (10°C) and winter (5°C).

A second objective of this study was to determine whether the mechanism for altering $\dot{Q}$ in exercising largescale suckers changes seasonally. Exercise-induced increases in $\dot{Q}$ are associated with increases in heart rate (f_H) and stroke volume (V_S). Although it has been suggested that the exercise-induced increase in $\dot{Q}$ is most often the result of a larger increase in V_S than in f_H in fish (Farrell and Jones, 1992), it is possible that the relative contribution of f_H and V_S to increasing $\dot{Q}$ may change seasonally.

A third objective of our study was to determine the distribution of blood flow in largescale suckers at rest and while swimming using a colored microsphere technique previously used with success on mammals (Kowallik *et al.* 1991). Distribution of blood flow in fish has previously been measured using radiolabelled microspheres in resting fish (*Thymalus arcticus*, arctic grayling, Cameron, 1974; rainbow trout, Barron *et al.* 1987; *Thunnus alalunga*, albacore tuna, White *et al.* 1988) and in swimming rainbow trout (Randall and Daxboeck, 1982; Neumann *et al.* 1983). The largescale sucker is not as strong a swimmer as the rainbow trout, and we were interested in comparing the blood flow distribution in these two species at rest and while swimming.

Materials and methods

Care and handling of the largescale suckers

Adult largescale suckers were collected from Cultus Lake, British Columbia, during May 1991 and 1992. The fish were transported to the laboratory, where they were held outdoors in fiberglass aquaria that held 2000l of dechlorinated water from the municipal water supply. Water exchange was approximately $5\,l\,min^{-1}$. Water temperature in the holding tank was allowed to vary seasonally in a manner consistent with that found in Cultus Lake (i.e. a summer maximum of 16°C and a winter minimum of 5°C). Fish rapidly resumed feeding during captivity and were fed commercial fish pellets three times per week.

Largescale suckers proved to be very docile and responded well to handling. Three different groups of fish were used in this study: intact fish, fish with a cannulated efferent branchial artery and fish with an ultrasonic flowprobe fitted around their ventral aorta. Each group of fish was treated differently. Intact fish used to determine critical swimming speed (U_{crit}) were removed from the holding tank, placed in the swim chamber and given 1.5h to habituate to a water velocity of $20\,cm\,s^{-1}$ before the swimming challenge. This habituation period has been used in other studies (e.g. Kolok, 1991; Griffiths and Alderdice, 1972) and it has been documented that the U_{crit} of fish given 1.5h to habituate to the swim chamber did not differ from that found in fish given a habituation period of 24 h.

The fish fitted with a cannula were given at least 4h to recover from surgery, while the fish fitted with an ultrasonic flowprobe were given at least 18h to recover. A 4h post-surgical period was used for the cannulated fish because: (1) the surgery was relatively minor and fast, (2) cannulated fish left for 4h could swim at water velocities essentially equal to the U_{crit} of intact fish, and (3) initial attempts to collect data on fish left to recover for 18h were unsuccessful. An 18h recovery period was used for the fish fitted with an ultrasonic flowprobe because this surgery was more time-consuming and invasive than the cannulation. To minimize unnecessary handling of the fish, all fish that underwent surgery and were to be challenged with a bout of swimming were allowed to recover in the swim chamber. During the recovery period, the water in the swim chamber was aerated and exchanged at a rate of approximately $0.5\,l\,min^{-1}$.

Procedures for measuring U_{crit}

U_{crit} is a measure of the prolonged swimming performance of a fish. U_{crit} for intact largescale suckers was determined during the summer and winter of 1991 and the spring of 1992 using a Brett-type swim chamber identical to that described by Gerhke *et al.* (1990). The water temperature during the swimming trials was the same as that in the holding tank (i.e. summer 16˚C, winter 5˚C and spring 10˚C).

The step-test protocol used to measure U_{crit} was similar to that of Kolok (1991). An intact fish was placed into the swim chamber and given 1.5h to habituate to a water velocity of $20cm\,s^{-1}$. At the end of the habituation period, the fish was subjected to a swimming challenge in which the water velocity was increased by $5cm\,s^{-1}$ every 20min until it fatigued. Fish used in this study were approximately 40cm in length, so the velocity increment used was approximately $0.10 body lengths\,s^{-1}$ ($BL\,s^{-1}$). A fish was judged to be fatigued when it could no longer be persuaded, by mild electrical stimulation, to swim off the grid at the rear of the swim chamber.

Procedures for measuring $\dot{Q}$

A Transonic ultrasonic flowprobe (Drost, 1978) was surgically implanted around the ventral aorta of the fish to measure $\dot{Q}$. The probe used in this study was 9mm long with a square sensing window $14.8mm^2$ in cross-sectional area and with a T-type cable connection. Prior to surgery, the fish was anesthetized with 2-phenoxyethanol (3:5000 v/v) until breathing stopped. The fish was then placed on a surgical table and its gills were irrigated with a 3:10000 solution of the anesthetic. An incision was made into the ventral midline of the fish immediately posterior to the operculum, to expose the ventral aorta. An ultrasonic flowprobe was loosely fitted around the ventral aorta, and the incision was closed using 3-4 silk sutures. The cable of the flow probe was sutured to the back of the animal near the anterior edge of the dorsal fin. The fish was then placed into the swim chamber, after which it quickly recovered (5–10min) from the anesthesia. The animals were given 18h to recover in still water from the effects of surgery before the experimental protocol was initiated.

The ultrasonic flowprobe proved to be very well suited for measuring the flow in the ventral aorta of suckers. The ventral aorta was located 5–10mm dorsal to the ventral surface of the fish and was surrounded by white muscle. When the muscle was retracted, a

small cavity formed around the ventral aorta, which was subsequently filled when the probe was fitted to the vessel. The ventral aorta never completely filled the cross-sectional area of the probe's sensing window, so electrically conductive gel was carefully squeezed into the air space between the probe and the vessel. Once the gel was in place, the signal from the probe became very strong and stable.

When the incision was sutured closed, the muscle lateral to the ventral aorta was drawn against the sides of the probe, rendering it essentially immobile. Occasionally, during this process, the flow probe twisted and the signal from the probe deteriorated. This was easily remedied by removing the sutures, repositioning the probe and completing the surgery. The signal from the probe was monitored while the fish recovered from surgery and immediately before the experiment on the next day. Abnormal flow signals were invariably the result of a change in the position of the probe, and these were excluded from analysis. The flow probe was never found to have shifted orientation in those fish that successfully completed the experiment.

Prior to purchase, the ultrasonic flowprobe was bench-calibrated by the manufacturer using blood at 10°C. These estimates of flow were sensitive to changes in temperature, and measures were taken to correct for this potential bias. In addition, preliminary data at each water temperature verified that a zero reading from the flowmeter corresponded with zero flow past the probe when it was fitted around the ventral aorta.

Experimental protocol for measuring $\dot{Q}$

Measurements of $\dot{Q}$ were made during September (16°C), November (10°C) and February (5°C) using six different fish on each occasion. The protocol was initiated by determining $\dot{Q}$ while the fish was resting in still water. The water flow through the swim chamber was then increased to 20cm s^{-1} and the fish was given 1.5h to habituate to the water flow before $\dot{Q}$ was remeasured. The water flow was incrementally increased in the same manner as for the U_{crit} test. At low water velocities, the fish did not swim and $\dot{Q}$ and heart rate (f_H) were recorded only during the final 2min of each 20min interval. When the fish began to swim, measurements were made continuously, with only the last 2min of each interval being used to quantify f_H and $\dot{Q}$. Maximum $\dot{Q}$ was defined as the highest $\dot{Q}$ value of the fish at any time during the swimming trial. In all fish tested, the f_H and $\dot{Q}$ of the continuously swimming fish was near maximum, with the maximum values usually occurring at some point within the last 5min of the swimming challenge. Stroke volume (V_S) was calculated from the values of f_H and $\dot{Q}$.

Blood flow distribution

In a separate set of experiments, during the summer and fall of 1992 (water temperature 14–16°C), blood flow distribution in largescale suckers was determined using colored microspheres (Kowallik *et al.* 1991). The microspheres were injected into the fish *via* a cannula (PE-50 tubing) inserted occlusively and downstream in the efferent branchial artery of the fourth gill arch (Farrell, 1986). The placement of the cannula in the efferent branchial artery meant that the microspheres were introduced into the most cephalad region of the dorsal aorta.

Preliminary data suggested that the cannula had to be securely anchored to prevent slipping, so it was secured to the gill arch with two sutures and to the ventral surface of

the fish with a third suture. A fourth suture was used to attach the cannula to the dorsal surface of the fish anterior to the dorsal fin. The fourth suture was particularly important in the swimming fish to keep the cannula from becoming entangled around the undulating tail. After surgery, the fish to be sampled at rest were placed into a black acrylic aquarium, while the fish to be sampled while swimming were placed directly into the swim chamber. As stated previously, fish were given at least 4h to recover from surgery before they were injected with microspheres or subjected to a bout of swimming.

For the swimming trials, fish were subjected to an initial water velocity of 25cm s^{-1} for 1.5h, a velocity at which all of the fish could maintain their position in the chamber without swimming. The water velocity was increased by 5cm s^{-1} every 10min until the velocity reached 50cm s^{-1}. At this velocity, all of the fish were swimming. After 10min of swimming at 50cm s^{-1}, the microspheres were injected into the fish.

The microspheres were 15 μm in diameter and were purchased from Triton Technology suspended in a solution of saline and 0.01% Tween 80 to a final concentration of 3×10^6 spheres ml^{-1}. Prior to injection, this solution was vigorously vortexed for 1min. A 500 μl sample of the solution was then injected into the fish, and the fish was killed 10min after injection. Since circulation time in fish is of the order of 1–2min, this period was viewed as ample for the microspheres to circulate. Immediately prior to killing the fish, a 1ml blood sample was taken and processed for the recovery of microspheres.

After the fish had been killed, four organs (the liver, spleen, kidney and heart) were dissected out, weighed and processed for the recovery of microspheres. The gill filaments were dissected from the gill arches and weighed. All of the gill filaments from the cannulated arch and a weighed subsample of the filaments from the other arches were processed. The gill filaments on the cannulated arch were assayed for spheres to quantify the number of spheres lodging locally as opposed to flowing unimpeded into the dorsal aorta. The intestine was removed, stripped of fat and food, weighed and subsampled for processing. One sample (approximately 2g) of red muscle and three samples of white muscle (anterior, middle and posterior regions of the epaxial muscle mass) were taken from each side of the fish and processed.

Tissue samples were cut into approximately 2g pieces, weighed, then immersed into 7 ml of 4mol l^{-1} KOH and left to digest overnight at room temperature. Although it was reported by the manufacturer that colored microspheres lodged in capillaries were freezer-stable, preliminary experiments indicated that this was not the case for all tissues, especially kidney. Therefore, data were collected only from fish in which the tissues had been immersed in KOH immediately after dissection.

Generally tissue digestion was completed overnight, and the following day the solution was filtered through a 8 μm polyester filter. The filter paper was then dried and immersed into 700 μl of dimethylformamide, to release the dye from the microspheres. The dye absorbed light at 448nm, and the absorbance was linear with respect to dye concentration. A standard curve was generated for dye released from known numbers of spheres (number of spheres$\times$1000=20.48$\times$absorbance$-$0.867, r^2=0.95). The standard curve was then used to estimate the number of microspheres in each tissue based upon the absorbance value for each sample.

The number of microspheres injected into each animal was calculated from the number of microspheres in 500 μl of solution (i.e. 1.5×10^6 spheres) minus the number of spheres remaining in the syringe after the injection. Microspheres found in the blood and gill filaments were assumed to be representative of spheres that did not lodge during the first pass through the fish (see Barron *et al.* 1987) and the number of these spheres was subtracted from the total number of spheres available to be lodged in the systemic circulation. Blood volume of the fish was estimated to be 5% of body mass. Blood flow to the heart could not be estimated using microspheres since blood did not enter the coronary artery from the cannulated vessel (i.e. the dorsal aorta). The numbers of microspheres estimated to be in the gill filaments, blood and heart ventricle were always very low and never exceeded 3% of the total.

The kidney and liver received blood from arterial and portal blood vessels; it was assumed that the microspheres found in these tissues were solely from arterial sources.

To determine the blood flow to the red or white muscle, it was necessary to estimate the mass of those muscles. The total muscle mass to body mass ratio was estimated to be 0.42 from the complete dissection of muscle from six fish. The percentage of red and white muscle in the axial muscle mass was estimated from the cross-sectional area of each muscle type found in six cross sections (behind the head, pre-dorsal fin, post-dorsal fin, pre-anal fin, post-anal fin, anterior caudal peduncle and posterior caudal peduncle) cut through the bodies of five fish. The cross sections were cut and photographed, then the photographic images were projected onto a digitizing board where the cross-sectional area of the vertebrae, the white muscle and the red muscle could be easily distinguished and quantified. The cross-sectional area of red muscle occupied approximately 4–7 % (weighted average 5%) of the total muscle cross-sectional area and, from these data, the total amounts of red and white muscle mass in the fish were estimated to be 2 and 40% of the body mass of the fish, respectively.

Statistical analysis

It is unlikely that U_{crit} values from a sample of fish would be normally distributed because the measurement is not arithmetic. In other words, the interval between a U_{crit} of 2.1 and 2.2 cm s^{-1} does not represent the same distance travelled by the fish as does the interval from 3.1 to 3.2 cm s^{-1}. For this reason, analyses of these data were conducted using nonparametric statistics. Differences in the U_{crit} values of fish at different water temperatures were established using the Kruskal–Wallis test along with the nonparametric Neuman–Keuls multiple-comparisons test. Differences in the U_{crit} values between the intact and surgically altered fish were tested using a Mann–Whitney *U*-test.

Differences in the values of $\dot{Q}$, f_H and V_S at different water temperatures were analyzed using analysis of variance (ANOVA) and Sheffe's-*F* multiple-comparison test. Increases in f_H, V_S or $\dot{Q}$ due to swimming activity were established by comparing the value at rest (still water) with the value at a given water velocity using a paired *t*-test. Differences in blood flow distribution and rates of perfusion at rest and while swimming were established using *t*-tests. Probabilities to establish significant differences were set at 0.05 for all statistical procedures.

Results

The fish (N=46) used in this study were of both sexes and had a mean (±S.E.) body mass of 660±21g and a mean length (±S.E.) of 39.7±0.5cm. There were no significant differences in either the fork length or body mass of the fish used for $\dot{Q}$ and U_{crit} determinations at 5, 10 or 16°C. Similarly, there were no significant differences in fork length or body mass of the fish injected with microspheres for experiments at rest and while swimming.

Resting fish

The resting values of $\dot{Q}$, f_H and V_S for fish at 5, 10 and 16°C are given in Fig. 1. The resting f_H of the fish increased significantly from 5 to 10°C, and again from 10 to 16°C, with the Q_{10} for f_H being 1.65 from 5 to 10°C, 2.01 from 10 to 16°C and 1.84 from 5 to 16°C. Resting $\dot{Q}$ in fish at 5°C was not significantly different from that at 10°C, but was significantly reduced relative to that at 16°C. Use of a Q_{10} relationship to express the effect of water temperature on resting $\dot{Q}$ from 5 to 16°C is unwarranted considering the thermal independence of resting $\dot{Q}$ between 5 and 10°C. The Q_{10} for resting $\dot{Q}$ between 10 and 16°C, however, was 2.01. Resting V_S was not found to be significantly different in resting fish at 5, 10 or 16°C.

Swimming fish

Largescale suckers were not strong swimmers, but were adept at holding station without swimming even at relatively high water velocities. Both intact and surgically manipulated fish at 5 and 16°C held station in water velocities up to 30cm s^{-1}, whereas fish at 10°C held station in water velocities up to 40cm s^{-1}. Holding station in this species did not involve any obvious changes in fin position or posture, nor did it involve the use of the mouthparts to suck onto the bottom of the chamber.

At 10 and 16°C, when fish were no longer able to hold station, they first adopted a burst-and-glide method of swimming. During this period (roughly the 45cm s^{-1} interval at 16°C and the 35 and 40cm s^{-1} intervals at 10°C) fish slowly slid to the back of the chamber, touched the downstream retaining screen, then swam to the front of the chamber to repeat the cycle. This method of swimming usually lasted until initiation of the next swimming interval (e.g. 45cm s^{-1} at 16°C), at which time the fish began to swim continuously.

At 5°C, the swimming performance of the fish was noticeably impaired. Four of the intact fish and one surgically altered fish refused to swim when subjected to a water velocity in which they could not hold station. These fish attempted a few bursts of swimming to regain position, then would not move off the downstream retaining screen. For these fish, the velocity at which they could no longer maintain position was considered to be equivalent to U_{crit}.

Surgically implanting a flowprobe into the fish did not significantly alter U_{crit} at 5, 10 or 16°C (Fig. 2). Water temperature had a significant effect on the U_{crit} of largescale suckers regardless of whether they had undergone surgery. U_{crit} at 5°C was significantly less than that at 10 or 16°C, but there were no significant differences in the U_{crit} values of the fish at 10 and 16°C.

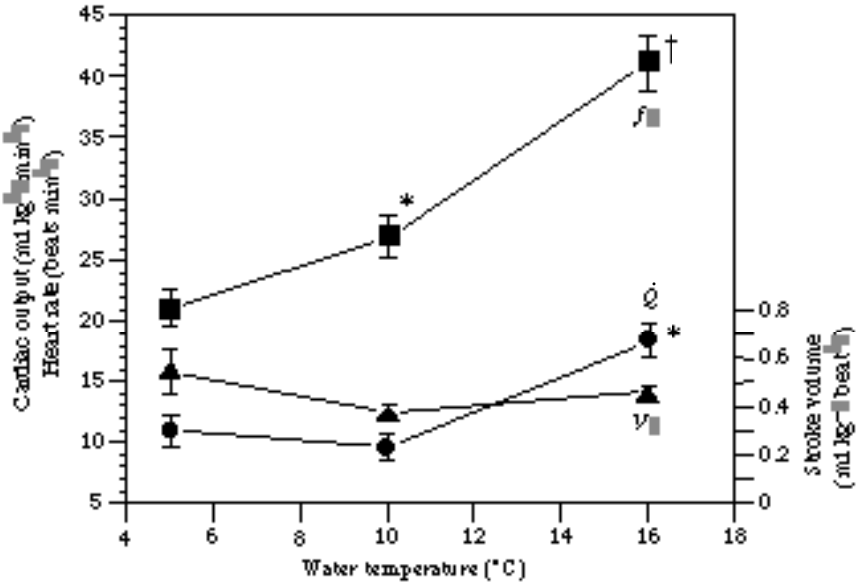

Fig. 1. The mean (±S.E.) cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_S) of six resting largescale suckers at 5, 10 and 16°C. An asterisk denotes a significant difference between the value at 5°C and that at 10°C or 16°C, whereas a dagger denotes a significant difference between the value at 10°C and that at 16°C.

The interactions between water velocity and the cardiovascular variables are illustrated in Figs 3, 4 and 5. At 5°C and 16°C, there were no significant increases in f_H, V_S or $\dot{Q}$ until the fish began to swim. However, at 10°C, f_H increased significantly when the fish held station at 25 and 30cm s^{-1} (Fig. 4). Once the fish began either continuous or burst swimming, f_H increased significantly regardless of water temperature. In all cases except fish swimming at 35cm s^{-1} at 16°C, the tachycardia was associated with a significant increase in $\dot{Q}$. Significant increases in V_S contributed to the increase in $\dot{Q}$ found in the fish swimming in a burst-and-glide fashion at 10°C, but not at 16°C. When the fish began to swim continuously, V_S increased significantly in the fish at 16 and 10°C, but not at 5°C (Figs 3, 4 and 5).

The maximum and resting values for $\dot{Q}$, f_H and V_S of fish at 5, 10 and 16°C are given in Fig. 6 to illustrate how the scope for increasing these variables changed as a function of water temperature. Maximum f_H increased significantly in a linear manner from 5 to 16°C, with a Q_{10} of 3.53 from 5 to 10°C, 2.08 from 10 to 16°C and 2.65 from 5 to 16°C. The factorial scope (maximum/rest) for f_H was 1.19 at 5°C, 1.74 at 10°C and 1.78 at 16°C. The scope for f_H (maximum minus rest) was 4beatsmin^{-1} at 5°C, 20beatsmin^{-1} at 10°C and 32beatsmin^{-1} at 16°C. The scope for f_H was significantly greater at 16°C than it was at 10°C, but this difference did not show up in the factorial scope because the resting f_H increased between 10 and 16°C (Fig. 6B).

The maximum $\dot{Q}$ of fish at 5°C was significantly less than that at 10°C (Q_{10} 2.96), but the maximum $\dot{Q}$ was unchanged between 10 and 16°C. The factorial scope for $\dot{Q}$ was 1.51 at 5°C, 3.84 at 10°C and 2.36 at 16°C. The large increase in factorial scope at 10°C

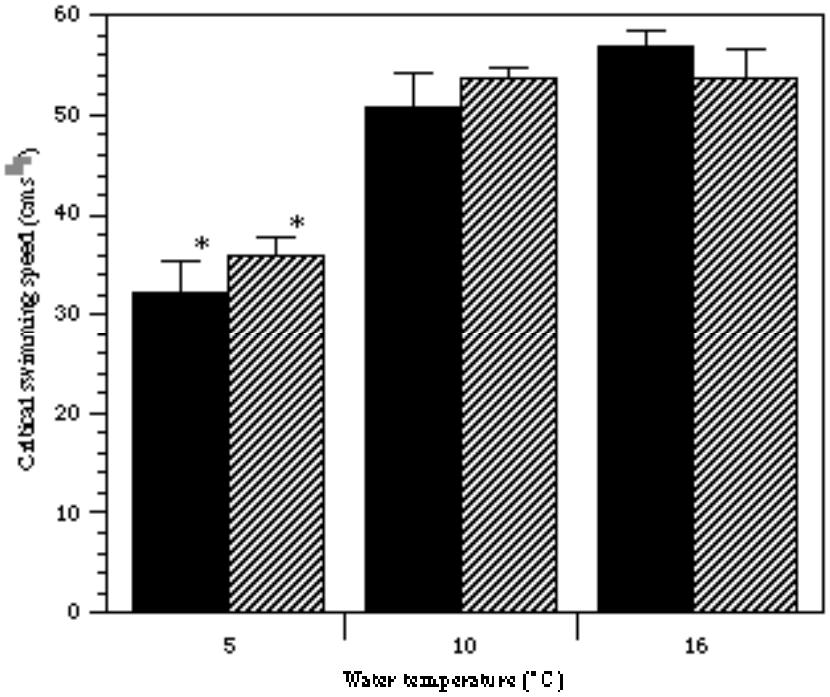

Fig. 2. The mean (+S.E.) U_{crit} of six largescale suckers at 5, 10 and 16°C that were either intact (black bars) or surgically fitted with an ultrasonic flowprobe (grey bars). An asterisk denotes a significant difference between the values at 5°C and the other temperatures. No significant differences were found between the intact and surgically altered fish at any of the three temperatures.

relative to 5°C reflected an increase in the maximum $\dot{Q}$ without a change in resting $\dot{Q}$. The scopes for $\dot{Q}$ at 10°C and 16°C were very similar (27.2ml$kg^{-1}$$min^{-1}$ and 25.1ml$kg^{-1}$$min^{-1}$, respectively) despite the difference in factorial scope between the two temperatures (Fig. 6A).

Maximum V_S was unaffected by temperature at 5, 10 and 16°C (Fig. 6C). The factorial scope for V_S was 1.02 at 5°C, 2.21 at 10°C and 1.34 at 16°C. The scope for V_S at 10°C (0.43ml$kg^{-1}$$beat^{-1}$) was significantly greater than that at 5°C or 16°C (0.12 and 0.15ml $kg^{-1}$$beat^{-1}$, respectively).

The relative contributions of tachycardia and increased V_S to maximum $\dot{Q}$ during exercise are compared for the largescale sucker at three experimental temperatures and for five other fish species in Fig. 7. The relative contributions of f_H and V_S to the exercise-induced increase in $\dot{Q}$ in the largescale sucker clearly changed with temperature, with f_H being most important at 16°C. The increase in V_S was responsible for 54% of the increase in $\dot{Q}$ at 5°C, 61% at 10°C and only 30% at 16°C.

Blood flow distribution

Blood flow distribution to six tissues was determined for largescale suckers at rest and while swimming. These six tissues constituted 46% of the total body mass of the fish, and

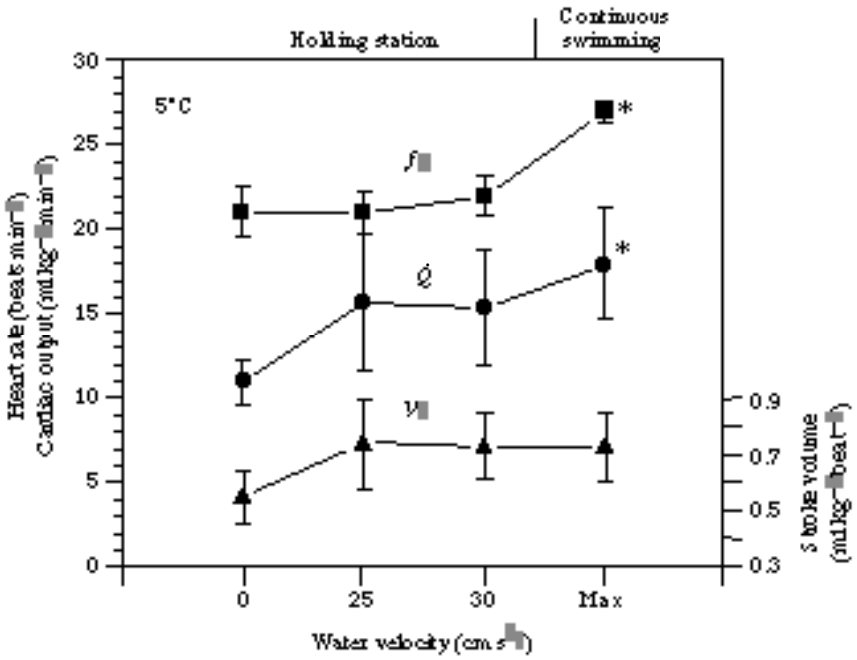

Fig. 3. The influence of water flow on the mean (±S.E.) cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_S) of six largescale suckers at 5°C. The average swimming speed of these fish whenswimming maximally (Max) was 32cm s^{-1}. Measurements were made on the fish at rest (zero water velocity), holding station against a water flow and swimming continuously. An asterisk denotes a significant difference between a trait at one water flow and that trait in fish at rest.

it was estimated that 58% of the spheres injected into the resting fish lodged in these tissues. When expressed in terms of blood flow distribution, the white muscle and the kidney accounted for virtually all of the blood flow to the tissues examined in the resting fish (Fig. 8A). Rates of perfusion in the resting fish were determined using the results of the perfusion experiments and a $\dot{Q}$ of 18.4ml kg^{-1} min^{-1}, the resting $\dot{Q}$ determined with the ultrasonic flowprobe. At rest, the highest mass-specific perfusion rates were found in the kidney, followed by spleen and white muscle (Fig. 8B).

Swimming significantly changed perfusion rates and blood flow distribution. When expressed as a percentage of total blood flow, the blood flow to the red muscle increased significantly from less than 1% to more than 11%, while the blood flow to the white muscle significantly decreased from 44% to 15% (Fig. 8A).

Rates of perfusion in the swimming fish were determined using the results of the perfusion experiments and a $\dot{Q}$ of 43.5ml kg^{-1} min^{-1}, the maximum $\dot{Q}$ determined using the ultrasonic flowprobe. This $\dot{Q}$ value was generated from suckers swimming at U_{crit}, which means that these fish were fatiguing at water velocities of 45, 50 or 55cm s^{-1}. As stated in Materials and methods, the cannulated fish were swimming at 50cm s^{-1}, which suggests that they were near U_{crit} and probably had a $\dot{Q}$ that was approaching 43.5ml kg^{-1} min^{-1}. In the swimming fish, the only rate of perfusion that changed

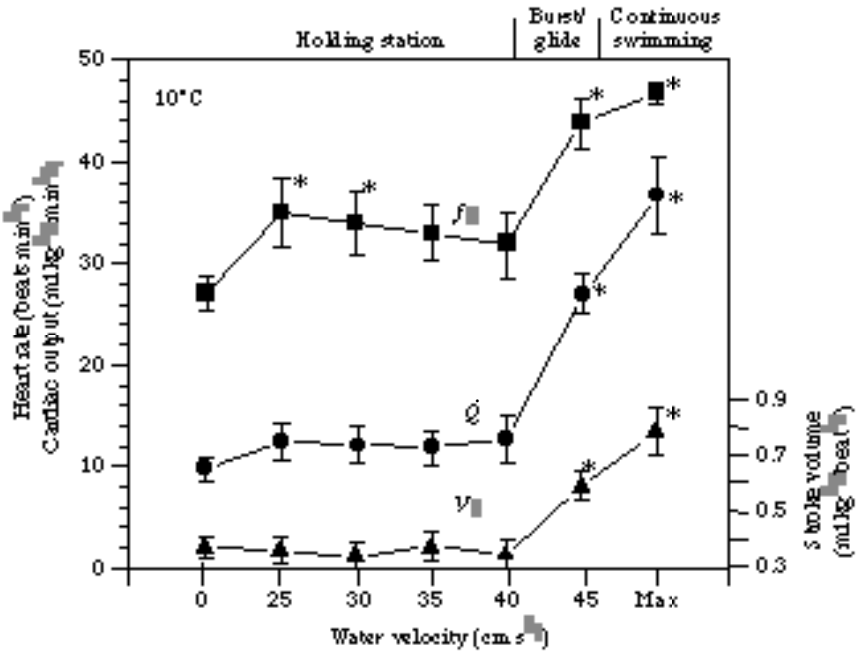

Fig. 4. The influence of water flow on the mean (±S.E.) cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_S) of six largescale suckers at 10°C. The average swimming speed of these fish when swimming maximally (Max) was 53cm s^{-1}. Measurements were made on the fish at rest (zero water velocity), holding station against a water flow, swimming in a burst-and-glide fashion and swimming continuously. An asterisk denotes a significant difference between a trait at one water flow and that trait in fish at rest.

significantly was the blood flow to the red muscle. In the swimming fish, red muscle blood flow increased significantly by 60-fold (Fig. 8B).

Discussion

Resting f_H, V_S *and* $\dot{Q}$

The resting $\dot{Q}$ values estimated for the largescale sucker can be put into context by comparing them with the $\dot{Q}$ values of other relatively inactive fishes (Table 1). Data included in this table are from studies that measured flow directly using a flowprobe or measured it indirectly using the Fick equation. Indirect measurements have not been found to estimate $\dot{Q}$ reliably (Peyraud-Waitzenegger and Soulier, 1989; Hughes *et al.* 1982) and a general trend in the data in Table 1 is that resting $\dot{Q}$ is often overestimated when measured using the Fick equation. Despite this bias, it seems that the resting $\dot{Q}$ for many relatively inactive fishes at 10°C is between 10 and 20ml kg^{-1} min^{-1}.

Increasing water temperature has been found to increase resting $\dot{Q}$ in virtually all fishes examined. Over the temperature range 5–15°C, resting $\dot{Q}$ was found to increase with water temperature with a Q_{10} of 2.70 for the winter flounder (Cech *et al.* 1976) and a Q_{10} of 2.60 for rainbow trout (Barron *et al.* 1987). In the largescale sucker, however, an

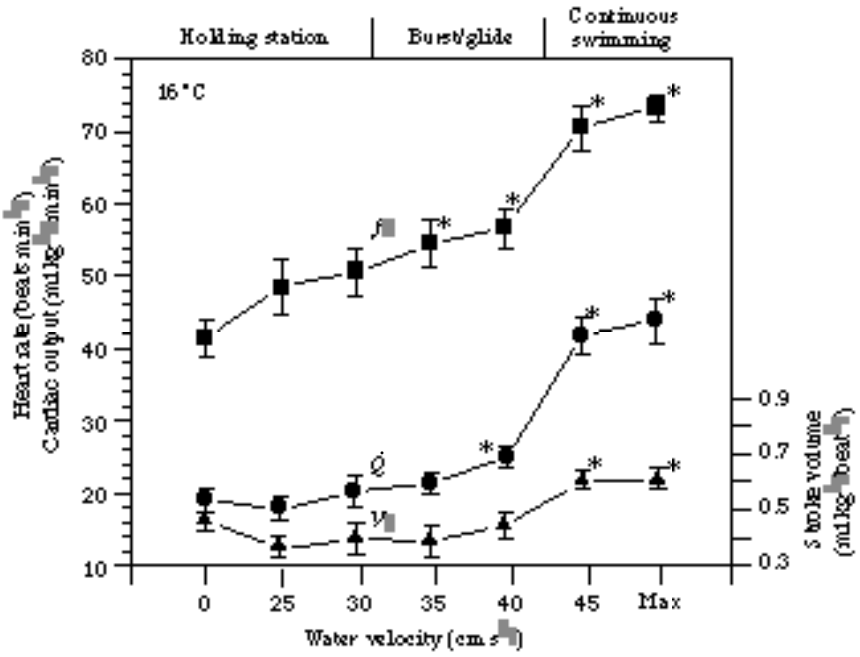

Fig. 5. The influence of water flow on the mean (±S.E.) cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_S) of six largescale suckers at 16°C. The average swimming speed of these fish when swimming maximally (Max) was 53cm s^{-1}. Measurements were made on the fish at rest (zero water velocity), holding station against a water flow, swimming in a burst-and-glide fashion and swimming continuously. An asterisk denotes a significant difference between a trait at one water flow and that trait in fish at rest.

increase in water temperature from 5 to 10°C did not increase $\dot{Q}$, although an increase in water temperature from 5 to 16°C led to a significant increase in resting $\dot{Q}$ (Q_{10} 2.01). Similarly, a degree of thermal insensitivity in resting $\dot{Q}$ apparently occurs in the eel *Anguilla anguilla*, which has a resting $\dot{Q}$ of 11–12ml kg^{-1} min^{-1} over the range 8.5–15°C (Table 1). Thus, a positive correlation between water temperature and resting $\dot{Q}$ cannot be assumed for all species over all thermal ranges because intervals of thermal insensitivity may occur, with the exact range of insensitivity being a characteristic of the species.

Temperature-induced changes in resting f_H appear to be consistent among different species of fish, while changes in V_S seem to be strongly species-specific. The resting heart rate of intact fish generally increases with a Q_{10} of less than 2.0 (1.5 for rainbow trout, from 6.5 to 15°C, Preide, 1974; 2.6 for rainbow trout from 5 to 12°C and 1.5 from 12 to 20°C, Wood *et al.* 1979; 1.2 for Atlantic eel from 15 to 25°C, Seibert, 1979; and 1.3–1.6 for winter flounder from 5 to 15°C, Cech *et al.* 1976). These increases with water temperature are consistent with what we observed in the largescale sucker (Q_{10} 1.84 from 5 to 16°C). With respect to resting V_S, the only data on the effect of water temperature are those for the winter flounder (Cech *et al.* 1976) and the largescale sucker. In the flounder, an increase in resting $\dot{Q}$ from 5 to 16°C was associated with a 64% increase in V_S. This

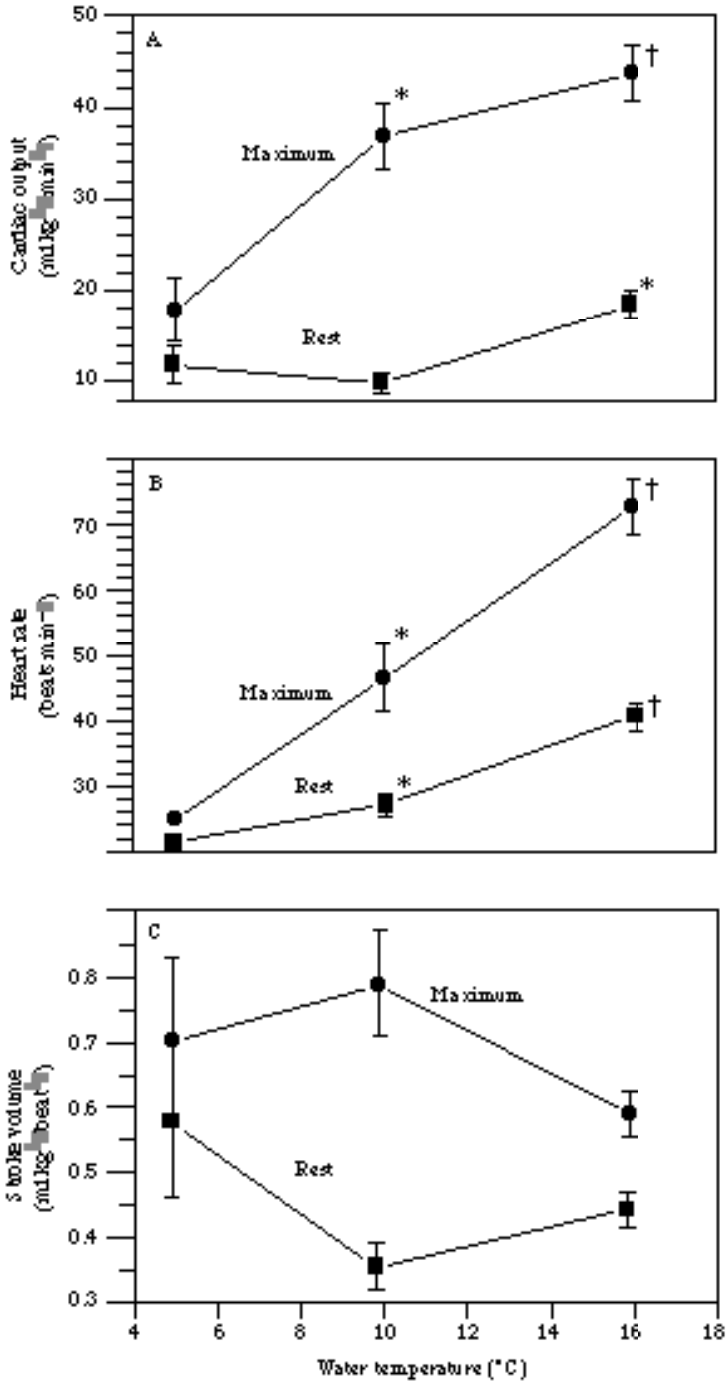

Fig. 6. The mean (±S.E.) maximum and resting cardiac output ($\dot{Q}$, A), heart rate (f_H, B) and stroke volume (V_S, C) for six largescale suckers at 5, 10 and 16˚C. An asterisk denotes a significant difference between the value at 5˚C and that at 10 or 16˚C; whereas a dagger denotes a significant difference between the value at 10˚C and that at 16˚C.

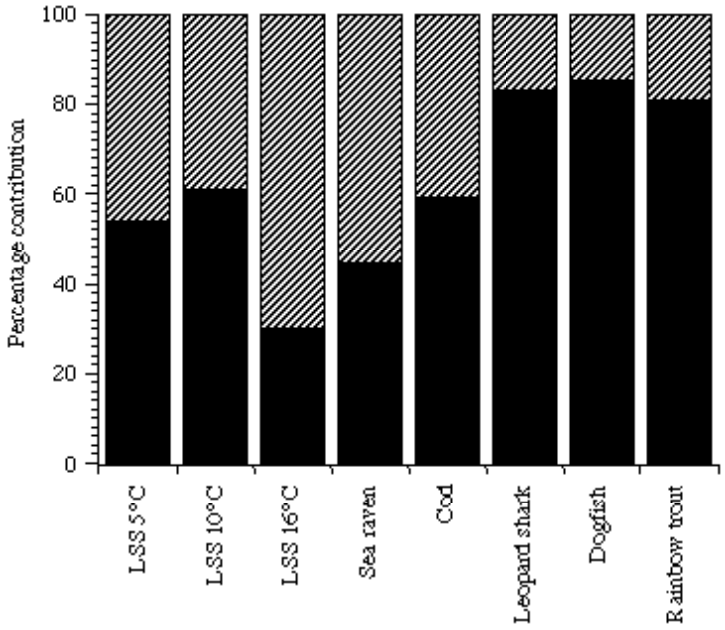

Fig. 7. The percentage contribution of heart rate and stroke volume to the exercise-induced increase in cardiac output in six fish species. The black shading represents the contribution of stroke volume, the grey shading that of heart rate. Data for the largescale sucker (LSS) are from this study; data for the other fish are from the literature (sea raven, 10–12°C, Axelsson *et al.* 1989; cod, 10–11°C, Axelsson and Nilsson, 1986; leopard shark, 14–24°C, Lai *et al.* 1989; dogfish, 18–19°C, Piiper *et al.* 1977; rainbow trout, 9–10.5°C, Kiceniuk and Jones, 1977).

was quite different from what was found in the largescale sucker, where an increase in water temperature from 5 to 16°C was associated with a decrease in V_S of almost 30%.

Exercise, f_H, V_S *and* $\dot{Q}$

Catostomid fishes, such as the largescale sucker, hold station against strong water currents by using fin appression (Lundberg and Marsh, 1976). This behavior does not appear to be very energetically demanding, since there were no significant increases in $\dot{Q}$ in largescale suckers while holding station. Our observation is consistent with the results of Facey and Grossman (1990), who found that the oxygen consumption of the mottled sculpin, *Cottus bairdi*, and the longnose dace, *Rhinichthys cataractae*, did not increase significantly while the fish held station without swimming against water velocities as high as $8\,BL\,s^{-1}$. At water velocities above $8\,BL\,s^{-1}$, the mottled sculpin fatigued while the longnose dace swam and oxygen consumption increased dramatically.

With one exception (*Anguilla australis*, Davie and Forster, 1980), $\dot{Q}$ increases with swimming in fishes. Variation in the factorial scope for $\dot{Q}$ appears to depend upon the species examined and on water temperature. The factorial scope for $\dot{Q}$ due to swimming ranges from 3 in rainbow trout at 11°C (Kiceniuk and Jones, 1977) to 1.5–1.7 in Atlantic cod *Gadus morhua* at 10–12°C (Axelsson, 1988) and sea raven *Hemitripterus americanus* at 10–12°C (Axelsson *et al.* 1989). The factorial scope for $\dot{Q}$ in largescale

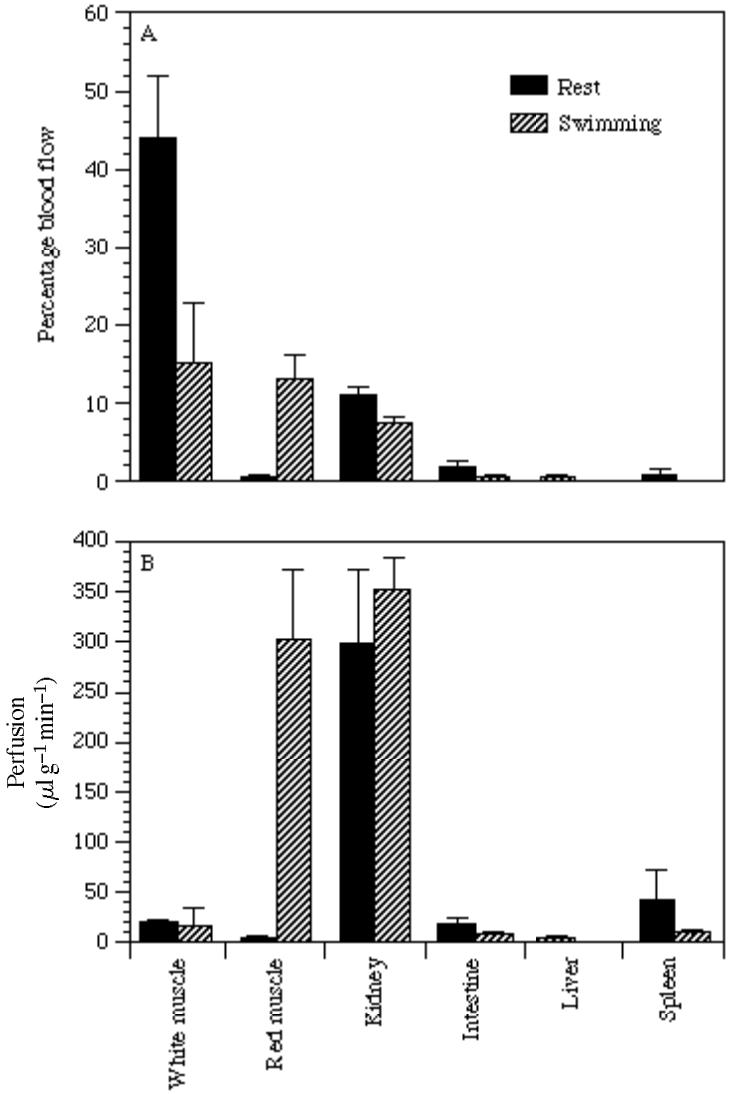

Fig. 8. The distribution of blood flow (percentage) (A) and rates of perfusion (μl g^{-1} min^{-1}) (B) to six tissues in the largescale sucker at rest (black bars) and while swimming (grey bars). Means (+S.E.) are based upon six fish at rest and four fish while swimming.

suckers spans the range found in other species and was clearly temperature-dependent, with factorial scopes of 1.51 at 5˚C, 3.84 at 10˚C and 2.36 at 16˚C. What is not known is whether swimming fish attain the highest possible $\dot{Q}$. Farrell and Jones (1992) argue that this is the case, since maximum $\dot{Q}$ for *in situ* heart preparations for the sea raven and rainbow trout are similar to *in vivo* values measured near U_{crit} in the same species.

The effect of water temperature on the U_{crit}, maximum $\dot{Q}$ and scope for $\dot{Q}$ was the same. Between 16 and 10˚C there were no significant differences among any of these traits, while from 10 to 5˚C there were significant reductions in the values for all three. From this

Table 1. *The resting cardiac output ($ml min^{-1} kg^{-1}$) for relatively sluggish fishes assayed at approximately 10 and 15°C*

Species	Cardiac output ($ml kg^{-1} min^{-1}$)	Water temperature (°C)	Method[a]	Source
Catostomus macrocheilus	9.6	10	d	1
	18.4	16		1
Ophiodon elongatus	10.9	10		2
	11.2	10		3
Anguilla anguilla	12.2	8.5–10.5		4
	11.5	15		5
Hemitripterus americanus	14.6	10.5		6
	18.8	10–12		7
Gadus morhua	17.3	10–11		8
	19.2	10–12		9
	17–26	9–10		10
	29.1	10	d	11
Cyprinus carpio	18.3	9–11	i	12
	20.7	15–16		13
Pseudopleuronectes americanus	23.1	10		14
	41.8	15	i	14
Anguilla australis	9.1	15.5–18.5	d	15
	6.2–10.2	16–20		16
	10.4			17
Anguilla australis schmidtii	10.9–11.3	16.6–17.0	d	18
Tinca tinca	14–18	12–14	i	19
Myoxocephalus scorpius	27.7	15–18	i	20

[a]i, indirectly measured; d, directly measured.

1, Kolok *et al.*, this study; 2, Farrell, 1981; 3, Farrell, 1982; 4, Hughes *et al.* 1982; 5, Peyraud-Waitzenegger and Soulier, 1989; 6, Farrell, 1986; 7, Axelsson *et al*. 1989; 8, Axelsson and Nilsson, 1986; 9, Axelsson, 1988; 10, Jones *et al.* 1974; 11, Pettersen and Nilsson, 1980; 12, Garey, 1970; 13, Itazawa, 1970; 14, Cech *et al.*, 1976; 15, Hipkins *et al*. 1986; 16, Hipkins and Smith, 1983; 17, Hipkins, 1985; 18, Davie and Forster, 1989; 19, Eddy, 1974; 20, Goldstein *et al.* 1964.

result it can be hypothesized that there may be a cardiovascular limit on swimming performance. This hypothesis is supported by the findings of Kolok (1992) in which the individual variation in heart enzymatic activity was significantly correlated with individual variation in U_{crit}. It is also supported by the results of Farrell *et al.* (1990), who found that training rainbow trout significantly increased their heart enzymatic activities and U_{crit}.

Between 10 and 16°C, maximum $\dot{Q}$, scope for $\dot{Q}$ and U_{crit} appear to be insensitive to changes in water temperature. A thermal insensitivity of U_{crit} between 15 and 19°C (Kolok, 1991) and 20 and 30°C (Beamish, 1970) has been shown for the largemouth bass. Patterns of thermal insensitivity such as these are important in that they suggest that the fish can tolerate variation in water temperature during the summer yet maintain the same level of cardiovascular and/or swimming performance.

To maintain a consistent scope for $\dot{Q}$ at 10 and 16°C, the fish altered their reliance upon f_H and V_S. At 5 and 10°C, the fish appeared to rely more on an increase in V_S to maintain

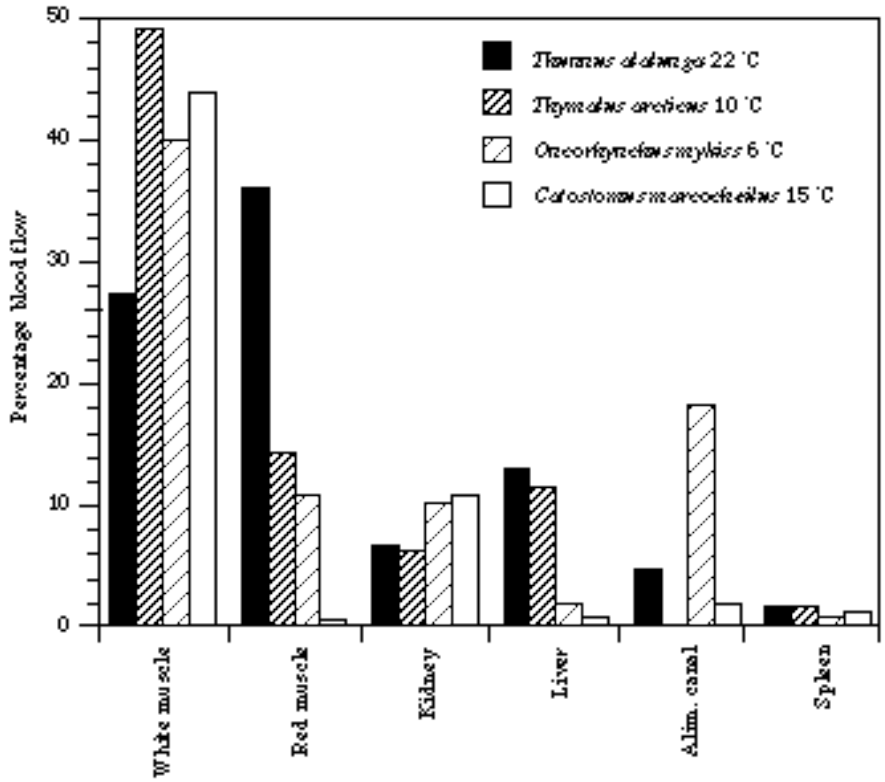

Fig. 9. The distribution of blood flow (percentage) to six tissues in four fish species. Data for the largescale sucker are from the current study, while data regarding the other fish are from the literature (*T. alalunga*, White *et al.* 1988; *T. arcticus*, Cameron, 1974; *O. mykiss*, Barron *et al.* 1987).

the scope for $\dot{Q}$, while at 16˚C the fish appeared to rely more upon an increase in f_H. At 5 and 10˚C, over 50% of the increase in $\dot{Q}$ was due to an increase in V_S, while at 16˚C, V_S only made a 30% contribution. The data from the largescale sucker at 5 and 10˚C were consistent with those for the relatively inactive sea raven and Atlantic cod (Fig. 7), while the increase in $\dot{Q}$ in rainbow trout and two shark species was much more dependent upon V_S than it was on f_H. The contribution of V_S to $\dot{Q}$ at 16˚C is the lowest recorded in a fish and suggests a profound reliance upon f_H at this temperature to increase $\dot{Q}$.

To maintain maximum $\dot{Q}$ from 16 to 10˚C, the suckers increased maximum V_S, while maximum f_H declined. An increase in maximum V_S at reduced temperatures has also been found using *in situ* heart preparations for rainbow trout (Graham and Farrell, 1989, 1990) and for sea raven at a preload pressure of 0.1kPa (Graham and Farrell, 1985). It has been suggested (Graham and Farrell, 1990) that increases in the maximum V_S of fish acclimated to cold water may be a result of ventricular hypertrophy and/or a reduced f_H, which may allow for greater ventricular filling. Heart mass was not measured in the fish fitted with ultrasonic flowprobes in our study, so it is not known which of these events was more responsible for the observed increase in maximum V_S from 16 to 10˚C.

As water temperature dropped from 10 to 5˚C, U_{crit}, maximum $\dot{Q}$ and scope for $\dot{Q}$ all dramatically decreased in the largescale sucker. This pattern was similar to the dramatic decreases found in the U_{crit} and spontaneous activity of largemouth bass when water temperatures approach 5˚C (Kolok, 1991; Lemons and Crawshaw, 1985). This dramatic decrease in activity is probably an important component of the overwintering strategy for these fish. When the temperature drops to near or below 5˚C, both species probably minimize their swimming and remain quiescent for long periods. It has been suggested that winter quiescence is a common overwintering strategy for many freshwater fish species (Ultsch, 1989) and our findings suggest that, at least for the largescale sucker, winter quiescence is associated with a limited scope for $\dot{Q}$.

Blood flow distribution

This is the first study in which the colored microsphere technique has been used to determine the blood flow distribution in a fish species. An advantage of this technique is that it provides quantitative data regarding blood distribution without the safety and waste management concerns associated with the use of radioactive isotopes. A disadvantage is that samples had to be completely digested with KOH prior to filtering. This precluded analysis of much of the carcass including the head, vertebral column, fins and skin with associated scales and, as a result blood flow determination was not possible on all tissues. Therefore, we concentrated our efforts on six major tissues.

The pattern of blood flow distribution in the largescale sucker at rest was consistent with that found in fish used in three other studies involving radioactive microspheres (Fig. 9). On a percentage basis, blood flow to the kidney was a consistent 6–11% in all species. Similarly, blood distribution to the spleen was roughly 1% of the total blood flow in all four species. Blood flow to the white muscle was between 40 and 50% for the three species other than the tuna.

At rest, blood flow distribution to the red muscle is quite variable among the four species in Fig. 9, with the species with increased capacity for prolonged swimming having more flow distributed to the red muscle. For example, tuna are the elite athletes among fishes, their red muscle is roughly 4% of their body mass and it receives roughly 36% of the blood flow at rest (White *et al.* 1988). Rainbow trout and arctic grayling are good prolonged swimmers, have red muscle masses between 1 and 2.5% of their body mass, and the red muscle in their bodies receives between 10 and 15% of the resting cardiac output (Barron *et al.* 1987; Cameron, 1974; Randall and Daxboeck, 1982). In contrast, the largescale sucker is a relatively poor swimmer and has a red muscle mass that is roughly 2% of body mass and a resting blood flow of less than 1% of the total $\dot{Q}$. If the data regarding blood distribution to the red muscle is expressed in terms of rates of perfusion ($\mu l\,g^{-1}\,min^{-1}$), the active swimming fish all have a rate of perfusion around $200\,\mu l\,g^{-1}\,min^{-1}$, while that of the sucker is a very low $5\,\mu l\,g^{-1}\,min^{-1}$.

Blood flow to the alimentary canal has only been estimated using microspheres in two studies other than our work on largescale suckers (Barron *et al.* 1987; White *et al.* 1988). In these studies, blood flow to the alimentary canal was found to be so variable that it is difficult to generalize about it. Barron *et al.* (1987) estimated blood flow to the intestine, stomach and pyloric caeca of rainbow trout to be 18% at 5˚C, 15% at 12˚C and 7% at

18°C, while blood flow to the intestine of the largescale sucker was estimated to be 3% and blood flow to the stomach and intestine of albacore tuna was estimated to be 0.2%. The variation in these data may be due to a number of factors including: (1) anatomical differences in the fish, e.g. largescale suckers do not have a stomach or pyloric caeca, (2) visceral vasoconstriction, and (3) inadequate mixing of the spheres into the blood before it enters the arteries supplying the alimentary canal (Barron *et al.* 1987). Stress may also be important. The tuna were collected and processed at sea without mention of a recovery period, while the trout and suckers were given periods of 1 and 4h after surgery, respectively, before they were injected with spheres.

When the sucker exercised, blood flow distribution to the red muscle increased significantly, while distribution of blood to the white muscle dropped significantly. When these data were expressed in terms of $\mu l\,g^{-1}\,min^{-1}$, the perfusion rate to the red muscle increased by 60-fold when the animal exercised, while the rate of perfusion of the other tissues did not change significantly. The only other published data from the muscles of resting and exercising fish are from two studies on rainbow trout. Neumann *et al.* (1983) found that the blood flow to the red muscle of trout was six times higher 5min after the fish had been electrically stimulated to exhaustion, while blood flow to the white muscle increased by more than threefold 30min after stimulation. In a study where the trout were exercised in a less stressful fashion (Randall and Daxboeck, 1982), a 15-fold increase in the perfusion of the lateral red muscle of exercising rainbow trout was found concomitant with a decrease in the absolute blood flow to the white muscle. It is interesting that the blood flow to the red muscle in the exercising sucker (13.1%) is similar to that in the resting rainbow trout (10%, Barron *et al.* 1987). This trend is also true for $\dot{Q}$; resting values of $\dot{Q}$ for the trout at 12°C ($38.7 ml\,kg^{-1}\,min^{-1}$; Barron *et al.* 1987) are approximately the same as the exercising values of $\dot{Q}$ that we estimated for the sucker at 10°C ($36.7 ml\,kg^{-1}\,min^{-1}$).

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