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CARDIAC OUTPUT AS A PREDICTOR OF METABOLIC RATE IN COD *GADUS MORHUA*

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Summary

Adult Atlantic cod (2 kg *Gadus morhua*) were fitted with Doppler ultrasonic flow-probes to measure ventral aortic outflow (i.e. cardiac output). The probes remained patent for upwards of 3 months, during which time detailed relationships between cardiac output ($\dot{Q}$), heart rate (f_H) and rate of oxygen consumption ($\dot{M}_{O_2}$) were determined as a function of swimming speed and temperature (5 °C and 10 °C). The rate of oxygen consumption increased linearly with $\dot{Q}$ and exponentially with swimming speed. A very good correlation was observed between $\dot{M}_{O_2}$ and $\dot{Q}$ (r^2=0.86) compared with the correlation between $\dot{M}_{O_2}$ and f_H (r^2=0.50 for all 10 °C data and r^2=0.86 for all 5 °C data). However, the $\dot{M}_{O_2}$ *versus* f_H correlation gradually improved over approximately 1 week after surgery (r^2=0.86). The relationship between $\dot{M}_{O_2}$ and $\dot{Q}$ was independent of temperature, while the relationship between $\dot{M}_{O_2}$ and f_H changed with temperature. Hence, calculating $\dot{M}_{O_2}$ from $\dot{Q}$ is simpler and does not require that temperature be recorded simultaneously. Variations in cardiac output were determined more by changes in stroke volume (V_S) than by f_H; therefore, f_H was a less reliable predictor of metabolic rate than was $\dot{Q}$. Given that $\dot{Q}$ can be used to estimate $\dot{M}_{O_2}$ so faithfully, the advent of a cardiac output telemeter would enable robust estimates to be made of the activity metabolism of free-ranging fish in nature, thereby strengthening one of the weakest links in the bioenergetic models of fisheries biology.

Key words: *Gadus morhua*, Atlantic cod, oxygen consumption, cardiac output, heart rate, stroke volume, bioenergetics, metabolism, fish.

Introduction

In fisheries biology, bioenergetic models for the analysis of fish production systems are quite advanced (e.g. Kitchell *et al.* 1977; Kerr, 1982; Krohn *et al.* 1997). Such models take the form of a steady-state energy equation in which the total amount of energy consumed is balanced by the total amount of energy expended through respiration, activity metabolism, food digestion and assimilation, together with waste losses and somatic and gonadal growth. Bioenergetic models have been shown to apply to a wide variety of ecological problems (Hansen *et al.* 1993; Hewett, 1989; Kitchell *et al.* 1977) and have been applied specifically to Atlantic cod (*Gadus morhua*) (Krohn *et al.* 1997). Whereas the growth component can be estimated with relative ease, the ration intake and metabolic components (standard and active metabolic rates) are difficult to estimate in the field and require numerous approximations and assumptions (Kerr, 1982; Kerr and Dickie, 1985; Boisclair and Leggett, 1989; Ney, 1993).

The ability to telemeter physiological information from free-ranging animals in nature promises to open up many new areas of research into animal energetics. There have been several attempts to estimate the activity metabolic rate of free-ranging animals by taking telemetered measures of their heart rate (f_H) and converting them into the rate of oxygen consumption ($\dot{M}_{O_2}$) using equations derived from calibration experiments (i.e. f_H *versus* $\dot{M}_{O_2}$) carried out in the laboratory (e.g. Butler, 1993; Lucas *et al.* 1993; Bevan *et al.* 1995). Such estimates are far more reliable for birds and mammals (Butler *et al.* 1995) than they are for most fish, since cardiac output ($\dot{Q}$) in endotherms is modulated primarily through changes in heart beat frequency (f_H) rather than stroke volume (V_S). Given that cardiac output in fish is determined largely by V_S (Stevens and Randall, 1967; Randall, 1968; Kiceniuk and Jones, 1977) and that:

$$\dot{M}_{O_2} = f_H \times V_S \times (C_{aO_2} - C_{vO_2}), \quad (1)$$

one could only expect robust relationships between f_H and $\dot{M}_{O_2}$ in circumstances where stroke volume (V_S) and the arterial-venous O_2 content difference (C_{aO_2}–C_{vO_2}) either remained constant or varied systematically. Indeed, this is probably why f_H and $\dot{M}_{O_2}$ can be so highly correlated in some species of free-swimming fish (Wardle and Kanwisher, 1974; Armstrong, 1986, 1998; Sureau and Lagardere, 1991; Lucas, 1994) and not in others (Priede and Tytler, 1977; Sureau and Lagardere, 1991; Claireaux *et al.* 1995*a*,*b*).

In terms of increased oxygen demands from swimming, f_H

is often not well correlated with metabolic rate and its control is not completely understood. Priede and Tytler (1977) observed a large range of $\dot{M}_{O_2}$ for a given f_H in rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and cod. This variability is presumably due to a large range of V_S for a given f_H. Heart rate has been used to estimate energy expenditure of brown trout (Priede and Young, 1977; Priede, 1983) and pike (*Esox lucius*) (Lucas *et al.* 1991, 1993) in nature but, according to Thorarensen *et al.* (1996*a*), the accuracy of these estimates is questionable. Thorarensen *et al.* (1996*a*) state that the relationship between $\dot{M}_{O_2}$ and f_H is described by numerous curves subject to the physiological condition of a fish and to environmental factors.

During steady-state swimming, the rate of oxygen uptake of fish increases considerably, to as much as 15 times the value at standard metabolic rate in sockeye salmon (*Oncorhynchus nerka*) (Brett and Glass, 1973). The cardiovascular system meets oxygen uptake demands by increasing $\dot{Q}$ and by utilizing more of the oxygen in the blood. Since $\dot{Q}$ is the product of the volume of blood pumped for each contraction (V_S) and the frequency of pumping (f_H), one would expect $\dot{Q}$ to be highly correlated with the rate of oxygen uptake. Although $\dot{Q}$ and $\dot{M}_{O_2}$ have been measured in fish a number of times, there have been no direct and long-term simultaneous measurements of both $\dot{Q}$ and $\dot{M}_{O_2}$ in actively swimming fish. Studies that have reported a relationship between $\dot{Q}$ and $\dot{M}_{O_2}$ use indirect determinations of $\dot{Q}$ or $\dot{M}_{O_2}$, through solution of the Fick equation (see above) (Goldstein *et al.* 1964; Lenfant and Johansen, 1966; Robin *et al.* 1966; Baumgarten-Schulmann and Piiper, 1968; Garey, 1970; Hanson and Johansen, 1970; Holeton, 1970; Cech *et al.* 1976; Kiceniuk and Jones, 1977; White *et al.* 1988; Takeda, 1993; Korsmeyer *et al.* 1997*b*).

The objectives of this study are to determine precisely the relationship between $\dot{M}_{O_2}$ and cardiac variables in cod, over a range of temperature, as a necessary step in developing an ultrasonic telemetering transmitter that allows the measurement of $\dot{Q}$ in free-swimming fish. The development of such a transmitter will permit more accurate estimates of the metabolic rate of free-swimming fish in nature, enabling quantification of the effects of external factors on fish growth and production. We describe laboratory results on the efficacy of cardiac output as a predictor of the metabolic rate of Atlantic cod as an essential prerequisite to the development of such a transmitter.

Materials and methods

Approximately 60 Atlantic cod (*Gadus morhua* L.) (mean mass 1.91 kg) were acquired from Eastern Passage, Nova Scotia (63°25′ W, 44°34′ N) in the autumn of 1993 and from nearby Sambro Fisheries in the autumn of 1995. The animals were transported in aerated fresh sea water to the laboratory at Dalhousie University and maintained in ambient and temperature-controlled sea water between 5 and 10 °C and at 30–32 ‰ salinity under a controlled photoperiod (12 h:12 h L:D). The cod were fed a diet of frozen mackerel (*Scomber scombrus*) and squid (*Loligo opalescens*), twice a week before experimentation. To eliminate the effect of feeding on metabolic rate, the cod were not fed for 1 week prior to surgery and before and during swimming trials. The cod were never fed to satiation, and a meal never exceeded 2.5 % of body mass. We limited the animals used here to a mass range of 1.6–2.2 kg to minimize any possible scaling effects of mass on the rate of oxygen consumption ($\dot{M}_{O_2}$), cardiac output ($\dot{Q}$) and heart rate (f_H).

Swimming metabolic rate

The experiments were conducted at 5 °C and 10 °C on individual fish using a 'Brett-style' swimming respirometer (Brett, 1964) described in detail in Webber (1985) and Webber and O'Dor (1986). The water volume was 89 l, and water currents were generated by a centrifugal pump capable of producing water velocities up to 2 m s^{-1}. A computerized control system enabled temperature control to ±0.05 °C. Chilled (2 °C) and heated (20 °C) seawater lines gave flexibility in setting temperatures. A brief description of the working system is as follows. Within the toroidal-shaped acrylic pipe, a fish confined to a straight section 0.2 m in diameter × 1.2 m in length was forced to swim against the current. Oxygen depletion was measured in a parallel external water circuit using a Radiometer P_{O_2} electrode (Radiometer Inc., Copenhagen) and Endeco/YSI, model 1125, pulsed oxygen analyzer and electrode (YSI, Yellow Springs Instruments, Ohio, USA). Changes in oxygen concentration were not detected in the absence of test fish. The water circuit provided a constant flow over the oxygen probes and facilitated calibration of the oxygen system. All sensor inputs (oxygen, temperature, cardiac output) were acquired in digital format and transformed into real units. Doppler blood flow inputs were sampled continuously at 50 Hz. The rate of oxygen consumption was adjusted to a standard body mass of 1 kg using the mass exponent of 0.8 determined by Saunders (1963):

$$\dot{M}_{O_2(1\,kg)} = (1/M_b)^{0.8}\dot{M}_{O_2}, \quad (2)$$

where M_b is body mass.

Many studies of swimming energetics use a 'critical speed' protocol defined by Brett (1964) in which the animal is swum, starting at 0 m s^{-1}, at stepwise increases in velocity until it fatigues. The problem with this technique is that the researcher cannot deviate from the fixed incremental protocol even if the animal appears stressed, overactive or inactive. Rather than following a fixed time procedure, our protocol involved swimming the animal at any water speed between 0.08 and 0.7 m s^{-1} for approximately 1–1.5 h at each speed. The cod were allowed to rest for 1–2 h between swimming trials and overnight. The water velocity was corrected for the acceleration of water around the fish according to the method of Webb (1974). The animal was monitored on a video monitor.

Approximately 1 week prior to surgery, the animal was tested in the respirometer. During this time, fish were exposed to various water currents, providing a control to compare with

post-surgery swimming performance and to acclimate the animal to the chamber. The rate of oxygen consumption, tailbeat frequency and water speed were measured during all swimming trials before and after surgery. Cardiac output and f_H were measured after surgery. Every effort was made to minimize stress. The animals were not exposed to an electric grid and were seldom prodded to encourage activity. Reported values are means ± standard error of the mean.

Surgery

Cod were anaesthetized with 0.05 g l^{-1} tricaine methanesulphonate (MS-222), weighed, measured and transferred to an operating sling immersed in oxygenated and temperature-controlled sea water. The gills were continuously bathed with aerated sea water and 0.025 g l^{-1} MS-222. The ventral aorta was exposed *via* a 1.5 cm long and approximately 3 cm deep incision. Cardiac output was measured using a Doppler ultrasonic flow probe and a directional pulsed Doppler flowmeter (Bioengineering, University of Iowa, USA). The Doppler crystal was pre-mounted at 45 ° in the flow probe. A flow probe (3–4 mm inside diameter) was chosen to match the diameter of the ventral aorta as closely as possible. The tubing was then fitted around the ventral aorta and fixed in place with silk or braided polyglycolic acid ligature. A 10 cm long 14 gauge needle was used to lead the wires under the skin from the incision, behind the pectoral fin, to emerge at the base of the dorsal fin. To minimize infection, antibiotics were applied to the wound before completely closing the incision using sutures.

Two calibration techniques were used to determine blood flow from the Doppler blood speed signal. One technique involved excising the heart and perfusing the atrium, ventricle and aorta with perfusate at known flow rates. The other technique, performed on four cod, involved calibrating the flow probe using latex tubing of known inside diameter and blood flow rate. Human or fish whole blood or Cephadex particles (20 µm diameter) were used for the calibration. The velocity of the blood was measured, at 45 ° to the direction of flow, at various distances across the tubing. This velocity profile was measured at different flow rates. Once the probe had been implanted, the velocity profile of the ventral aortic blood was measured daily, thus calibrating blood flow (Fig. 1A). Both techniques yielded comparable flow/voltage ratios. A comparison of the two techniques is illustrated in Fig. 1B. The equation relating blood flow (ml min^{-1}) for the two techniques was linear with a slope near 1 and the coefficient of determination (r^2) was 0.90 (N=4; P<0.0001).

After the 30–60 min operation, the cod were placed in the swimming respirometer to commence recovery. Data were collected immediately after surgery; however, fish were not exercised until the next day. Extreme care was taken to avoid disturbing the animal. To measure metabolic rates and $\dot{Q}$ over the long term, 60 % (N=4) of the animals were kept in the respirometer for up to 3 months. The remainder (N=3) were kept for 2 weeks to 1 month. Initially, cod were tested at 10 °C for 2 weeks, acclimated to 5 °C over a 3 week period and then tested at 5 °C. Over this time, other acute temperature and feeding experiments were performed. It is important to note that the same animals were used for both temperature trials.

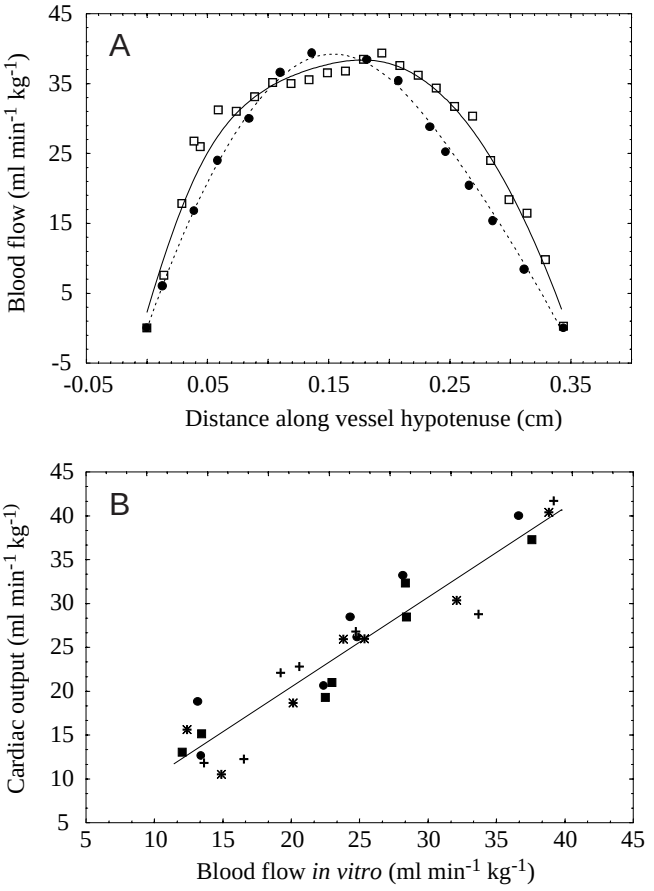

Fig. 1. (A) Comparison of blood flow ($\dot{V}$b) through a tube (filled circles) and through the ventral aorta (open squares) of a cod (*Gadus morhu*) *in vivo*. The lines were fitted using distance-weighted least-squares smoothing. (B) The relationship between cardiac output ($\dot{Q}$) calculated *in vivo* in resting or swimming fish and $\dot{Q}$ measured using *in vitro* ventral aortic perfusion. Different symbols are for different fish. $\dot{Q}$(*in vivo*)=–0.049+1.026$\dot{V}$b(*in vitro*); r^2=0.90; P<0.0001; N=4.

Results

The cod were able to maintain an upright position after 1 h of recovery from surgery in sea water. Physiological recovery was judged to take from 2 to 7 days after surgery on the basis of the resting heart rate (f_H) values. During the first 24 h after surgery, f_H generally exceeded the maximum values observed during swimming (46 *versus* 40 beats min^{-1}). After 1 week of post-operative recovery, the f_H for resting cod decreased to approximately 28.3±0.53 beats min^{-1} (N=6) and remained consistently at that level for the duration of the experiment at 10 °C. Also, most of the animals accepted food after 1 week, suggesting that recovery was near complete. The condition factor (mass/length^3) of the cod used in this study was higher than that observed in cod in nature. Condition factor is

generally accepted as an indication of the general health of an animal. The level of activity or observed spontaneous movements of cod in the respirometer did not appear to be different before and after surgery.

Oxygen consumption, swimming speed, cardiac output, stroke volume and heart rate

Cod were exercised in the respirometer at 5 and 10 °C. All animals were able to maintain their positions in the swimming chamber and swam steadily at low speeds. At higher speeds, water velocity was lowered if animals began to 'burst swim' (i.e. to show sporadic rapid accelerations). In no instances did the animals become exhausted. We refer to this level of activity as 'maximum activity'. The linear relationship between swimming speed and tailbeat frequency suggested that the animals were swimming smoothly (Fig. 2A; r^2=0.85; P<0.0001). This relationship confirmed that the respirometer provided reproducible swimming exercise. The rate of oxygen consumption was also correlated with tailbeat frequency (Fig. 2B; r^2=0.67; P<0.0001).

The rate of oxygen consumption ($\dot{M}_{O_2}$) as a function of swimming speed for fish equipped with cardiac output ($\dot{Q}$) flow probes is shown in Fig. 3. A regression line of the form $\dot{M}_{O_2}=ab^U$ was fitted, where a is the value of ($\dot{M}_{O_2}$) at zero velocity (the standard metabolic rate; μmol kg^{-1} min^{-1}) and U is the swimming speed in m s^{-1}. The correlation coefficient for this relationship for each individual was always higher when fitted to logarithmic than to linear coordinates, and the pooled data also gave a slightly higher correlation for a logarithmic model than for a linear model. The predicted values for standard metabolic rate and active metabolic rate were 35±1.2 and 97±2.3 μmol O_2 kg^{-1} min^{-1} at 10 °C (r^2=0.9; P<0.0001; N=9) and 22±2.0 and 72±5.0 μmol O_2 kg^{-1} min^{-1} at 5 °C (r^2=0.91; P<0.0001; N=7). These values correspond to swimming at 0 and approximately 1 body length s^{-1} and they can be used to calculate the metabolic facultative scope for activity; i.e. the difference between maximum and standard $\dot{M}_{O_2}$ (62 μmol O_2 kg^{-1} min^{-1} at 10 °C and 50 μmol O_2 kg^{-1} min^{-1} at 5 °C). The Q_{10}, between 5 and 10 °C, for metabolic rate was 2.5 at rest and 2.1 at maximum activity.

The interactions between $\dot{M}_{O_2}$, temperature and the cardiovascular variables are illustrated in Figs 4–6. At 10 °C, from rest to maximum activity, $\dot{Q}$ increased by 192 % from 12±0.74 to 35±2.6 ml kg^{-1} min^{-1} (N=7) (Fig. 4), f_H by 43 %

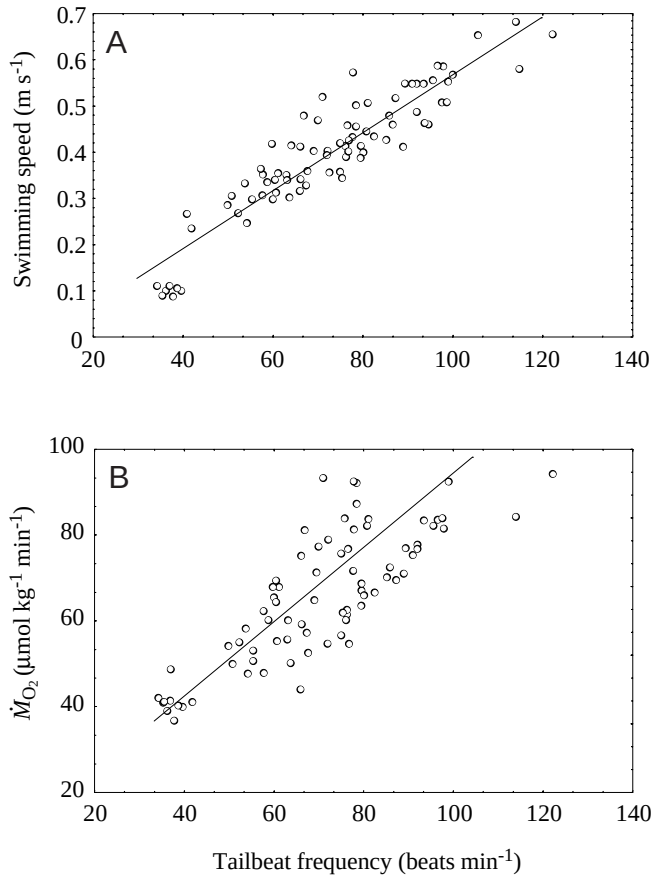

Fig. 2. (A) The relationship between swimming speed (U) and tailbeat frequency f_{TB} in cod (U=0.02+0.0053f_{TB}; r^2=0.85; P<0.0001; N=9). (B) The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and tailbeat frequency ($\dot{M}_{O_2}$=20+0.648f_{TB}; r^2=0.67; P<0.0001; N=9) in cod *Gadus morhua* at 10 °C.

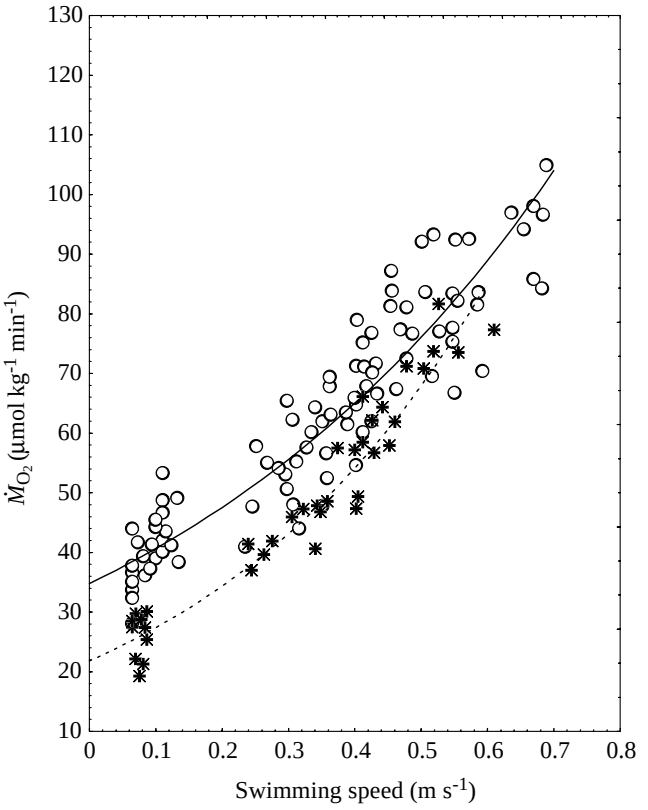

Fig. 3. The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and swimming speed (U) in cod *Gadus morhua* at 5 °C (asterisks) ($\dot{M}_{O_2}$=21.8×9.72^U; r^2=0.91; P<0.0001; N=7) and 10 °C (open circles) ($\dot{M}_{O_2}$=34.8×4.79^U; r^2=0.90; P<0.0001; N=9).

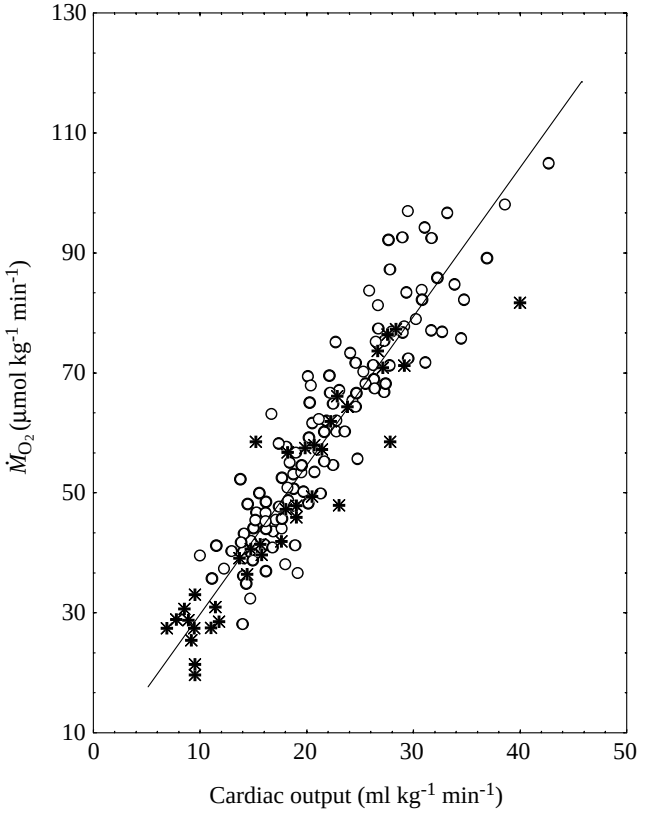

Fig. 4. The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and cardiac output ($\dot{Q}$) in cod *Gadus morhua* at 10 °C (open circles) and 5 °C (asterisks) ($\dot{M}_{O_2}$=5.6+2.46$\dot{Q}$; r^2=0.86; P<0.0001; N=7). An analysis of variance with regression for the 5 and 10 °C data revealed that the slopes and intercepts were not significantly different (P>0.6).

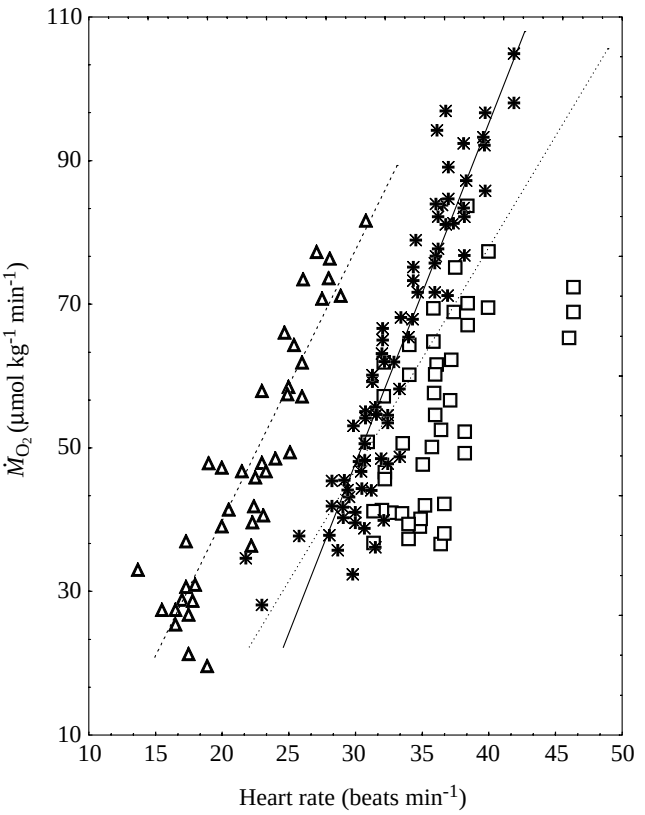

Fig. 5. The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and heart rate (f_H) in cod *Gadus morhua* at 5 °C (open triangles, dashed line) ($\dot{M}_{O_2}$=–35.5+3.75f_H; r^2=0.86; P<0.0001; N=5) and at 10 °C (open squares and asterisks). The open squares represent data collected less than 7 days after surgery, and the asterisks represent data collected more than 7 days after surgery. The solid line drawn through the asterisks is represented by the equation $\dot{M}_{O_2}$=–92.7+4.69f_H; r^2=0.86; P<0.0001; N=7. The combined data at 10 °C represented by the asterisks and open squares (dotted line) yield the relationship $\dot{M}_{O_2}$=–44.7+3.07f_H; r^2=0.50; P<0.0001; N=7).

from 28±0.84 to 40±0.81 beats min^{-1} (N=7) (Fig. 5) and stroke volume (V_S) by 100 % from 0.45±0.03 to 0.88±0.047 ml kg^{-1} (N=7) (Fig. 6). At 5 °C, $\dot{Q}$ increased by 240 % from 9±0.87 to 31±2.9 ml kg^{-1} min^{-1}, f_H by 72 % from 16±0.41 to 28±1.0 beats min^{-1} and V_S by 120 % from 0.515±0.04 to 1.12±0.06 ml kg^{-1}. From rest to maximum activity, V_S constituted 69 % of the increase in $\dot{Q}$ at 10 °C and 62 % at 5 °C. The $\dot{M}_{O_2}$ *versus* $\dot{Q}$ relationship had a much higher correlation (r^2=0.86) at 10 °C (Fig. 4) than the $\dot{M}_{O_2}$ *versus* f_H relationship (r^2=0.50) (Fig. 5; dotted line).

When the $\dot{M}_{O_2}$ *versus* f_H relationship was examined on a day-to-day basis following surgery, the slope of the line remained constant but the line shifted to the left such that, over time, $\dot{M}_{O_2}$ increased for the same f_H. Indeed, heart rate decreased from approximately 46 to 28 beats min^{-1} (at rest) over the first week following surgery. The downward trend in f_H is clearly illustrated in Fig. 7. Immediately after surgery, f_H was higher than that observed at maximum levels of swimming activity (approximately 40 beats min^{-1}); f_H took upwards of 8–10 days to stabilize eventually at 28 beats min^{-1}. As the fish recovered, heart rate decreased progressively as $\dot{Q}$ increased, corresponding to a gradual increase in V_S. On any given day, the relationship between $\dot{M}_{O_2}$ and f_H can be very highly correlated, and if the f_H data from 'recovered' (greater than 7 days post-surgery) cod only are related to $\dot{M}_{O_2}$ (Fig. 5, asterisks), the relationship (r^2=0.86; P<0.0001) is as strong as that between $\dot{M}_{O_2}$ and $\dot{Q}$. During the recovery period, the $\dot{M}_{O_2}$ *versus* $\dot{Q}$ relationship did not change appreciably.

High-resolution measurements of activity

Fig. 8 illustrates the $\dot{Q}$ of a resting cod that initially gently beat its pectoral fins, then stopped for 15 min before restarting again at 0.63 Hz. Generally, while resting, cod spontaneously beat their pectorals or caudal fin for short periods and, as a result, true resting rates of $\dot{Q}$ are difficult to measure. The response of $\dot{Q}$ to the beating pectoral fins was similar to that observed when a cod flexes its tail and starts to swim. Cardiac output did not rise immediately after the onset of exercise but took up to 10 min before stabilizing. If we assume that the drop in $\dot{Q}$ when pectoral fin beating stopped was related to the

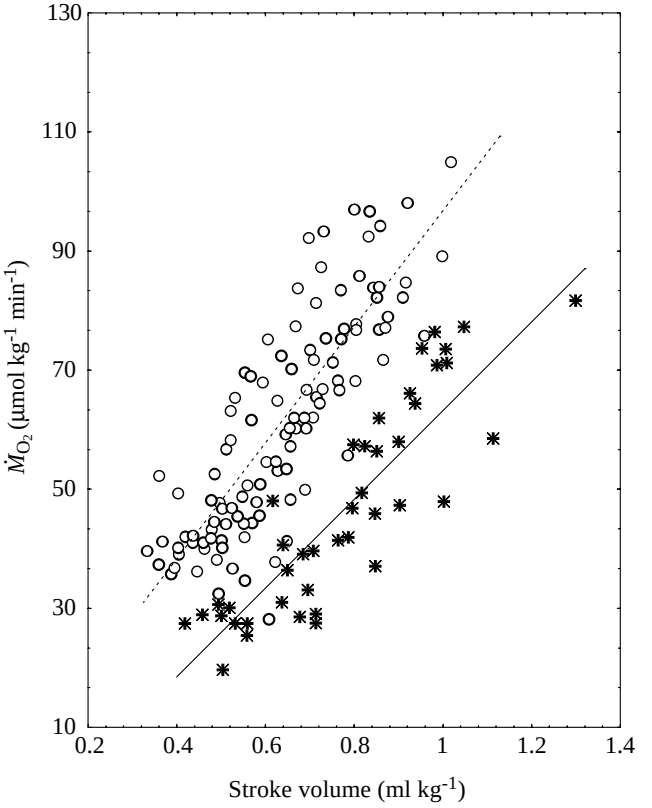

Fig. 6. The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and stroke volume (Vs) in cod *Gadus morhua* at 5 °C (asterisks; solid line) ($\dot{M}_{O_2}$=−12.4+75.3Vs; r^2=0.71; P<0.0001; N=5) and at 10 °C (open circles; dashed line) ($\dot{M}_{O_2}$=−2.6+98.7Vs; r^2=0.70; P<0.0001; N=7).

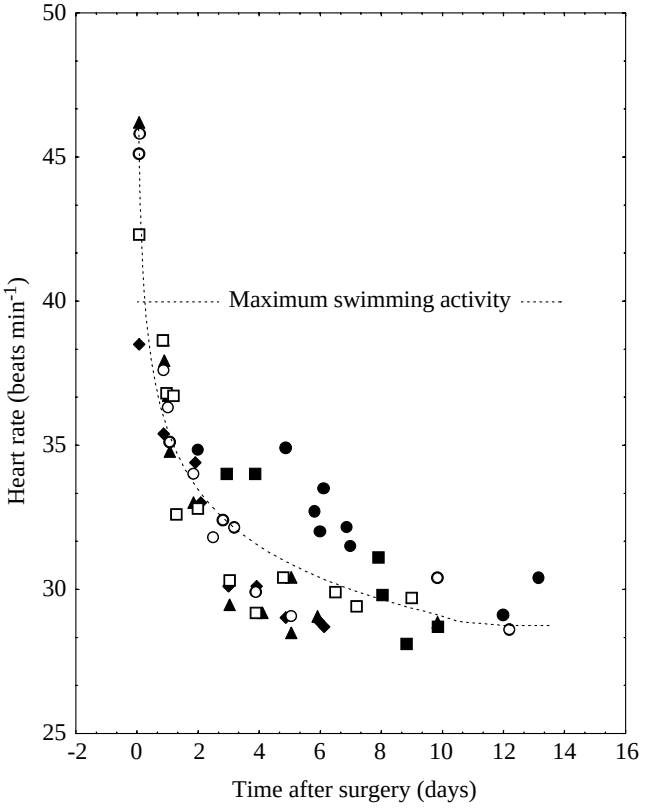

Fig. 7. Heart rate (f_H) plotted against time (t) after surgery for resting cod *Gadus morhua* at 10 °C (f_H=35.6$t^{-0.873}$; r^2=0.81; P<0.0001; N=6). Each different symbol represents a different fish. The mean value of f_H at maximum swimming activity is indicated.

drop in $\dot{M}_{O_2}$, then the cost of pectoral fin beating can be estimated from the $\dot{M}_{O_2}$ *versus* $\dot{Q}$ relationship. We used 12.7 ml kg^{-1} min^{-1} as a baseline value of $\dot{Q}$, estimated the drop in $\dot{Q}$ from Fig. 8 and calculated $\dot{M}_{O_2}$ (from Fig. 4) to be 3.4 % of total metabolic rate or 5.6 % of the aerobic scope.

Discussion

$\dot{M}_{O_2}$ *and cardiac variables*

Our data indicate that cardiac output ($\dot{Q}$) shows great promise as a tool to estimate metabolic rate for use in bioenergetic models of free-swimming fish in nature. Certainly, $\dot{Q}$ is a much more robust and reliable indicator of $\dot{M}_{O_2}$ than is f_H. For example, the $\dot{M}_{O_2}$ *versus* $\dot{Q}$ relationship was independent of time after surgery and of temperature and was linear over a range of activity levels (Fig. 4). In contrast the $\dot{M}_{O_2}$ *versus* f_H relationship depended on both temperature and time after surgery in terms of intercept but not slope (Fig. 5).

We stress that, by using a $\dot{M}_{O_2}$ *versus* f_H calibration from the recovery period, $\dot{M}_{O_2}$ can be overestimated by as much as 100 %. Thorarensen *et al.* (1996*a*) state that the relationship between $\dot{M}_{O_2}$ and f_H is subject to change depending on the physiological condition of a fish and environmental factors. They present evidence that several $\dot{M}_{O_2}$ *versus* f_H curves exist in the literature for the same species and, as a result, they have proscribed the use of f_H as a useful tool for measuring the metabolic rate of fish in nature. However, we would like to point out that, if fish are allowed to recover fully from surgery and if individuals are calibrated before release into nature rather than predicting $\dot{M}_{O_2}$ from f_H at a population level, then many of the concerns of Thorarensen *et al.* (1996*a*) can probably be ameliorated. Our results demonstrate that the relationship between $\dot{M}_{O_2}$ and f_H, for cod, is temperature-dependent and are in agreement with Claireaux *et al.* (1995*a*). Claireaux *et al.* (1995*a*) also observed that the $\dot{M}_{O_2}$ *versus* f_H relationship varied with seawater oxygen saturation. Temperature can easily be measured in the laboratory and telemetered in the field. We suggest that, for cod, f_H can be useful under known conditions of temperature, oxygen level and salinity in fully recovered animals; however, the use of f_H as a predictor of $\dot{M}_{O_2}$ will be subject to uncertainty for a free-ranging animal patrolling large volumes of water through changing fields of temperature and changing oxygen and salinity levels.

Clearly, surgically induced trauma affects f_H. Presumably, earlier studies that report poor correlations between $\dot{M}_{O_2}$ and f_H have not considered the physiological effects of surgical

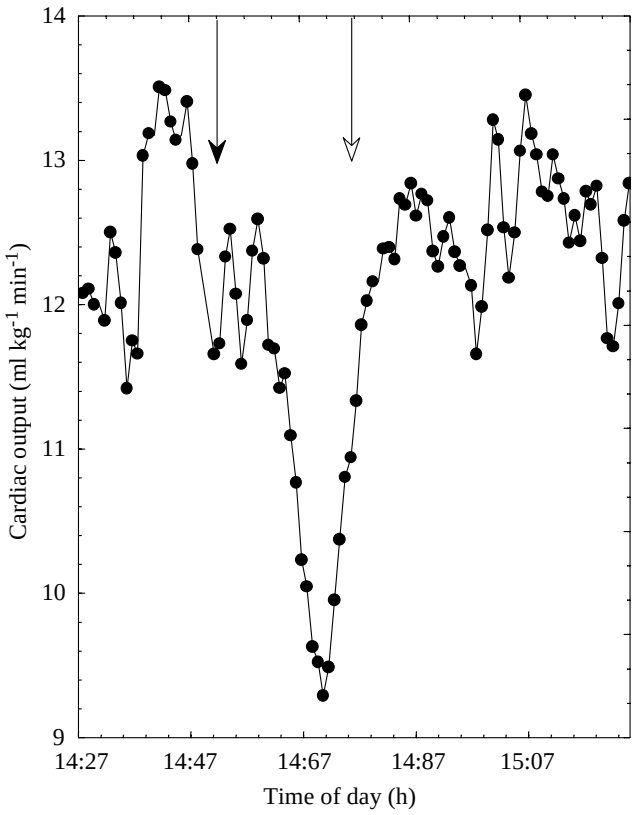

Fig. 8. Cardiac output ($\dot{Q}$) plotted against time of day for a resting cod *Gadus morhua* at 10 °C. The solid arrow indicates the time when the cod stopped beating its pectoral fins and the open arrow indicates when it started to beat its pectoral fins at 0.63 Hz. A mean cardiac output was calculated 15 min before and 15 min after the arrows ($\dot{Q}_{base}$, 12.7 ml kg^{-1} min^{-1}) and between the arrows ($\dot{Q}_{drop}$, 11.2 ml kg^{-1} min^{-1}). The difference ($\dot{Q}_{base}$–$\dot{Q}_{drop}$) of 1.5 ml kg^{-1} min^{-1} was used to estimate the rate of oxygen consumption ($\dot{M}_{O_2}$) (3.3 μmol kg^{-1} min^{-1}) from Fig. 4. The percentage of metabolic costs of the total metabolic scope and activity scope were determined to be 3.4 % and 5.6 % respectively.

trauma. A detailed time course following surgery has not been previously reported; accordingly, f_H may still be a very useful tool for estimating metabolic rate if full post-operative recovery is achieved before calibration and experimentation. Heart rate is the conventional cardiovascular variable recorded but, except for a study on frogs (Dumsday, 1990), there are few data describing its long-term variation. A number of factors influence the metabolic *status quo*, and it has long been postulated that confinement and external stimuli can be major causes of stress to animals. Gamperl *et al.* (1994) have shown that cannulated trout in 'black-box' confinement have elevated levels of plasma cortisol and catecholamines, and the time course to reach resting levels is 6–8 days post-confinement. In our study, a similar time course was observed for the $\dot{M}_{O_2}$ *versus* f_H relationship (Figs 5, 7), with f_H taking upwards of 8–10 days to stabilize. As f_H is influenced adrenergically in cod (Farrell, 1984; Fritsche and Nilsson, 1990), our f_H results are consistent with the hormonal data of Gamperl *et al.* (1994). An obvious implication is that fish must be given approximately 1 week to recover from surgical procedures before meaningful measurements can be made. Most earlier studies have acclimated fish for 24 h or less before measuring $\dot{M}_{O_2}$ and other cardiac variables, and this is clearly not sufficient in cod. For instance, a study designed to examine the relative contributions of f_H and V_S to increases in $\dot{Q}$ during exercise might, on the basis of abnormally high values of f_H 24 h after surgery (Fig. 7), erroneously conclude that f_H plays little or no role.

Very few studies have reported $\dot{Q}$ for controlled levels of exercise (Stevens and Randall, 1967; Kiceniuk and Jones, 1977; Davie and Forster, 1980; Kolok *et al.* 1993; Keen and Farrell, 1994; Thorarensen *et al.* 1996*b*; Korsmeyer *et al.* 1997*a*), and no studies have measured $\dot{Q}$ directly in relation to oxidative metabolism in fish. Our measurements show that $\dot{Q}$ increased by almost 200 % at 10 °C from rest to near-maximum exercise, a rise similar in magnitude to that in $\dot{Q}$ during a swim to the critical swimming speed (U_{crit}) in the rainbow trout (*Oncorhynchus mykiss*) (Kiceniuk and Jones, 1977). The percentage increase in $\dot{Q}$ at 10 °C as swimming speed increased from 0 to 0.67 body length s^{-1} in cod was 100 % in this study compared with 47 % in a study by Axelsson and Nilsson (1986) and 57 % in a study by Axelsson (1988). There are, however, a number of differences between these studies. The cod used by Axelsson and Nilsson (1986) and Axelsson (1988) weighed 0.5–0.6 kg compared with the 2 kg cod used in the present study, and the scaling effect between mass and $\dot{Q}$ is unknown. Also, their cod were from North Sea populations considered genetically distinct from the Scotian Shelf cod used in this study (Pogson *et al.* 1995). North Sea cod inhabit water of warmer annular temperatures. Also, our highest $\dot{Q}$ values were recorded at least 2 or 3 days after surgery.

With few exceptions, increases in $\dot{Q}$ were brought about by increases in both stroke volume (V_S) and f_H, with V_S predominating. In the present study, the percentage contributions to $\dot{Q}$ by f_H and V_S for all 5 °C and 10 °C data were 35 % and 65 %, respectively (42 and 58 %, respectively, calculated from Axelsson and Nilsson, 1986; Axelsson, 1988). Our measurements of V_S at rest (0.45 ml kg^{-1}) and during exercise (0.88 ml kg^{-1}) are consistent with values reported in other studies (Hart, 1943; Kiceniuk and Jones, 1977; Kolok *et al.* 1993; Takeda, 1993; Keen and Farrell, 1994; Thorarensen *et al.* 1996*b*). This means that V_S largely accounts for the differences in $\dot{Q}$ during exercise in cod. Thus, the predominantly V_S-modulated increase in $\dot{Q}$ in cod contrasts markedly with the situation in higher vertebrates, where increases in $\dot{Q}$ are primarily frequency-modulated. Frequency modulation is thought to be an advantage in higher vertebrates. Amphibians and reptiles have two atria and one ventricle. With this design, arterial and venous blood depend on laminar flow to keep them from mixing, and large increases in V_S might cause undesirable mixing of the venous and arterial bloodstreams (Satchell, 1991).

Data on rates of oxygen consumption and cardiac output for cod from this study and for other fish species are shown in Fig. 9. Although cardiac output in the other studies was

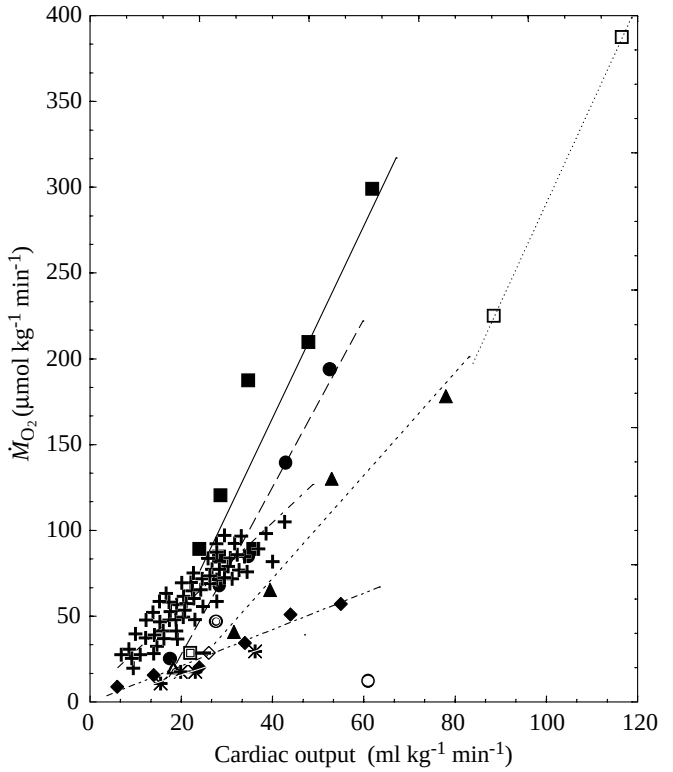

Fig. 9. The relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and cardiac output ($\dot{Q}$) for various fish species. Cardiac output values in this study were measured directly using a Doppler ultrasonic probe. The remaining data were estimated using the Fick principal, based on arterial-venous blood oxygen content difference and whole-body oxygen consumption or by combining data for a species from different publications. Data were taken directly or calculated from other studies. Open squares, yellowfin tuna (*Thunnus albacares*) at 24–25 °C (Korsmeyer *et al.* 1997*a*,*b*; Dewer and Graham, 1994) ($\dot{M}_{O_2}$=–286.8+5.8$\dot{Q}$, N=2); filled squares, albacore tuna (*Thunnus alalunga*) at 22 °C (White *et al.* 1988) ($\dot{M}_{O_2}$=–54.9+5.61$\dot{Q}$, N=7, r^2=0.81, P<0.05); filled circles, rainbow trout (*Oncorhynchus mykiss*) at 9–10.5 °C (Kiceniuk and Jones, 1977) ($\dot{M}_{O_2}$=–68.6+4.84$\dot{Q}$, N=5, r^2=0.97, P<0.001); filled triangles, carp (*Cyprinus carpio*) at 24–25 °C (Takeda, 1993) ($\dot{M}_{O_2}$=–96.9+4.24$\dot{Q}$, N=5, r^2=0.94, P<0.05); open triangle, carp (*Cyprinus carpio*) at 10 °C (Garey, 1970); plus signs, Atlantic cod (*Gadus morhua*) at 5 and 10 °C (this study) ($\dot{M}_{O_2}$=5.6+2.46$\dot{Q}$, N=206 from seven cod, r^2=0.86, P<0.0001); asterisks, winter flounder (*Pseudopleuronectes americanus*) at 5–10 °C (Cech *et al.* 1976) ($\dot{M}_{O_2}$=0.469+0.831$\dot{Q}$, N=5, r^2=0.93, P<0.01); filled diamonds, Pacific dogfish (*Squalus suckleyi*) at 6–10 °C (Hanson and Johansen, 1970) ($\dot{M}_{O_2}$=0.09+1.052$\dot{Q}$, N=6, r^2=0.96, P<0.001); open diamond, Pacific dogfish (*Squalus suckleyi*) at 11 °C (Lenfant and Johansen, 1966); double open square, dogfish (*Scyliorhinus stellaris*) at 16 °C (Baumgarten-Schulmann and Piiper, 1968); minus sign, dogfish (*Squalus acanthias*) at 12 °C (Robin *et al.* 1966); double circle, shorthorn sculpin (*Myoxocephalus scorpius*) at 15–18 °C (Goldstein *et al.* 1964); open circle, haemoglobinless Antarctic fish (*Chaenocephalus aceratus*) at 0.3–1.4 °C (Holeton, 1970).

calculated using the Fick equation, it is clear that cardiac output can be used to estimate the rate of oxygen consumption for both sluggish and active fish species. Animals that have high metabolic requirements have high cardiac outputs (Farrell and Jones, 1992), and their $\dot{M}_{O_2}$ *versus* $\dot{Q}$ slopes are steeper (Fig. 9).

High-resolution measurements of activity

An advantage of the Doppler flow measurement technique is that small and low-frequency metabolic events can be detected. Generally, while resting, cod spontaneously beat their pectoral or caudal fins for short periods and, as a result, a true resting heart rate is difficult to measure. The response of $\dot{Q}$ to the beating pectoral fins was similar to that observed when a cod flexes its tail and starts to swim. Oxygen measurements, at the level of resolution described here, would not reveal subtle activities of this kind. If our assumptions are correct, this means that for free-swimming fish it will be possible to measure $\dot{Q}$ in low-frequency events and to estimate with high accuracy the metabolic cost to the animal. This, in turn, will mean more accurate bioenergetic estimates. It may also prove useful in this and other species in determining the energetic costs of various behaviours (e.g. reproduction, courtship, territorial defence).

The effects of temperature on $\dot{M}_{O_2}$ *and cardiac variables*

Cardiac and swimming performance is greatly affected by temperature (Keen and Farrell, 1994), and if exercise is linked to cardiac performance one would expect similar trends between metabolic rate and cardiac variables. The increased metabolic demands during exercise are met through complex interactions between ventilation volume and frequency, blood oxygen content and $\dot{Q}$. Both standard and active metabolic rates were lower at 5 °C than at 10 °C. The Q_{10} for $\dot{M}_{O_2}$ from 5 to 10 °C was 2.5 at rest and 2.1 at high levels of activity. Acute and chronic changes in temperature are expected to affect $\dot{Q}$; a Q_{10} of 1.0 would indicate complete temperature compensation and a Q_{10} of 2.0 would indicate temperature conformity. The Q_{10} values of $\dot{Q}$ and f_H from 5 to 10 °C were 1.65 and 2.93 for rest and 1.22 and 1.97 for near-maximum activity, respectively. In both cases, f_H was the more temperature-dependent component of $\dot{Q}$. Therefore, the reduction in $\dot{Q}$ with lower temperature was attributable mostly to a large reduction in f_H. This sensitivity of f_H to temperature has been observed in other studies (Armstrong, 1986; Gehrke and Fielder, 1988; Kolok *et al.* 1993; Keen and Farrell, 1994; Claireaux *et al.* 1995*a*), and there is considerable variability among species. The large reduction in f_H from 10 to 5 °C was partially compensated by an increase in V_S (i.e. stroke volume was higher at 5 °C than at 10 °C).

Temperature is a strong determinant of the $\dot{M}_{O_2}$ *versus* f_H relationship. Heart rate demonstrated a greater thermal dependence, compared with V_S, and it is well known that temperature affects f_H directly through changes in pacemaker rate and response to modulators (Farrell, 1984). Therefore, one should not necessarily expect f_H to follow metabolic rate if pacemaker rate is influenced by the interplay of a variety of effector mechanisms. Claireaux *et al.* (1995*a*) also report $\dot{M}_{O_2}$ *versus* f_H relationships for cod at 5 °C and describe left and right shifts in the intercept when cod are exposed to 2.5 and

7.5 °C, respectively. The $\dot{M}_{O_2}$ *versus* f_H relationship at 5 °C for cod from this study closely matches the data from Claireaux *et al.* (1995*a*) for cod from the same geographical region.

The Q_{10} for $\dot{Q}$ from 5 to 10 °C (1.65) was lower than the Q_{10} for $\dot{M}_{O_2}$ (2.5). According to the Fick equation, this would mean that, at the lower temperature, the arterial-venous oxygen content difference is smaller. With lower metabolic demands at lower temperatures, cod probably maintain a high arterial blood oxygen content but remove less oxygen from the blood while lowering $\dot{Q}$ by only a small amount.

Stroke volume at rest was only slightly lower at 10 °C (V_S=0.45 ml kg^{-1}) than at 5 °C (V_S=0.52 ml kg^{-1}) (P>0.05; N=7). At high activity, the lower V_S at 10 °C (V_S=0.88 ml kg^{-1} *versus* 1.12 ml kg^{-1} at 5 °C; P<0.05; N=7) can simply be interpreted as either cardiac filling time being compromised because of higher contraction frequency or a reduction in the force of contraction at high frequency (Farrell *et al.* 1989).

$\dot{M}_{O_2}$ *and swimming speed*

Our observations also permit conclusions about the relationship between swimming speed and the rate of oxygen consumption ($\dot{M}_{O_2}$) in cod, which has been described by a number of authors. Our $\dot{M}_{O_2}$ values for standard (22 and 35 μmol O_2 kg^{-1} min^{-1}) and active (72 and 97 μmol O_2 kg^{-1} min^{-1}) metabolic rate at 5 and 10 °C for cod of 2 kg closely match the $\dot{M}_{O_2}$ observed by Saunders (1963) (20.2 and 35 μmol O_2 kg^{-1} min^{-1} at 3 and 10 °C, respectively) and Edwards *et al.* (1972) (33.3 μmol O_2 kg^{-1} min^{-1} at 12 °C) for routine metabolism of unfed Bay of Fundy cod and fall between the values reported by Nelson *et al.* (1994, 1996), Reidy *et al.* (1995) and Tang *et al.* (1994) at 2, 5 and 15 °C for the same stock of fish (16.1, 31.5 and 60 μmol O_2 kg^{-1} min^{-1} for standard metabolic rate and 54, 76 and 108 μmol O_2 kg^{-1} min^{-1} for active metabolic rate at 2, 5 and 15 °C respectively). The major difference between the present study and the others is that the cod in our study spent up to 8–16 weeks in the respirometer. This may account for the observed low resting and standard $\dot{M}_{O_2}$ values. In the present study, $\dot{M}_{O_2}$ increased exponentially with swimming speed (Fig. 3). An exponential relationship between swimming speed and $\dot{M}_{O_2}$ is expected because, in fish, the predominant inertial drag forces opposing forward momentum increase as a power function as swimming speed increases linearly (Webb, 1978). Some studies report linear relationships, probably because $\dot{M}_{O_2}$ measurements at low swimming speeds are inflated due to animal stress or hyperactivity. Although we did not determine maximum $\dot{M}_{O_2}$ at the critical swimming speed, the observed facultative scopes for activity were 50 and 62 μmol O_2 kg^{-1} min^{-1}, and active metabolic rate was 3.3 and 2.8 times the extrapolated standard metabolic rate values at 5 and 10 °C respectively.

To conclude, our results clearly show that cardiac output rather than f_H is the physiological correlate that will permit the most accurate estimates of metabolic rate in free-swimming fish. Cardiac output very accurately represents the metabolic rate of exercising cod and will be useful as a measure of metabolic costs resulting from other physiological processes. We are currently designing an ultrasonic cardiac output transmitter that will make it possible to estimate the metabolic rates of free-swimming fish in the field.

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