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PHYSIOLOGY AND BEHAVIOUR OF FREE-SWIMMING ATLANTIC COD (*GADUS MORHUA*) FACING FLUCTUATING TEMPERATURE CONDITIONS

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Summary

Atlantic cod (*Gadus morhua* L.), acclimated to 5 °C, were equipped with ultrasonic transmitters which allowed the continuous monitoring of their vertical movements and heart rate. Fish were then placed in a 125 m^3 tower tank in which the various thermal conditions they encounter in their natural environment were reproduced. Physiological and behavioural responses of cod were followed in parallel to the induced environmental changes. The experimental conditions studied in the tower tank were also reproduced in a swimming respirometer, where oxygen consumption and heart rate could be monitored within the activity range of a free-swimming animal.

In a homogeneous water column, a rise in temperature induced marked increases in fish swimming activity, heart rate and heart beat-to-beat variability. In a thermally stratified environment, voluntary activity also increased when the thermal structure of the water column was altered, though no temperature-dependent changes in heart rate were observed. In this case, fish avoided the new temperature conditions, exhibiting distinct thermoregulatory behaviour. Stratification of the water column also prompted daily cyclic changes in fish distribution, animals tending to be in deeper and colder water layers during the day and in shallower and warmer layers at night.

Respirometry experiments revealed that the thermoregulatory behaviour observed in free-ranging fish was probably driven by the energetic expedient of maintaining the physiological *status quo* – i.e. avoiding bioenergically costly reacclimation processes. Indeed, acute temperature increases or decreases of 2.5 °C led to marked differences in oxygen consumption, with metabolic rate changes of 15 and 30 %, respectively. The persistent linear relationship between heart rate and oxygen consumption allowed us to estimate, from the heart rate recorded in free-swimming fish, the entire range of metabolic responses that cod underwent voluntarily while experiencing a thermally stratified water column. The most profound metabolic effect, however, was observed with feeding, when oxygen consumption increased by as much as 80 %, resulting in an estimated 90 % reduction in their subsequent scope for activity.

Key words: temperature, heart rate, depth, oxygen consumption, exercise, ultrasonic transmitter, thermocline, cod, *Gadus morhua*.

Introduction

It can be argued that animals optimize their energy expenditure in nature (Arnold, 1988). This continuous 'optimization' process requires that fish be able to monitor and integrate key environmental variables, and then quickly and efficiently react through a number of regulatory behavioural responses. Temperature is almost certainly one of the most important environmental effectors of energy expenditure because of its direct impact on the metabolic rate. Numerous studies have shown a close link between the distribution pattern of cod and the thermal structure of the surrounding waters (Jean, 1964; Templeman, 1966; Nakken and Raknes, 1987; Rose and Leggett, 1988*a*,*b*, 1989; Perry and Neilson, 1988). However, the nature of the processes that allow animals to respond accurately to alterations in environmental temperature remains unclear. The lack of information concerning such processes arises because of the necessity of tracking physiological responses of animals moving about freely in their natural environment. For example, fish will adopt a distribution pattern according to the various thermal 'options' available. Knowing what these options are is essential if one wants to understand and/or predict the dissemination of fish in a given environment. It follows that not only must the immediate surroundings of the fish be taken into account, but also the thermal history of the animal. A second problem in studies of this kind results from the need to obtain an image of the physical environment which is as close as possible to the one being integrated by the animal. This means that the environment must be sampled at the appropriate

scale. To keep 'up to date' with their surroundings, fish actively and continuously patrol a certain volume of water, in particular when environmental conditions are changing. This volume is probably a good approximation of the scale at which individuals integrate their environment (i.e. tens to hundreds of metres). The third problem is linked to the fact that, in nature, simultaneous changes in several environmental factors are frequent, making the evaluation of their respective impacts difficult.

To study some aspects of the process that determines their distribution pattern, we reproduced, in a 125 m^3 tower tank, various thermal conditions encountered by cod in their natural environment. In this 'mesocosm', the environmental variables were monitored and controlled very accurately and on a very fine scale. Using ultrasonic transmitters carried by the animals, the physiological and behavioural responses associated with each experimental condition were continuously followed. In this manner, the fish heart rate and vertical movements were recorded for extended periods up to 3 weeks. Moreover, in order to establish whether the behavioural responses observed in free-ranging animals were governed by the necessity to meet specific metabolic requirements and/or to maintain a physiological *status quo*, some of these environmental conditions were also reproduced in a swimming respirometer where the relationship between heart rate and oxygen consumption could be accurately determined.

Materials and methods

Fish holding

Adult Atlantic cod *Gadus morhua* L. of both sexes, weighing 1.2–1.5 kg (55–65 cm), were captured by local fishermen off the shore of Nova Scotia (NAFO fishing area 4W). They were brought to our rearing facilities, where they were allowed to acclimate for 2 months in 2 m^3 indoor tanks supplied with running sea water at 5 °C. The artificial lighting followed the natural photoperiod. Fish were fed 2–3 times a week with a mixed diet consisting of frozen squid, capelin, herring, smelt and/or mackerel. Feeding was discontinued 48 h prior to any manipulation of the animals.

Ultrasonic transmitters

The ultrasonic transmitters and a model VR-15 ultrasonic receiver were manufactured by VEMCO Ltd (RR#4 Armdale, Halifax, Nova Scotia, Canada B3L 4J4). The transmitters were approximately 10 cm long, 1.5 cm in diameter, weighed 35 g in air and had presumably no impact on the hydrodynamic characteristics of the animal. Indeed, according to Arnold and Holford (1978), a 40 cm cod fitted with an external transmitter of similar size would have to increase its power output by less than 1 % to achieve the same acceleration as an untagged fish.

Heart rate (f_H) was monitored by recording the pulse rate emitted by an electrocardiogram transmitter. Signal detection was *via* two differential silver-tipped input electrodes surgically placed approximately 0.5–1 cm apart in the pericardial cavity, while the reference electrode was exposed to sea water. Individual pulse duration was 15 ms and the pulse rate was equal to the heart signal rate for signals above a threshold of approximately 120–150 μV.

Pressure transmitters were of the semiconductor strain gauge bridge type. The transmitter electronics converted a bridge voltage to a pulse rate that was linearly proportional to the pressure (depth). These transmitters were calibrated prior to the experiment and depths were measured with an accuracy of ±5 cm.

The ultrasonic receiver was interfaced with a computer using an RS-232 serial port. The data acquisition system allowed the computer to monitor up to three fish (i.e. six transmitters) during each trial. The transmitters used separate frequencies ranging from 50.0 to 76.8 kHz. The data acquisition system alternately recorded the signal from each transmitter for a period of approximately 30 s. The number of transmitters actually scanned during these experiments varied but never exceeded four and, therefore, successive samples from the same transmitter were never more than 2 min apart. To evaluate the vertical distance covered by fish during a given time interval, the distance the fish swam between two scanning periods was extrapolated from the swimming activity recorded during the preceding 30 s scanning period. Because only changes in pressure (depth) were detected, swimming activity measurements reported here underestimate the actual movements of the fish. However, because the tank was 10.5 m deep and only 4 m in diameter, our estimates of fish activity are expected to index actual activity accurately.

Surgical and instrumentation procedures

Cod were anaesthetized in a solution of ethyl-*m*-aminobenzoate (0.25 $g\,l^{-1}$; MS222) and their mass and length were measured. Throughout the surgical procedure, the gills were perfused with oxygenated water containing a small dose of MS222 (0.01 $g\,l^{-1}$). They were fitted with a ventral harness holding only a heart rate transmitter (respirometry) or both types of transmitter (tower tank). The electrocardiogram electrodes were inserted through a small incision in the skin (<1 cm long). The entire procedure took 30 min to complete. Fish resumed ventilatory activity within 5 min when ventilated with anaesthetic-free water and were then transferred to a holding tank, where they were allowed to recover for 24 h.

Oxygen consumption measurements and swim tunnel respirometry

Respirometry experiments were performed in a 62 l Brett-type swim tunnel. Oxygen tension and water velocity in the swimming chamber were continuously recorded on a computer through a 12-bit analog-to-digital integrating converter. The computer also monitored and controlled the water temperature in the swim tunnel (±0.05 °C). Fifty measurements of water P_{O_2}, temperature and velocity were made every 30 s, and the averaged values were stored for later analysis. Water oxygen tension was measured using a

Radiometer PHM-73 meter connected to an oxygen electrode (E-5046) mounted in a cuvette (D616) thermostatted at the experimental temperature. A constant water flow over the electrode membrane was achieved using an Ismatec peristaltic pump. The oxygen electrode was calibrated with a zero-P_{O_2} solution and air-saturated water. Oxygen consumption ($\dot{M}_{O_2}$) was expressed in μmoles of oxygen consumed per minute per kilogram of fish.

Having recovered from surgery, fish were transferred into the swim tunnel respirometer and acclimated for another 72 h at low speed (7 cm s^{-1}) prior to any data collection. The respirometer was supplied continuously with well-aerated 5.0 °C sea water. During this second acclimation period, fish were trained to swim at speeds of up to 37 cm s^{-1} (i.e. 0.67 body lengths s^{-1}). These training sequences never lasted more than 15 min and later allowed us to swim the animals without having to resort to any kind of external stimulation. Following the acclimation and training procedure, the aerated seawater supply flow to the respirometer was periodically stopped and the reduction in the water oxygen partial pressure was continuously monitored. Oxygen consumption was first measured with the water flow set at 7 cm s^{-1} (0.13 body lengths s^{-1}) and then at successively increasing water speeds (18, 27, 37, 48 and 57 cm s^{-1}, i.e. 0.33, 0.49, 0.67, 0.87 and 1.05 body lengths s^{-1}). At each step, fish swam for approximately 30 min but only the last 15 min period was used to calculate $\dot{M}_{O_2}$. Though heart rate was continuously monitored during the whole swimming sequence, only the averaged value measured over the final 15 min period was used in the data analysis. After 30 min, the water speed was increased and the oxygen level brought back to saturation before the next measurement period began. At the end of the trial (4 h), water speed was reduced to 7 cm s^{-1} and the fish were allowed to recover overnight. The next day, a control oxygen consumption measurement was performed at the lowest speed. The temperature of the water in the respirometer was then quickly reduced (<30 min) by 2.5 °C and the swimming sequence previously described was repeated on the same individual. At the end of the trial, water temperature in the respirometer was brought back to 5.0 °C. On the third day, the effect of an increase in water temperature from 5.0 to 7.5 °C was tested according to the same protocol.

$\dot{M}_{O_2}$ and f_H recorded during swimming in the respirometer were expressed as mean ± standard error of the mean (S.E.M.). They were compared with the low water speed reference condition using an unpaired Student's *t*-test (P<0.05).

Tower tank

Following placement of the transmitters, fish were transferred into a 125 m^3 'tower' tank (Fig. 1) and monitoring of depth and heart rate was initiated. The tank was filled from the same water supply as the acclimation and recovery tanks. The artificial lighting also followed the natural photoperiod. The light intensity was controlled such that the slow changes in light intensity occurring at sunset (08:30–08:45 h) and sunrise (18:30–18:45 h) could be reproduced. The temperature profile of the tank was measured regularly every 50 cm starting 50 cm below the surface down to the bottom of the tank (i.e. 10.5 m). To ensure that the transmitters had only a minor impact on the behaviour of the 'tagged' fish, 10 unequipped fish were also transferred to the tank and observed as controls. These fish also allowed us to assess the impact of the hierarchical organisation existing within a small school on the behaviour of individuals. Animals were fed at various depths through polyvinylchloride pipes of different length hanging on the side of the tank. Feeding depths were randomized to avoid the possibility of inadvertently 'training' the fish to feed at a particular station in the water column.

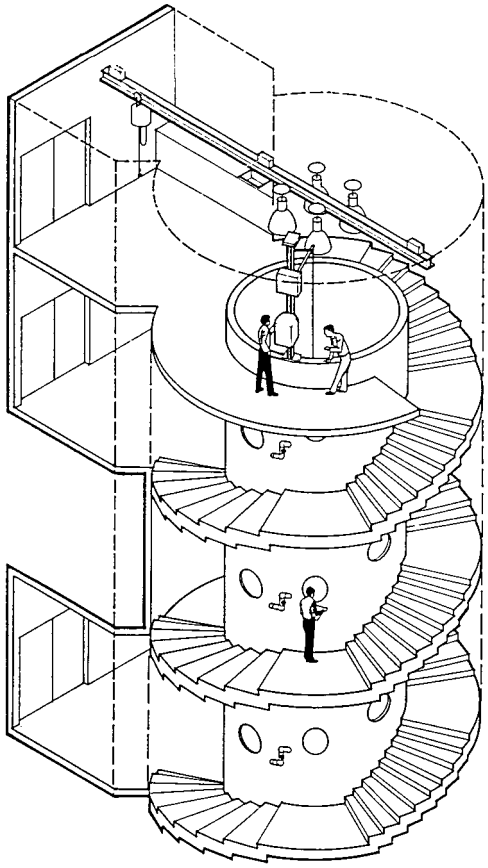

Fig. 1. Diagram of the tower tank used for the telemetry experiments. The tank is 10.5 m deep and 4 m in diameter. Windows displayed all around the tank allowed observation of the fish. Water inlets placed at regular intervals in the tank were used to achieve the thermal stratification of the water column.

Temperature regulation and stratification of the water column were achieved by flowing known volumes of heated or chilled sea water through inlets placed at various depths in the tank. The temperature of the water was further controlled using two titanium heat-exchange coils immersed at different levels in the tank. To check against the possible development of hypoxic layers in the tank, an oxygen profile was measured daily.

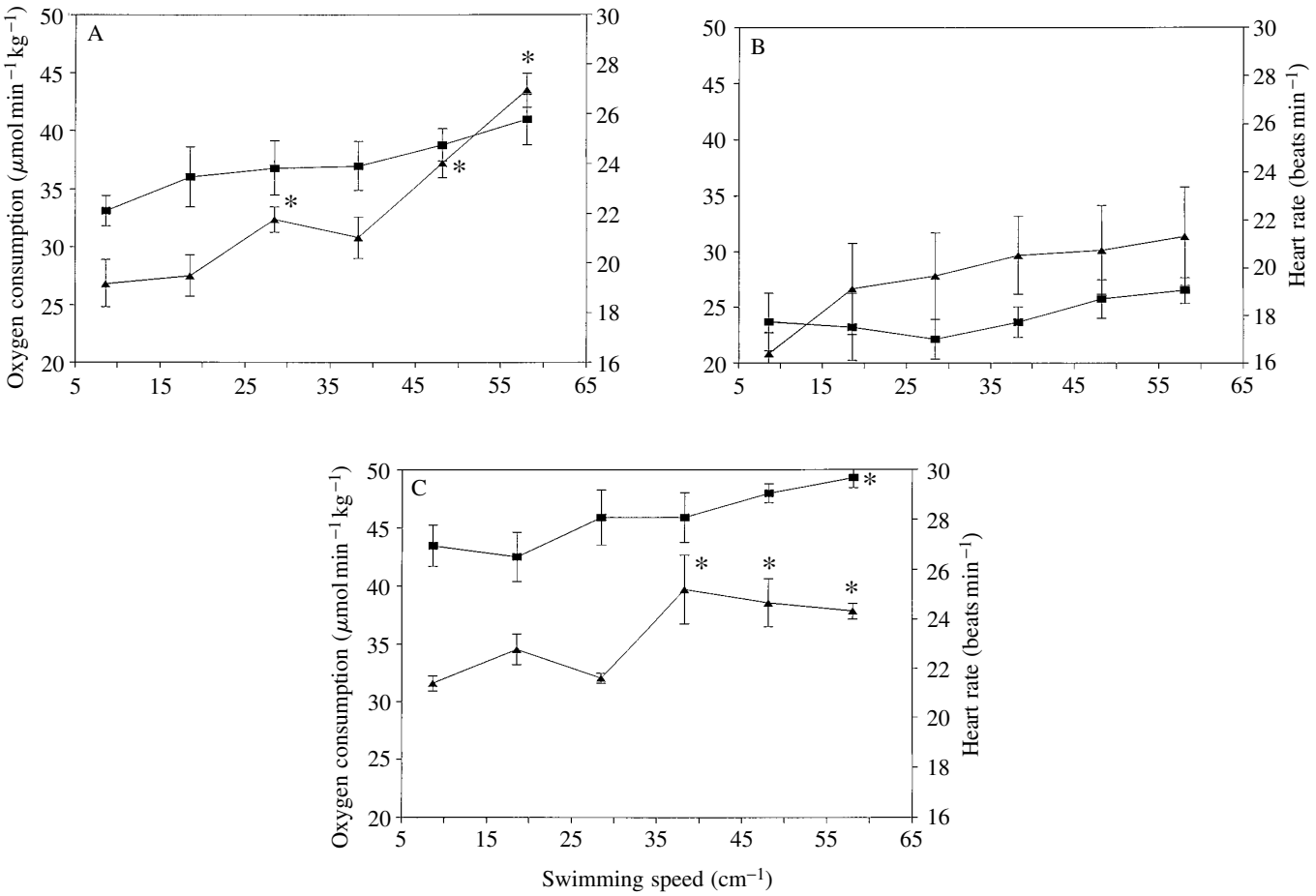

Fig. 2. Mean (± S.E.M.) oxygen consumption (triangles) and heart rate (squares) of 5.0 °C-acclimated cod swimming at speeds ranging from 7 to 57 cm s^{-1} (N=5). In A, fish were exercised at their acclimation temperature. In B, water temperature in the respirometer was dropped from 5.0 to 2.5 °C within 30 min prior to the swimming trial. In C, water temperature was increased from 5.0 to 7.5 °C within 30 min prior to the exercise sequence. Each fish was successively exposed to all three experimental conditions. Between each swimming sequence, water temperature was brought back to 5 °C and the fish allowed to recover overnight. Asterisks indicate a significant difference from resting values.

Results

Swim tunnel respirometry

These experiments were designed to evaluate the extent of the metabolic perturbations associated with small changes in water temperature and so to establish whether the thermoregulatory behaviour reported in this species is part of a global conservative strategy aimed at maintaining a physiological *status quo* in changing environmental conditions. The heart rate (f_H) and oxygen consumption ($\dot{M}_{O_2}$) of five cod (60 cm) swimming at increasing speed in three different thermal conditions are shown in Fig. 2. At their temperature of acclimation (i.e. 5 °C), fish $\dot{M}_{O_2}$ rose steadily with increasing exercise intensity, reaching a value of 43 μmol min^{-1} kg^{-1} at 57 cm s^{-1} (Fig. 2A). However, the concomitant increase in f_H (approximately 2 beats min^{-1}) was not significant. When water temperature in the swim tunnel was quickly reduced (within 30 min) to 2.5 °C, we observed a 20 % and 16 % drop in resting f_H and $\dot{M}_{O_2}$ respectively (Fig. 2B). During the subsequent exercise sequence, however,

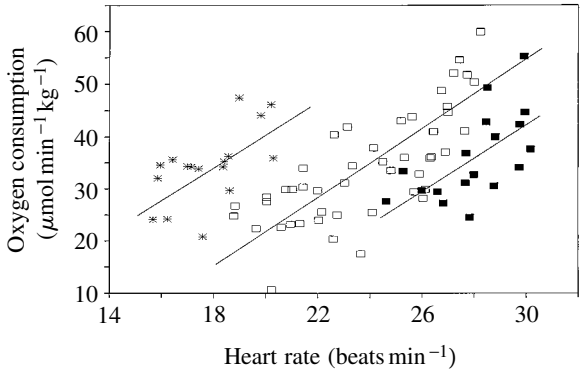

Fig. 3. Oxygen consumption *versus* heart rate relationships of 5.0 °C acclimated cod, at 5.0 °C (open squares), following a drop of the water temperature from 5 to 2.5 °C (asterisks) and following an increase of the temperature from 5 to 7.5 °C (filled squares). In this graph, data were plotted irrespective of the swimming speed. 5.0 °C, $\dot{M}_{O_2}$=2.98f_H−37.34, r=0.761; 2.5 °C, $\dot{M}_{O_2}$=2.96f_H−18.50, r=0.622; 7.5 °C, $\dot{M}_{O_2}$=3.12f_H−52.46, r=0.624

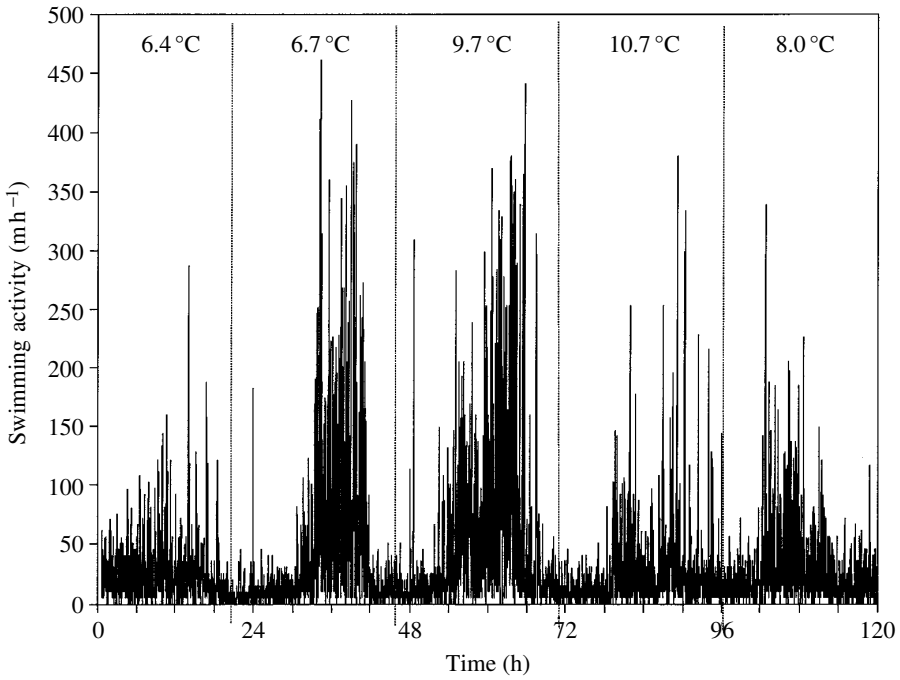

Fig. 4. Voluntary swimming activity of a 5.0 °C acclimated cod facing temperature fluctuations. Temperatures reported here are the daily mean values. During day 1, the total distance travelled by the fish was 1.94 km, during day 2 it was 3.69 km, on day 3 the fish covered 4.52 km, 2.14 km on day 4 and 2.36 km on day 5. In this experiment, only the average depth measured during each 30 s scanning period was stored by the data acquisition system.

the changes in f_H and $\dot{M}_{O_2}$ were not significant. When water temperature was increased from 5 to 7.5 °C (Fig. 2C), resting f_H and $\dot{M}_{O_2}$ rose sharply (+22 % and +28 % respectively). Subsequent changes in f_H with increasing swimming speed were minor and only became significant at the last speed tested. In contrast, $\dot{M}_{O_2}$ increased quickly between 27 and 37 cm s^{-1}, but thereafter remained constant.

The physiological and bioenergetic consequences of such temperature disturbances were even more apparent when the f_H values were plotted against their respective $\dot{M}_{O_2}$, irrespective of the swimming speed (Fig. 3). When compared with Fig. 2, the relatively strong correlations observed between f_H and $\dot{M}_{O_2}$ (Fig. 3) suggest that, within the speed range investigated, the intensity of the swimming activity was probably not the main factor determining cod heart rate and oxygen consumption. Temperature, however, appeared to be a strong determinant of the f_H *versus* $\dot{M}_{O_2}$ relationship. When compared with data obtained for 5 °C acclimated fish, the decrease in temperature induced a leftward shift in the f_H *versus* $\dot{M}_{O_2}$ relationship, a given oxygen consumption corresponding to a lower heart rate. Conversely, a 2.5 °C increase in temperature resulted in a rightward shift of this relationship.

Telemetry

Fig. 4 shows the vertical swimming activity of one fish (initially acclimated to 5 °C) monitored over a 5 day period during which the water temperature in the tower tank was altered in a homogeneous fashion. This record shows the existence of a daily cycle in the vertical distance travelled by the fish, the activity being low at night and high during the day. Moreover, the swimming activity increased sharply as the daily mean temperature rose from 6.4 °C on day 1 to 9.7 °C on day 3. On days 4 and 5, swimming activity returned to near reference levels, although the water temperature rose to 10.7 °C during day 4. The total daily distances covered by the fish were 1.94 km on day 1, 3.69 km on day 2 (+86 %), 4.52 km on day 3 (+128 %), 2.14 km on day 4 (+8 %) and 2.36 on day 5 (+19 %).

Fig. 5 is the record of the fish's heart rate during this period. As temperature increased from 6.4 to 10.7 °C, we observed a twofold increase in heart rate, f_H increasing from 20 beats min^{-1} on day 1 to 42 beats min^{-1} on day 4. As the temperature of the water was returned towards its initial value during day 5, the heart rate followed the same trend. From day 1 to day 3, the temperature-related decrease in water oxygen

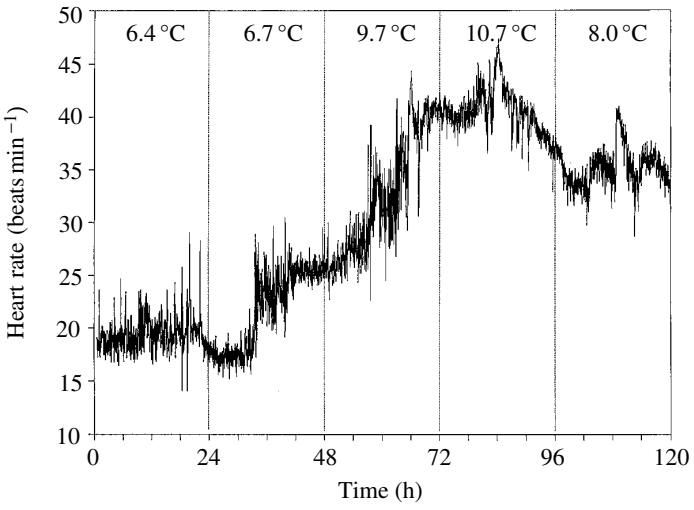

Fig. 5. Heart rate recording of the 5.0 °C acclimated fish considered in Fig. 4.

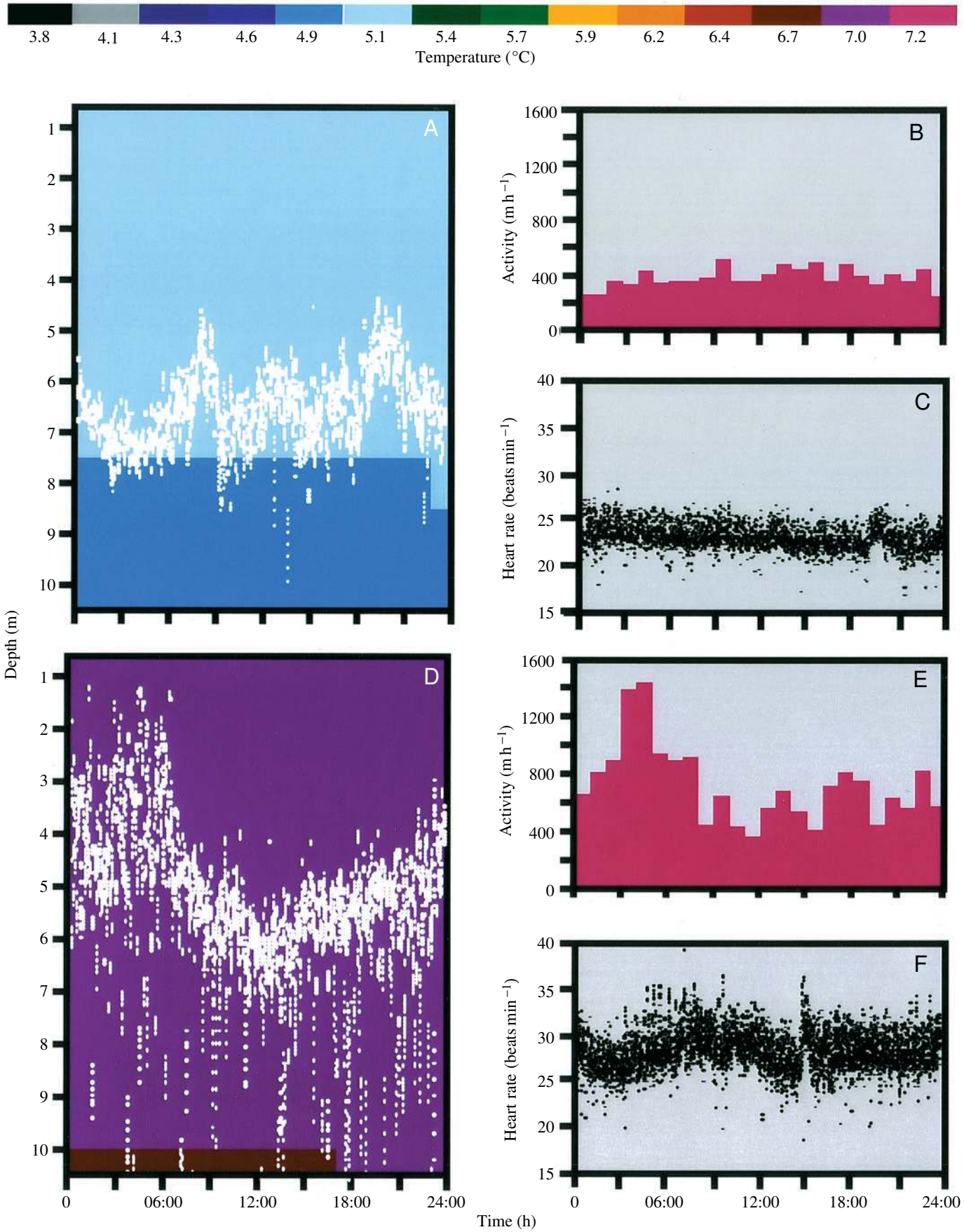
3.8
4.1
4.3
4.6
4.9
5.1
5.4
5.7
5.9
6.2
6.4
6.7
7.0
7.2
Temperature (°C)
A
B
C
D
E
F
Depth (m)
Activity (m h⁻¹)
Heart rate (beats min⁻¹)
Time (h)
0
06:00
12:00
18:00
24:00

Fig. 6

Fig. 6. Depth (A,B, white circles), swimming activity (B,E) and heart rate (C,F) of a 5.0 °C acclimated cod, monitored over 24 h, in two stable thermal situations (A,B,C, 5 °C; D,E,F, 7 °C). The temperature in the tank is given by the colour codes at the top of the figure. In this case (unlike Fig. 4), we recorded all vertical movements made by the animal while its transmitter was scanned.

content was approximately 10 % and was thus insufficient to explain the extent of the cardiac response recorded.

The relationship between the water temperature and the swimming activity of fish observed in Fig. 4 is further illustrated in Fig. 6. The vertical movements of an animal were monitored over a 24 h period in two different isothermal depth profiles. At its temperature of acclimation (Fig. 6A,B), the voluntary activity of the animal was relatively low, adding up to approximately 9.4 km per day. Note that the fish spent the great majority of its time at the nominal 5.1 °C level, as opposed to the 4.9 °C temperature also available. However, once the temperature was increased to 7 °C, this fish exhibited a dramatic increase in activity. Indeed, Fig. 6D,E shows that 24 h after being exposed to these new thermal conditions, the activity was still very high, totalling 17.9 km day^{-1}. A 2 °C increase in water temperature was thus sufficient to induce a twofold increase of the voluntary swimming activity. Note that the apparently greater distance covered by the animal in this experiment compared with the data reported in Fig. 4 is due only to the fact that, at the time of the experiment reported in Fig. 4, only the average depth measured during each 30 s scanning period was stored by the data acquisition system. The fish swimming activity was thus underestimated. In the case of the data reported in Fig. 6, every vertical movement exceeding the detection threshold of the system (i.e. movements greater than ±0.05 m) made by the animal over these 30 s intervals was recorded, and the distance the fish swam between two scanning periods was extrapolated from the distance recorded from the preceding 30 s scanning period.

In 5 °C water (Fig. 6C), heart rate was 25 beats min^{-1} and very little variation around this value was observed throughout

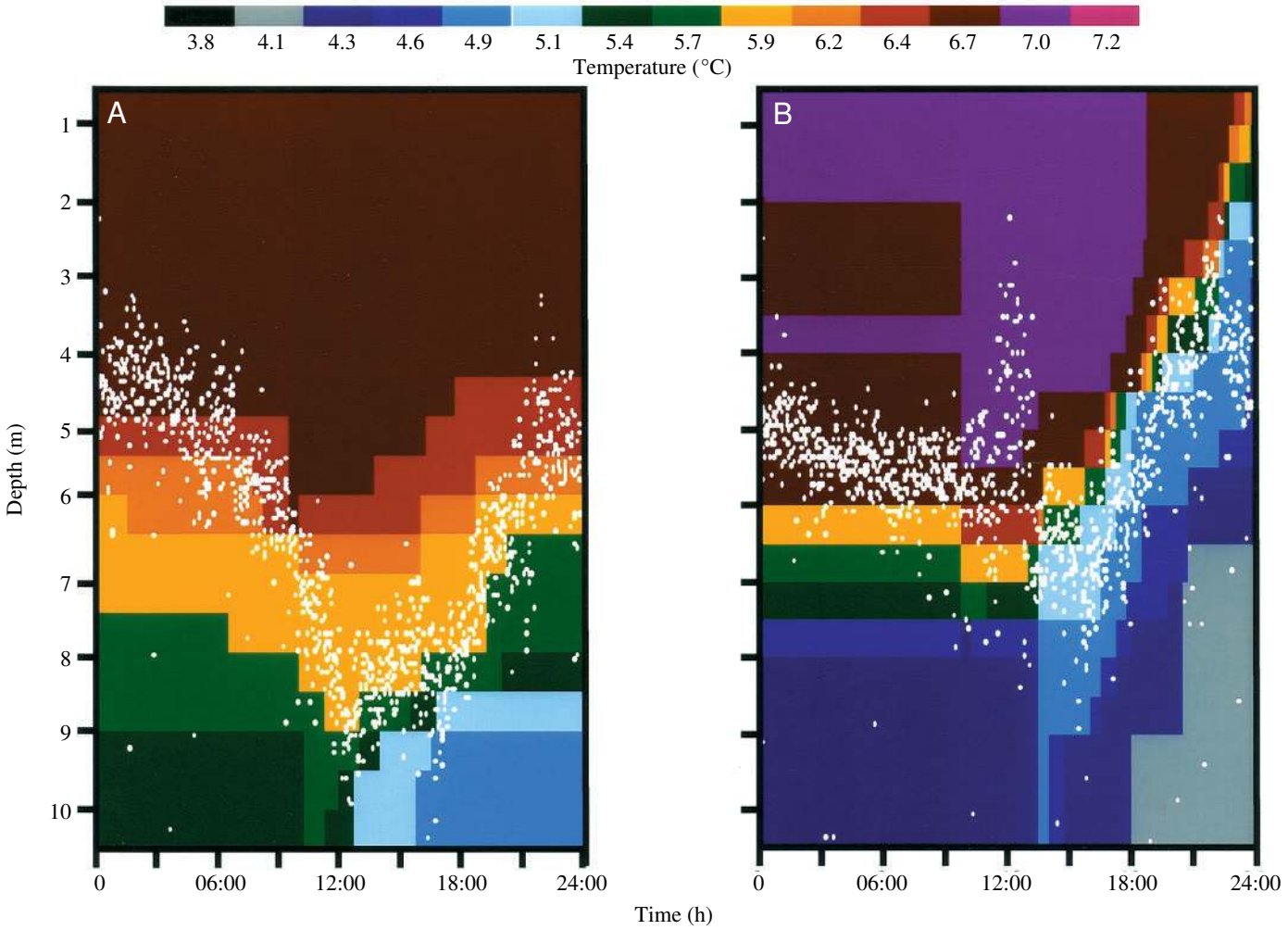

Fig. 7. (A) Example of the daily cycle displayed by a 5 °C acclimated cod in a thermally stratified water column. (B) Behavioural response of a 5 °C acclimated cod during an alteration of the water column thermal structure. Cold water flowed in at the bottom of the tank starting at 11:00 h. Water temperature is given by the colour codes at the top of the figure. The depth of the fish is shown by the white circles.

the day. In 7 °C water (Fig. 6F), in contrast, a higher (30 beats min^{-1}) and more variable heart rate was recorded. Indeed, the beat-to-beat variation around the mean heart rate value increased from ±2–3 beats min^{-1} at 5 °C to ±5–6 beats min^{-1} at 7 °C. As the fish's swimming activity and heart rate were not monitored simultaneously, we were unable to establish whether the greater variability of the fish's heart beat at 7 °C was due to the observed frequent and short-term pulses of activity or was the result of temperature-dependent changes in the heart function. However, since such irregular heart beats were not observed in the respirometer during similar temperature fluctuations, the former explanation seems more likely.

The behaviour of cod in response to changing thermal fields is illustrated in Fig. 7A,B. Following a period of stable but stratified thermal conditions, the temperature profile of the water column was slowly altered (within 12 h) in order to estimate how accurately the cod perceive their immediate environment. When the various water layers were moved upwards by introducing cold water into the bottom of the tank, the monitored animal, together with the 10 untagged fish, accurately tracked temperatures reasonably near the acclimation temperature of 5 °C. As previously observed, this phase of perturbation and adjustment was usually accompanied by increased swimming activity. This is quite obvious in Fig. 7B, where significant movement of the temperature fields induced an initial high activity response at 12:00 h followed by high activity as the temperature fields moved upwards. When the temperature layer they followed came too close to the surface, fish were finally compelled to swim down into colder water. The temperature response was sometimes difficult to separate from nocturnal and diurnal patterns; however, it was clear that, when cold water was added in the tank, the fish immediately responded and occupied warmer temperature layers in the top half of the tank. The cod also responded to temperature change between 12:00 and 18:00 h, approximately 6 h before nocturnal migrations would normally occur.

A few hours after the stratification was completed, fish began to display marked daily cycles in their distribution, although remaining very close to their acclimation temperature. This behaviour, monitored over a 5 day period, is summarized in Fig. 8. Together with the untagged fish, both telemetered animals tended to be deeper in the tank during the day, swimming up into warmer water during the night. Fish number 1 (dark bars) spent most of the day in the 5.8–6.3 °C layer and came up to the 7 °C layer at night. Irrespective of the time of the day, fish number 2 (open bars) was always found below fish number 1. During the day, fish number 2 was mostly reported in the 5.1 °C layer, but it moved up during the night, occupying the 6 °C water layer liberated by fish number 1 (see Fig. 7A for the temperature *versus* depth profile). Periodic visual checks showed that untagged fish also displayed similar behaviour.

Feeding periods were also correlated with important changes in heart rate. Two phases could be distinguished in the cardiovascular responses observed (Fig. 9). The first phase was

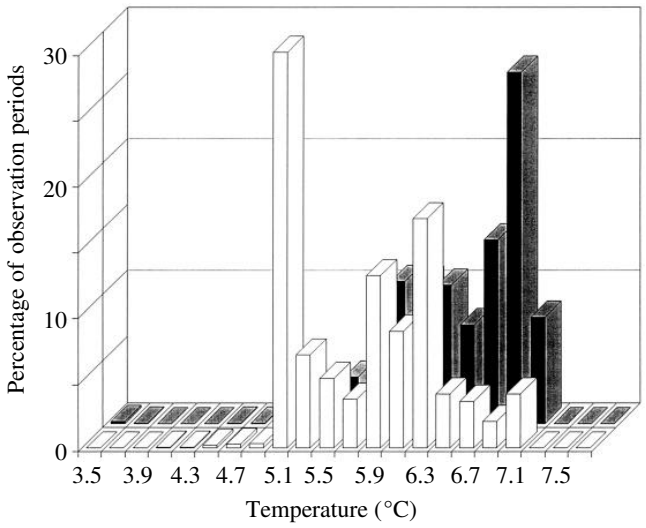

Fig. 8. Positioning (percentage of the 5 day monitoring period spent at each temperature interval) of two 5.0 °C acclimated fish in a thermally stratified water column. The thermal structure is described in Fig. 7A. For both animals, the distribution is bimodal, fish spending the day in colder and deeper layers, but moving to shallower and warmer waters at night.

a marked and short-lived overshoot of f_H, presumably corresponding to an excitation period. Indeed, during this period, the fish had not yet reached the food but had certainly perceived the odour and/or seen the food. The second, extended phase was evidently related to the specific dynamic action (SDA), as food ingestion was accompanied by a rapid increase in heart rate above the pre-fed level. As digestion proceeded, f_H reached a peak after 2.5 h and then gradually returned to pre-feeding values over a period whose duration was linked to the size of the meal. In the case of the example presented in Fig. 9, using the f_H *versus* $\dot{M}_{O_2}$ relationship established in the swim tunnel (Fig. 3), and integrating the postpandrial heart rate elevation over the digestion period (16 h), we estimated the oxygen consumption to be increased by approximately 30 % when compared with the unfed level. At maximal heart rate elevation (2.5 h post-feeding), the estimated oxygen consumption was 55 μmol min^{-1} kg^{-1} (+80 % above unfed level). However, as the amount of food ingested by the fish could not be estimated, we were unable to establish a relationship between the energetic cost of digestion and the meal size or energetic content.

Discussion

Respirometry experiments showed that the heart rate of Atlantic cod responded quickly to changes in water temperature. Indeed, $\dot{M}_{O_2}$ of resting cod was reduced by 16 % when exposed to a 2.5 °C drop in temperature and was increased by 28 % in the case of a temperature rise of the same amplitude. However, within the swimming speed range tested, f_H remained mostly unchanged during exercise, except at the

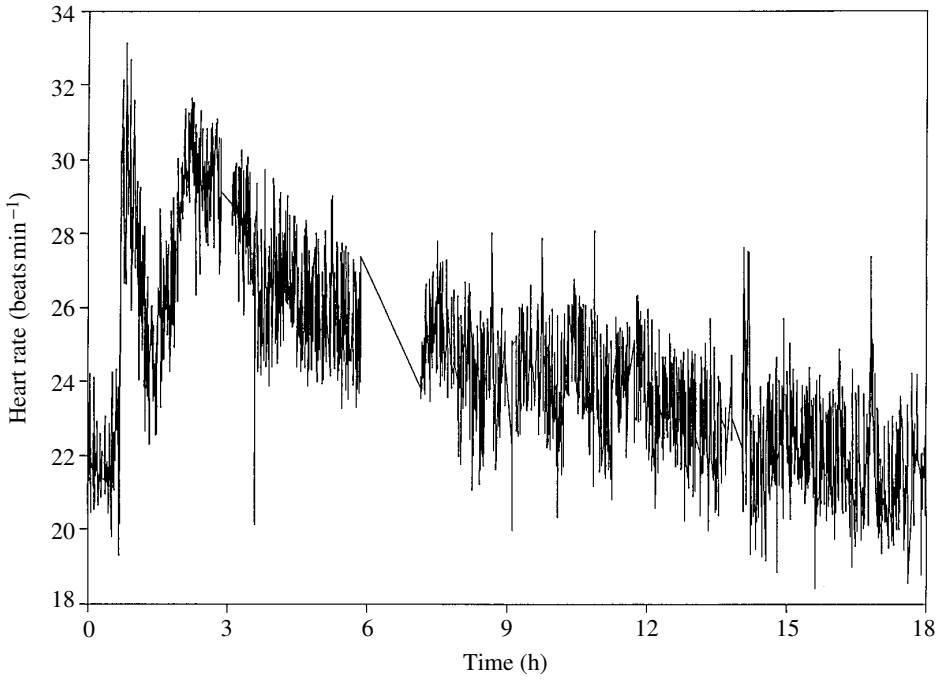

Fig. 9. Post-feeding cardiac response of a 5.0 °C acclimated cod at 5.0 °C. Food was introduced into the tank at 00:45 h.

highest speed in the 7.5 °C trial (Fig. 2). Wardle and Kanwisher (1974), Priede and Tytler (1977) and Priede (1983) also showed that cod displayed very little change in heart rate during exercise. The mechanisms responsible for the regulation of heart rate during exercise in fish are still not completely understood (Farrell, 1991). Axelsson and Nilsson (1986) suggested a mainly humorally based regulation of the excitatory adrenergic tonus in Atlantic cod. Furthermore, these authors also showed that, as in other species (Butler *et al.* 1986; Primmett *et al.* 1986; Axelsson *et al.* 1987), plasma catecholamine concentrations do not increase appreciably during non-exhaustive exercise in cod, indicating that the humorally based cardiac stimulation is probably minimal during aerobic swimming (Axelsson and Nilsson, 1986; Axelsson, 1988). During the respirometer experiments, fish were swum below their critical swimming speed (75–100 cm s^{-1}: He, 1991; 60 cm s^{-1}: Tang *et al.* 1994), presumably keeping the contribution of anaerobic metabolic pathways to a minimum and thus avoiding the release of catecholamines into the bloodstream. The observation that the only significant increase in heart rate was observed during exercise at high temperature (Fig. 2C) corroborates results reported by Priede (1974) on rainbow trout. This author observed that lowered temperature resulted in a decrease of the maximum heart rate induced by swimming activity.

The resting $\dot{M}_{O_2}$ values we report (Fig. 2A,B,C) are in agreement with those reported for adult Atlantic cod by Saunders (1963) and Tang *et al.* (1994) and for Pacific cod *Gadus macrocephalus* by Paul *et al.* (1988). During exercise in 5 °C acclimated fish, we observed an increase in $\dot{M}_{O_2}$ which was correlated with swimming speed (Fig. 2A). When temperature was altered, however, this relationship became less straightforward. In cold water, we observed no significant increase in $\dot{M}_{O_2}$ with increasing swimming speed, while in warmer conditions, $\dot{M}_{O_2}$ rose sharply at approximately 30 cm s^{-1} and thereafter remained constant.

G. A. Rose (personal communication) tracked northern Newfoundland cod during their spring migrations to the feeding grounds using high-resolution sonar techniques. The cruising ground speeds recorded during this migration were typically 10–20 cm s^{-1} and seldom exceeded 30–40 cm s^{-1}. Interestingly, when significant changes in $\dot{M}_{O_2}$ were observed during exercise in the swim tunnel, they always occurred at swimming speed above 30 cm s^{-1} (Fig. 2A,C).

When plotted irrespective of the swimming speed tested, f_H and $\dot{M}_{O_2}$ are related to each other, suggesting that, in the swimming conditions tested, muscular activity *per se* was probably not the only cause for the f_H and $\dot{M}_{O_2}$ responses reported in Fig. 2. Fig. 3 also underlines the impact of a 2.5 °C fluctuation in the water temperature on the f_H *versus* $\dot{M}_{O_2}$ relationship. This relationship describes the contribution of the heart rate to changes in the metabolic rate, and its susceptibility to temperature perturbations validates the bioenergetic relevance of an accurate thermoregulatory behaviour in Atlantic cod.

Figs 4 and 5 illustrate how a slow but steady increase in water temperature can affect the physiology and behaviour of cod swimming freely in the tower tank. As observed in the respirometer, f_H responded sharply to the increasing temperature, paralleled by a marked change in the animal's swimming activity. Two phases could be distinguished in the behavioural response. In the first phase, days 1, 2 and 3, activity rose quickly with increasing temperature, denoting an avoidance reaction as the fish tried to escape the adverse conditions. Indeed, on the basis of a conservative extrapolation of the data reported in Fig. 3, $\dot{M}_{O_2}$ probably rose of the order

of 50–75 % between days 1 and 3. In the second phase, days 4 and 5, the swimming activity dropped, even though water temperature kept rising on day 4. At this stage, escape being impossible and physiological adaptation taking over, the fish apparently reduced unnecessary energy expenditure. Peterson and Anderson (1969) observed similar responses in Atlantic salmon facing fluctuating temperature conditions. These authors also distinguished two stages in the response, a period of transient overshooting of the activity, followed by a stabilizing phase as the fish became reacclimated. If the return of voluntary swimming activity towards control values is taken as an index of the thermo-acclimation procedure, our data suggest that reacclimation of Atlantic cod to a 2 °C temperature change could take up to 3–4 days. In addition to the amplitude of the perturbation itself, the value at which the temperature stabilizes is also important and probably explains why the fish activity remained elevated at days 4 and 5. Brown *et al.* (1989) reported that the voluntary activity of cod could be reduced by as much as 75 % when temperatures fell from 8.3 to 0.6 °C. He (1991) also observed a 50 % reduction in cod swimming speed (for the same endurance) when water temperature was decreased by 5 °C. According to Jean (1964), Scott (1982) and Rose and Leggett (1988*a*), 10.7 °C is outside the temperature range where cod are usually found in the Atlantic off the coast of Canada (−0.5 to 8.5 °C). These observations emphasise the impact of acute changes in water temperature on cod physiology and substantiate the importance of a thermoregulatory behavioural response.

The influence of water temperature on the activity of cod is further illustrated in Fig. 6. Again, the voluntary movements of the animal were considerably increased even 24 h after being exposed to a 2 °C temperature rise. In the experiments presented in Fig. 5, only the averaged heart rate data, measured over each 30 s scanning period, were stored by the data acquisition system. During the experiments reported in Fig. 6, however, every heart beat was recorded, highlighting another dimension of the physiological consequences of temperature fluctuations. Besides the well-documented temperature-dependent increase in the mean heart rate, the 2 °C rise in temperature was also accompanied by a greater irregularity in the cardiac rhythm. Whereas the heart rate variance was approximately 2–3 beats min^{-1} at 5 °C, it increased to 5–6 beats min^{-1} at 7 °C. During the respirometry experiments, muscular activity was continuous, and a very regular heart beat was recorded. In the tower tank, in contrast, the apparently scattered heart signal was most probably linked to the numerous short-lived pulses of activity displayed by the animal during this period (see Fig. 6B). These observations illustrate the time scale within which the cardiovascular system can respond to increased energetic demand. They also suggest, together with the changes in the $\dot{M}_{O_2}$ *versus* f_H relationship reported in Fig. 3, that overlooking these short-term metabolic adjustments will, in instances such as those described here, lead to erroneous estimates of the animal's true energy expenditure.

So far, we have only considered fish in homogeneous temperature conditions. When the tank was thermally stratified, we started to observe daily cycles in the vertical distribution of the fish (Fig. 7A). Animals tended to be in the deeper and colder layers during the day, and moved up into warmer water at night. This behaviour, monitored over 5 days, is summarized Fig. 8. At any time of the day, fish number 1 was always found above fish number 2, i.e. in warmer water, but their vertical daily migrations always occurred in parallel. There are numerous examples of temperature and light intensity serving as stimuli affecting the movements and distribution of fish. However, the functions of these rhythms are still not fully understood. Levy (1990) investigated these cycles in wild sockeye salmon and examined three possible selective advantages, i.e. foraging efficiency, predator avoidance and bioenergetics. In effect, the animal is faced with a three-way compromise in which it must optimize its growth and reduce the risk of predation by continuous trade-offs between foraging (swimming only when and where food is present), predator avoidance (avoiding being seen by predators, but being able to see potential prey) and bioenergetics (digesting food in warmer water to maximize growth rate, but reduced energy expenditure otherwise). Perry and Neilson (1988) analyzed the vertical distribution of age-zero cod. They showed that young cod occurred in deep water during the day and moved to shallower water at night. These vertical migrations were observed at both thermally mixed and stratified sites. Extensive vertical migrations have also been reported in adult cod by Brunel (1965), Beamish (1966) and Woodhead (1966). If the adaptative relevance of this behaviour is confirmed, this would substantiate the suggestion by Ward (1976) that very constant thermal environments are unusually rigorous for animals adapted to varying temperature. Fig. 8 also supports the suggestion by Magnuson *et al.* (1979) that, within a certain temperature range, a thermal structure may also serve as a proximate or directive factor regulating the interactions between individuals, the occupation of a thermal niche being tied to social dominance in a manner analogous to food.

To examine the efficiency of the sensory mechanism by which fish position themselves in a stratified environment, the thermal structure of the water column was altered by flowing cold water into the bottom of the tank. As shown in Fig. 7B, fish quickly perceived the upward movement of the water layers and, presumably to avoid the bioenergetic cost associated with reacclimation, accurately adjusted by following the thermal water stratum they normally occupied at this time of the day. Again, the adjustment phase was accompanied by an increase in the swimming activity of the animal. This result illustrates the extreme sensitivity of the fishes thermal 'decision-making process' which enables these animals to position themselves in the water column so as to turn the physical environment to their advantage. For example, as fish got closer to the surface, the inhibitory effect of the light intensity was presumably offset by the potential cost of temperature acclimation (Fig. 3). Fish moved up into shallower water and were found at depths at which they were seldom observed during the day.

Although the intention of these experiments was to investigate cod thermoregulatory behaviour through the monitoring of heart rate and depth, we also observed marked fluctuations in f_H associated with feeding and digestion (Fig. 9). Two stages could be distinguished in the heart rate response to feeding. During the initial stage, there was a marked and short-lived increase in heart rate that occurred a few minutes after food was put in the tank. In the second phase, the elevation of f_H was directly associated with the postprandial apparent specific dynamic action (SDA), heart rate peaking approximately 2.5 h after food ingestion. Since the amount of food ingested by each animal was not controlled, we could not relate the meal size to the magnitude of the heart rate elevation (Soofiani and Hawkins, 1982; Lucas and Amstrong, 1991). However, on the basis of the results obtained in the respirometer, the integration of f_H over the digestion period allowed us to estimate that an average meal resulted in a 30 % increase of $\dot{M}_{O_2}$ above pre-feeding level. At the peak of the heart rate elevation (2.5 h post-feeding), the oxygen consumption was estimated to be approximately $55\,\mu mol\,min^{-1}\,kg^{-1}$, corresponding to a 80 % increase above pre-feeding level. Any increase in metabolic rate resulting from the need to assimilate food will tend to reduce the metabolic scope, restricting the amount of energy available for other purposes. On the basis of data from Tang *et al.* (1994), we estimate that the SDA-related increase in metabolic rate observed in Fig. 9 could result in an 80–90 % decrease in the scope for activity. Such a reduction in the metabolic scope for activity was also reported for juvenile cod by Soofiani and Hawkins (1982). The amplitude of the cardiovascular response during digestion suggests that, as already shown in pike by Lucas and Amstrong (1991), heart rate telemetry could be a useful tool in estimating the meal size of cod. The thermal structure of the tank appeared to have no impact on the feeding behaviour of cod. When smelling food, animals patrolled the entire tank until no more food was available, but then returned to their respective water layers for the period of digestion.

In conclusion, the present experiments illustrate the complexity of the interactions existing between the Atlantic cod and the thermal structure of the water column. Our data indicate that the distribution pattern of cod may be based on a highly accurate temperature-sensing ability which allows the animal quickly and reliably to adjust to any thermal perturbations occurring in its environment. Moreover, the study illustrates that thermoclines of only a few degrees Celsius can constitute fairly impenetrable boundaries, presumably contributing to the segregation of cod populations in nature.

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