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The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*

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Summary

Anaesthesia and minor surgery to place electrocardiogram recording electrodes in the short-horned sculpin caused a decrease in mean normal beat (R–R) interval and heart rate variability (HRV), measured as the standard deviation in the R–R interval (SDRR). Mean R–R interval increased to a steady state value (1.9±2.9 s) 72 h post-surgery, but SDRR took 120 h to stabilise (0.56±0.09 s). Power spectral analysis applied to recordings of instantaneous heart rate showed no spectral peaks immediately after surgery, with the development of twin peaks (at 0.02 and 0.05 Hz) that also became stable 120 h post surgery. Bilateral cardiac vagotomy abolished the variability in beat-to-beat interval, and both the high and low frequency peaks, suggesting that much of the regulation of heart rate and HRV in sculpin was under parasympathetic, cholinergic control that was withdrawn as a result of surgical and handling stress. Rate of oxygen consumption ($\dot{M}_{O_2}$) and heart rate (f_H) were monitored simultaneously and $\dot{M}_{O_2}$ showed a good correlation with both mean R–R interval (r^2=–0.89) and SDRR (r^2=0.93), although a more significant (ANCOVA, P=0.02) covariance existed between the post-surgical decrease in $\dot{M}_{O_2}$ and increase in SDRR. These data suggest that sculpin use f_H as a way of moderating oxygen consumption, fine-tuned on a beat-to-beat basis by cholinergic control. We conclude that power spectral analysis is a useful method of determining HRV in fish, and that HRV is a more sensitive measure of recovery from disturbance than f_H alone.

Key words: oxygen consumption, teleost fish, *Myoxocephalus scorpius*, electrocardiogram, heart rate variability, vagal control, power spectral analysis.

Introduction

The single circulatory system of teleost fish consists of a four-chambered heart (sinus venosus, atrium, ventricle and bulbous arteriosus), in series with the branchial and systemic vascular beds (Farrell and Jones, 1992). The matching of rates of water and blood flow over the functional counter-current at the gills, according to their relative capacities for oxygen, is essential for effective respiratory gas exchange and must be capable of rapid adjustment to varying metabolic rates. As both blood and water flow over the gills are pulsatile, it is likely that cardiac output and the respiratory cycle are coordinated or even synchronised in order to optimise respiratory gas exchange. A combination of central and peripheral control of cardiorespiratory interactions has been shown to be capable of generating synchrony between the respiratory pump and the heart, with a respiratory related component in the vagal outflow to the heart (Taylor, 1992; Taylor et al., 1999).

Direct measurement of cardiac output in active teleost fish species has shown that the increase during swimming in sub-carangiform mode is chiefly dependent on increases in stroke volume (V_S) rather than heart rate (f_H) (Stevens and Randall, 1967; Farrell, 1981). The resultant increase in pulsatility may increase lamellar recruitment, thereby sustaining oxygen uptake (Farrell et al., 1980; Altimiras et al., 1995). However, Lucas (1994) found f_H to be a useful indicator of changes in metabolic rate in Atlantic salmon *Salmo salar* at low swimming speeds, and Armstrong (1986) found that f_H provided a precise estimate of apparent specific dynamic action (SDA) and meal size in pike *Esox lucius* when rate of oxygen consumption ($\dot{M}_{O_2}$) was standardised between fish. The importance of f_H modulation as a response to changes in oxygen supply or demand is also emphasised by a characteristic bradycardic response when fish are exposed to hypoxic conditions (Axelsson et al., 1990) or startled (Priede, 1974). It may therefore be concluded that in resting fish or at low swimming speeds, reflex changes in heart rate may be a rapid and sensitive method of regulating cardiac output. Although the hypoxic bradycardia may be compensated by increased stroke volume to preserve cardiac output, reflex slowing of the heart is initiated by oxygen-sensitive chemoreceptors and is largely under vagal control (Taylor, 1992).

Histological and pharmacological studies have shown that

the teleost heart receives inhibitory innervation *via* the vagus (Xth cranial) nerve that stimulates muscarinic, cholinoceptors on the cardiac ganglion and myocardium (Laurent et al., 1983). This regulates heart rate by setting a resting vagal tonus (Axelsson et al., 1987). There is less clear evidence for adrenergic excitatory control *via* sympathetic innervation of the teleost heart, although adrenergic nerve fibres have been found in a few species and endogenous or circulating catecholamines may exert a stimulatory affect on cardiac function. However, the relative importance of these neural and humoral influences remains unclear (Taylor et al., 1999). Most studies of this dual mechanism affecting heart rate have been conducted by pharmacological blockade (Axelsson et al., 1987; Axelsson, 1988; Altimiras et al., 1997). Atropine, a muscarinic receptor antagonist introduced *via* cannulae, blocks cholinergic post-synaptic receptors, thus reducing inhibitory parasympathetic drive resulting in a tachycardia. Alternatively, β-adrenoceptor antagonists such as propranolol or sotalol competitively antagonise endogenous catecholamines and result in a bradycardia. A few studies have observed the degree of vagal tone by sectioning the nerve, and observed that the apparent beat-to-beat variation present in intact fish was greatly reduced in vagotomised animals (Priede, 1974; Altimiras et al., 1995).

A fluctuation in the instantaneous heart rate on a beat-to-beat basis is termed the heart rate variability signal (HRVS). Simple measurement of the variation in normal beat interval taken from electrocardiogram (e.c.g.) records (R–R interval) in the time domain (standard deviation of R–R interval, SDRR) provides an index of overall heart rate variability (HRV), but as a measure of changes in sympatho-vagal balance it is limited. Frequency domain analysis is preferable, whereby mathematical dissection of the signal reveals the amplitude of the oscillatory components hidden in the variability of the e.c.g. signal as distinct peaks in spectral amplitude (Malik, 1996). In mammalian systems, Akselrod (1981) demonstrated that random process analysis of the HRVS provided a sensitive, quantitative measure of rapidly reacting cardiovascular control mechanisms, revealing three distinct components. These were the high frequency component (0.3–0.4 Hz) associated with central respiratory drive and solely vagally mediated, a mid (0.1–0.3 Hz) and low (0.07–0.1 Hz) frequency component associated with blood pressure control systems, and thermal vasomotor activity, respectively. Both of these latter components had a mixture of sympathetic and vagal contributions (Bootsma et al., 1994). In the reptile (*Gallotia gallot*) two separate peaks were revealed (Gonzalaz and Porcell, 1988). Although these did not show the beat-to-beat cardiorespiratory synchrony present in mammals, they were thought to correspond to cutaneous vasomotor thermoregulation (0.032 Hz at 20°C to 0.07 Hz at 35°C) and endogenous pressure vasomotor activities (0.039 Hz at 20°C to 0.1 Hz at 35°C).

The few power spectral studies undertaken in fish have found interesting interspecific differences. A dual spectral peak was observed for rainbow trout (DeVera and Priede, 1991) and sea bream *Sparus aurata* (Altimiras et al., 1995), whilst only a single main component was found in pike, brown trout (Armstrong et al., 1988), ballen wrasse (Altimiras et al., 1995) and Atlantic salmon (Altimiras et al., 1996). This disparity between species in HRV may reflect differences in the degree of cholinergic inhibition and/or adrenergic excitation, with a predominance of vagal control generating high frequency components in the HRVS (Taylor et al., 1999). It has previously been shown, through pharmacological blockade and nerve transection, that the degree of cardiac vagal or adrenergic tone on the fish heart varies greatly between species, and with activity levels, temperature or oxygen level within species (Taylor, 1992). Consequently, a systematic study of variations in tonic control of the heart in fishes, at a range of temperatures and activity levels, may serve to clarify the mechanisms of beat-to-beat control of the fish heart.

In this study we explored the use of power spectral analysis further in examining the neural influences on the teleost heart, adapting the general methodology and application of spectral techniques for teleost e.c.g. as described previously (Altimiras et al., 1994, 1995; Altimiras, 1999). The short-horned sculpin *Myoxocephalus scorpius* was chosen as an example of a labriform swimmer of limited aerobic scope associated with sit-and-wait predation. It has not previously been examined by spectral analysis techniques, although pharmacological estimates of cholinergic and adrenergic control (Axelsson et al., 1987) facilitated this comparative study.

Materials and methods

Animals

Adult short-horned sculpin *Myoxocephalus scorpius* L. (200±28 g, *N*=9) were caught by creel in the North Sea off the east coast of England, and held at the CEFAS laboratory in Lowestoft for 28 days. The fish were then transported to the University of Birmingham, and held in a 2000 l seawater aquarium at 8°C, pH 8.1, and fed a diet of squid to satiation twice weekly. Fish were given 21 days acclimation before commencing experiments; after recordings fish were killed by MS222 overdose. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act of 1986.

Instantaneous heart rate

Two 30 cm lengths of 0.02 mm diameter, multi-strand Teflon coated stainless steel wire (A-M Systems, USA) were utilised as e.c.g. recording electrodes. A 2 mm section of insulation was removed from the end of the wire, and this was implanted into anaesthetised (MS222 0.5 g l^{-1}) fish (*N*=7) by inserting a 0.5 mm×25 mm hypodermic needle, carrying the wire, just behind each pectoral fin and advancing it a few millimetres underneath the pectoral girdle. The needle was then extracted and each wire was anchored into the pharyngeal skeleton. The electrodes were inserted at different rostro–caudal positions either side of the heart to maximise the gain from the vectored electrical signal. A loop was put into each wire close to the site of body entry, and secured to the fish underside by a single suture (7/0 braided silk); both wires

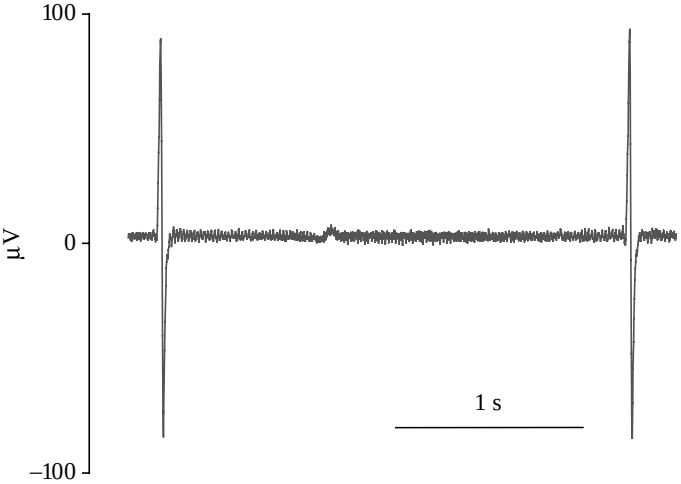

Fig. 1. Electrocardiogram of the instantaneous heart beat in the short-horned sculpin.

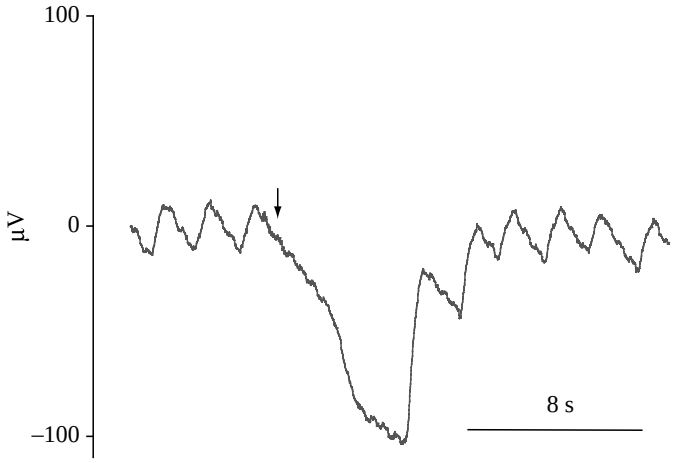

Fig. 2. Blood pressure trace (nominal values given as transducer output) obtained by ventral aortic cannulation, and showing the drop in pressure due to stimulation of the cardiac branch of the vagal nerve trunk by hook electrodes (arrow).

were brought around the left flank of the fish, twisted together and sutured in front of the dorsal fin. The whole surgical procedure took <8 min, after which the fish recovered in the holding aquarium and was then placed into a respirometry chamber (see below). The fish was unrestrained within the respirometry chamber. The electrode wires were passed up through an extended chimney in the lid of the chamber, such that recordings of e.c.g. (PowerLab 4e with animal bio-amp) could be taken with minimal disturbance to the fish (Fig. 1). A 30 min e.c.g. recording was taken 10 min after surgery, and thereafter every 24 h for 144 h.

Identification of the cardiac vagus

Two fish were anaesthetised in 0.5 g l^{-1} MS222, placed ventral side up on a small operating table and the gills irrigated with aerated, seawater at 8°C, pH 8.1, containing 0.1 g l^{-1} MS222. A polyethylene cannula (PE50, Smiths Medical, Hythe, UK) was inserted into the ventral aorta *via* a third gill arch using the method described by Axelsson and Fritsche (1994). Once a regular blood pressure trace was established a small incision was made through the body wall along the line of the opercular flap to expose the underlying vagal nerve trunk. Hook electrodes were placed around the putative cardiac branch of the vagus nerve, identified by dissection, and the branch was stimulated for a 3 s period using a Biostimulator 1000 (Searle, Birmingham, UK) with pulses of 0.4 ms duration at an intensity of 3 V and a frequency of 1.1 Hz. A PowerLab 4e, bridge amplifier (AD Instruments, Chalgrove, UK) and a Capto SP 844 pressure transducer (AD Instruments) was used for detection and amplification of the signal, and the resulting trace was recorded with Chart 4.1.2 software (ADI) for the PC. Positive identification of the branch was confirmed by cessation of the heartbeat, accompanied by a drop in ventral aortic pressure (Fig. 2).

Surgical intervention

After the final e.c.g recording five of the fish were anaesthetised (MS222, 0.5 g l^{-1}) and underwent bilateral cardiac vagotomy. A small incision was made behind the fourth gill arch and the cardiac branch of the vagus (identified previously) was exposed on either side. A 1 mm section was removed using bow scissors, and a single suture used to close each wound. The animal was replaced into the holding chamber. Recordings of e.c.g. were made as previously for a 144 h period. To validate the vagotomy procedure two fish underwent sham operations where the vagal nerves were exposed but left intact.

Pharmacological intervention

Relative parasympathetic (vagal), cholinergic (chol.) or sympathetic, adrenergic (adr.) tonus were calculated as follows:

$$\text{chol.}(\%) = \frac{(\text{R–R})_{\text{IR}} - (\text{R–R})_{\text{VR}}}{(\text{R–R})_{\text{IR}}} \times 100 \quad (1)$$

$$\text{adr.}(\%) = \frac{(\text{R–R})_{\text{VR}} - (\text{R–R})_{\text{VS}}}{(\text{R–R})_{\text{VR}}} \times 100 \quad (2)$$

where: $(\text{R–R})_{\text{IR}}$ = intact rest (chol. tonus ↑: adr. tonus ↓), $(\text{R–R})_{\text{VR}}$ = vagotomised resting (no chol. tonus: adr. tonus ↓) and $(\text{R–R})_{\text{VS}}$ = vagotomised stressed (no chol. tonus: adr. tonus↑).

In this study fish were both rested and undisturbed using our method of recording, such that the degree of adrenergic tonus is assumed to be minimal. In addition, data from other species has shown very similar calculated values for vagal tone on the heart determined after either vagotomy or pharmacological blockade (Taylor et al., 1977; Taylor, 1992)

Respirometry

Rate of oxygen consumption ($\dot{M}_{O_2}$) was measured to indicate aerobic metabolic rate in fish whilst undertaking f_H measurements. Six respirometry chambers were constructed

out of airtight PVC boxes (25 cm×25 cm×12 cm, volume 6.5 l). Each chamber was supplied with filtered aerated seawater, which was circulated by a small submersible pump (Interpet, Lancaster, UK). Each chamber had a 2.5 h closed period and a 25 min flush period at a flow rate of 100 l h^{-1}. The five fish with e.c.g. electrodes attached were monitored simultaneously, leaving one chamber to determine background (bacterial) $\dot{M}_{O_2}$. Water was sampled from each chamber by a rotor valve (Omnifit, Cambridge, UK) every 2.5 min and the water passed through a flow cell containing a 1302 Strathkelvin (Glasgow, UK) oxygen probe, monitored by a 781 Strathkelvin meter and logged *via* a channel interface (949 Strathkelvin) onto a PC computer. Using this method water oxygen tension within each chamber could be sampled every 15 min. Each fish was starved for 96 h before commencement of the experiment, and once the fish was placed inside the chamber oxygen measurement commenced immediately and ran continuously for 144 h.

Statistical analysis

Geometrical analysis of e.c.g. parameters was carried out on periods of recording selected as having no ectopic beats or signal artefacts. Student's two-tailed *t*-test for non-paired samples and the *F*-test were utilised, and data subjected to log transformation when distributions were not normally distributed. Suitability of line fits were tested by analysis of variance (ANOVA) and comparison of fitted regression lines by analysis of covariance (ANCOVA). Frequency domain analysis methods have been described previously (Altimiras et al., 1994; Altimiras, 1999), with the f_H data being converted to R–R interval (ms). A dataset of 256 consecutive R–R intervals was selected in an e.c.g. trace and tested for stationarity using the run test; subtracting the mean heart rate normalised the data. A Fast Fourier Transformation was then applied using a Hanning window to minimise spectral leakage, and the resulting output plotted graphically.

Results

Analysis of instantaneous heart rate (expressed as overall percentage change due to inter-individual variability in the resting f_H) showed that the mean R–R interval (distance between successive QRS complexes) was reduced to 1.9±0.1 s after surgery (anaesthetic and attachment of electrodes). It then increased by 40% over the following 72 h to reach a resting mean interval of 2.8±0.13 s, after which it did not significantly increase over the next 24 h of measurement (*t*-test, $P>0.05$). The resultant bradycardia (change in mean f_H from 31.5 to 20.8 beats min^{-1}) was significant (*t*-test, $P<0.05$). The standard deviation in R–R interval (SDRR) increased 4.2-fold immediately after surgery, then took a longer period of ~120 h to reach a steady level (0.52±0.05 s) which did not significantly increase thereafter (*t*-test, $P>0.05$; Figs 3 and 4). Elevated metabolism due to disturbance was estimated *via* continuous measurements of rate of oxygen consumption (Fig. 4). Mean $\dot{M}_{O_2}$ decreased from 1.65±0.17 mmol O_2 kg^{-1} h^{-1} immediately post-surgery to a steady, resting rate of 0.78±0.07 mmol O_2 kg^{-1} h^{-1} by 120 h. The mean R–R interval showed a clear negative correlation (r^2=–0.89) with $\dot{M}_{O_2}$, although SDRR showed an even better correlation with $\dot{M}_{O_2}$ (r^2=0.93). ANCOVA showed that the fitted regression lines of the two parameters had significantly different slopes ($P<0.05$), implying that R–R interval and SDRR scaled differently with respect to $\dot{M}_{O_2}$.

Power spectral analysis of the instantaneous heartbeat from individual sculpin, undisturbed for 120 h after surgery (Fig. 6),

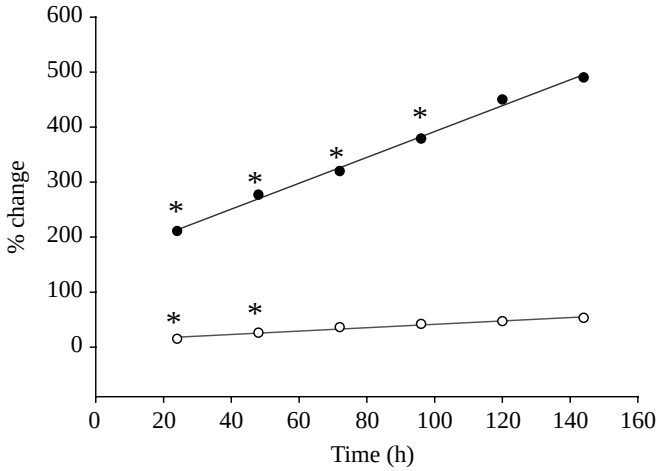

Fig. 3. Comparison of the mean R–R interval (open circles) and standard deviation of the R–R interval (closed circles) of 256 consecutive beats. Data expressed as a mean percentage change from e.c.g. recordings taken 1 h after surgery, and every 24 h for 144 h. Values are means ± S.E.M., N=5. *Data significantly different (*t*-test, $P<0.05$) from measurements taken 24 h previously.

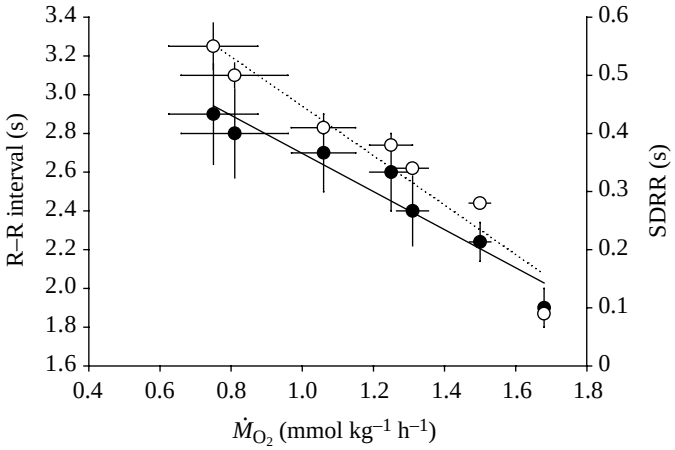

Fig. 4. $\dot{M}_{O_2}$ *vs.* R–R interval (closed circles) fitted with a solid linear regression line (y=–0.98x+3.67 r^2=0.89), and $\dot{M}_{O_2}$ *vs.* SDRR (see text; open circles) fitted with a dotted linear regression line (y=–0.42x+0.87, r^2=0.93). Data are determined from 256 consecutive R–R intervals in the instantaneous heart beat of the short-horned sculpin recorded 1 h after surgery and every 24 h for 144 h. $\dot{M}_{O_2}$ decreases with time from surgery, measurements taken every 24 h. Values are means ± S.E.M., N=5.

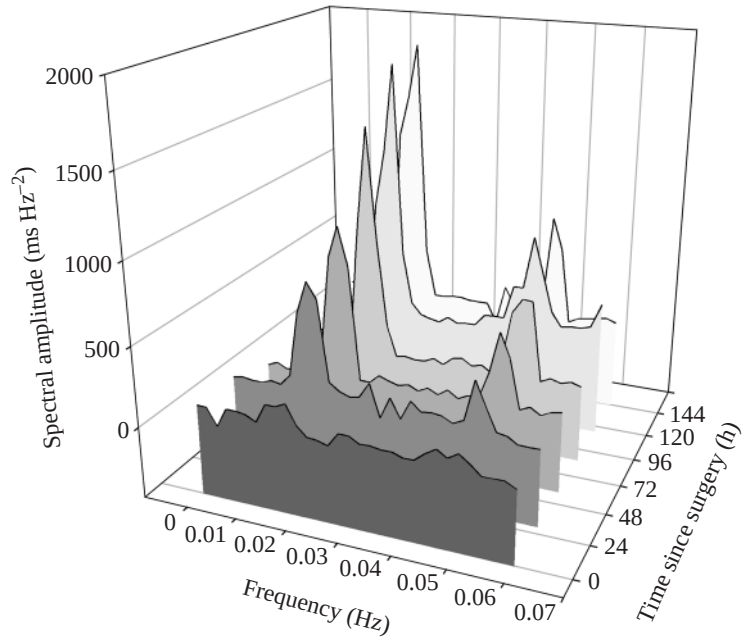

Fig. 5. Dynamic changes in spectra calculated from instantaneous e.c.g. recordings from a short-horned sculpin 1 h after implantation of electrodes and then every 24 h for 144 h.

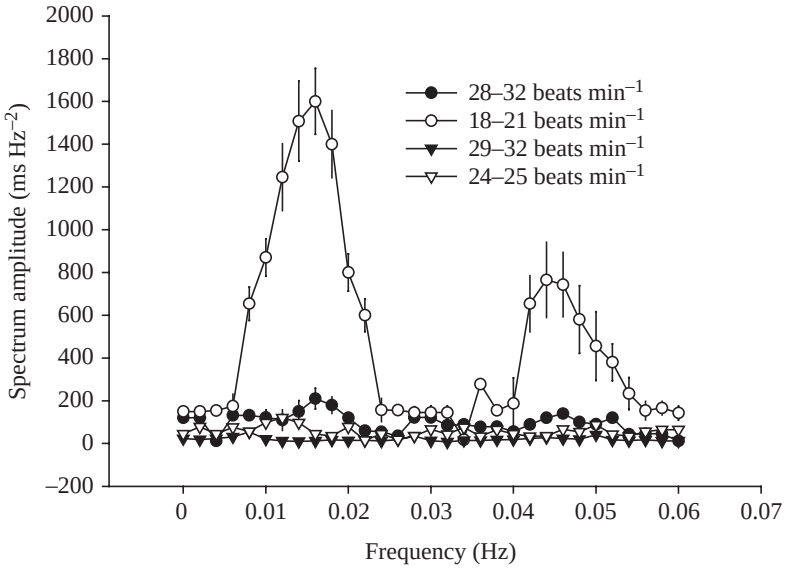

Fig. 6. Changes in spectra calculated from instantaneous e.c.g. recordings from five short horned sculpins. Data are plotted as means ± S.E.M. at 5 mHz bandwidth. Closed circles represent calculations from e.c.g. recordings taken 1 h after surgery and open circles represent data taken 144 h later. Closed triangles represent data calculated from recordings from the same fish 1 h after bilateral vagotomy and open triangles from recordings taken 144 h later.

revealed a dual peaked spectrum. The frequency of the larger spectral amplitude peak was 0.018 Hz and the smaller spectral peak 0.048 Hz, with the power at both peaks increasing with time after surgery (Fig. 5). All fish measured showed an abolition of beat-to-beat variability immediately post-surgery, with an increase in spectral power at mean frequencies of 0.018–0.2 Hz and 0.046–0.5 Hz (Fig. 6), on recovery. This gives an oscillatory period for the low frequency of 55.5–50 s and the higher frequency peak of 21.7–20 s. Using mean resting R–R interval (2.9 s), this corresponds to an oscillatory period in heart rate approximately every 7 and 17 beats. This corroborates the geometric statistics, showing that there is very little HRV after surgery, and implies that on recovery this variability has an oscillating waveform of fixed periodicity.

The effects of bilateral vagotomy on the instantaneous heart rate were dramatic, with an elevation in f_H of approximately 50% after 144 h rest and abolition of the beat-to-beat variability evident in the R–R interval (Fig. 7). Immediately after vagotomy both spectral peaks were abolished, and spectra showed a similar pattern to that previously obtained in a fish stressed due to anaesthesia and placement of electrodes. The beat-to-beat interval did increase with time post-surgery, increasing by 25% 144 h later. This was still 25% higher than the R–R interval of the intact fish, and no increase in spectral amplitude developed with time after surgery. The two sham-operated animals showed an increase in R–R interval and SDRR with time post surgery, and after 144 h a dual spectral peak was evident in the f_H trace, similar to that seen for intact fish.

The relative cholinergic and adrenergic tonus on the heart was estimated from recordings of heart rate taken from stressed, resting and vagotomised fish. Cholinergic tonus was ≥41%, calculated using Equation 1, and adrenergic tonus was ≤20%, using Equation 2.

Discussion

Although heart rate has a poor correlation with rate of O_2 consumption ($\dot{M}_{O_2}$) in some fish species (Thorarensen, 1996), the results in the present study for *M. scorpius* show that mean heart rate (f_H) varied directly with $\dot{M}_{O_2}$. For all experimental fish in this study, minor surgery or the placement of fish into respirometers caused an immediate and prolonged increase in heart rate and rate of oxygen consumption that took 96–120 h to reach steady values. Measurement of $\dot{M}_{O_2}$ and f_H 1 h after surgery (anaesthesia and attachment of electrodes only) showed values 62% and 50% greater than those found in fish measured 144 h post-surgery. The effects of surgery also abolished the beat-to-beat variability (HRV) in instantaneous heart rate, which was re-established with time after surgery, following a time course of recovery similar to $\dot{M}_{O_2}$. While mean R–R interval recovered at a faster rate following disturbance than $\dot{M}_{O_2}$, SDRR recovered at the same

rate as $\dot{M}_{O_2}$, thus reflecting better the decrease in $\dot{M}_{O_2}$ associated with recovery and acting as a more sensitive indicator of stress than f_H. This process is clearly finely tuned at the beat-to-beat level with a close correlation between HRV and $\dot{M}_{O_2}$, implying that HRV may serve to optimise respiratory gas exchange (Taylor, 1992).

Some of the contrasts between the results of this study and earlier work may have arisen as most studies have looked at variations in f_H and $\dot{M}_{O_2}$ whilst exercising the fish in a flume. *M. scorpius* is a labriform swimmer and would not readily swim in a steady flow. Consequently, the current observations were made not on an exercising fish. Instead a range of f_H and $\dot{M}_{O_2}$ was measured whilst metabolic rate recovered from its elevated rate following the disturbance of surgical and handling stress. Few studies have taken this approach so it is not clear whether the same would be true for other species, although Armstrong (1986) found a good correlation between f_H and $\dot{M}_{O_2}$ in pike *Esox lucius* after feeding. Similarities between *M. scorpius* and *E. lucius* may reflect the similar mode of life of the two species. While one is marine and one freshwater, both species are not subject to prolonged periods of activity, but are sit-and-wait predators. Therefore, elevated oxygen consumption will tend to be associated with digestion of food (specific dynamic action, SDA) or recovery from exhaustive exercise. Heart rate changes that can be up- or down-regulated quickly may be preferable for this mode of life rather than changes in stroke volume, which may be little affected by cholinergic innervation but more by an increase in venous return (Starling relationship) in the exercising animal due to muscle pumping (Lillywhite et al., 1999).

Steffensen et al. (1994) observed an $\dot{M}_{O_2}$ of 1.4±0.27 mmol O_2 kg^{-1} h^{-1} at 4.5°C in *M. scorpius*, which is similar to the value recorded from our recently disturbed fish. Thus, these high values may have been the result of handling stress, as their fish were measured immediately after placement in the respirometry chamber. In contrast, Johnston and Battram (1993) left their fish for a minimum of 72 h before recording O_2 consumption and obtained a mean $\dot{M}_{O_2}$ of 0.82± 0.044 mmol O_2 kg^{-1} h^{-1} at 5°C, which was almost identical to our $\dot{M}_{O_2}$ values for resting *M. scorpius* of 0.78±0.07 mmol O_2 kg^{-1} h^{-1} at 8°C. Results in the present study show that *M. scorpius* reduced $\dot{M}_{O_2}$ by approximately 60% after handling stress, and only stabilized 96 h post handling. Although $\dot{M}_{O_2}$ measurements in the present study at 72 h were similar to those of Johnston and Battram (1993) our fish were measured at a higher temperature, and it would be expected that this would increase oxygen demand. Clearly, we had achieved resting levels in our fish, despite the fact that they had undergone anaesthesia and surgery to insert e.c.g. electrodes prior to placement in respirometer chamber, and this may have induced a greater stress response then placement in a chamber alone.

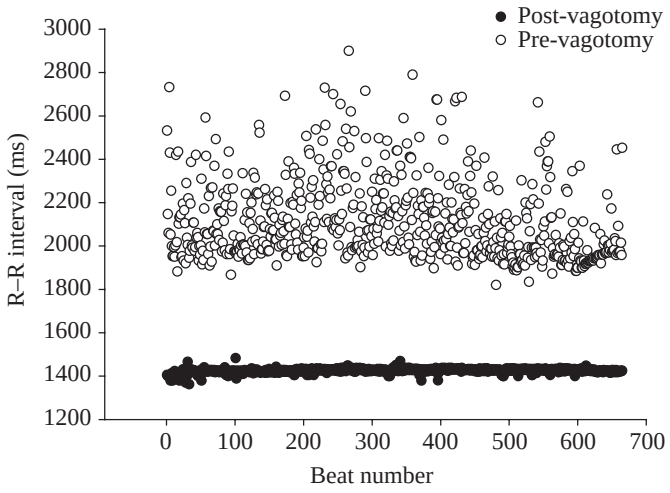

Fig. 7. Tachogram of 665 successive heart beats immediately before (open circles) and 144 h after bilateral vagotomy (closed circles). Mean R–R interval was significantly altered by vagotomy (*t*-test, t=96, P<0.01), as was data distribution (*F*-test, F=278, P<0.01).

Immediately after bilateral vagotomy the f_H parameters (a range of 29–32 beats min^{-1} among individual fish) were similar to those observed in post-surgery intact fish (28–32 beats min^{-1}). Over the next 144 h, consistent with withdrawal of sympathetic tone, f_H did decrease slightly (24–25 beats min^{-1}) although not to resting levels observed previously in intact animals (18–21 beats min^{-1}). No distinguishable increase in HRV was detectable with time post-vagotomy, suggesting that cholinergic control was the major influence on the cardiac pacemaker generating HRV in intact *M. scorpius*. The permanent abolition of HRV after cardiac vagotomy indicates that cholinergic innervation of the cardiac pacemaker regulates f_H on a beat-to-beat basis. Confirmation of this was obtained by power spectral analysis (PSA) of the instantaneous heart rate. When expressed graphically it is evident that immediately after surgery no peaks were present, implying that, in intact fish, extrinsic neural influences were having little effect on f_H, which was determined either by intrinsic cardiac pacemaker activity, or an adrenergic tachycardia influenced by circulating catecholamines. As mean f_H decreased with time after surgery, two separate peaks became apparent and increased in amplitude over time, reaching a maximum 120–144 h after surgery. The complete absence of HRV post-surgery implies that there was no cardiac vagal tone on the heart and the progressive return of HRV, illustrated in Fig. 5, may reflect its re-establishment.

The two peaks observed for *M. scorpius* (0.02 Hz and 0.048 Hz) were fourfold lower in frequency compared to those observed in rainbow trout at 10°C (0.084 Hz and 0.171 Hz) (DeVera and Priede, 1991). Thus, rainbow trout at 10°C have an oscillatory period in f_H of 11.9 s and 5.8 s, which is 4–5 times shorter than *M. scorpius* (50 s and 21 s). However, when oscillatory period was calculated per number of heart beats, the higher f_H observed in rainbow trout (52.4 beats min^{-1}) means an oscillatory period every 10.4 and 5.2 beats, which is comparable to the oscillatory period of 17.2 and 7.1 beats, observed for *M. scorpius*. A dual spectral peak (0.13 Hz and 0.25 Hz) has also been observed in the sea bream (Altimiras et al., 1995), whilst only a single spectral peak was found in pike

(0.014 Hz) and brown trout (0.027 Hz) (Armstrong et al., 1988). In these studies mean f_H was not given, so that the oscillations cannot be related to number of beats. Altimiras et al. (1996) found that Atlantic salmon males (0.027 Hz) had a lower frequency spectral peak compared to females (0.065 Hz), corresponding to an oscillatory period every 13.8 beats for male and 5.8 beats for female salmon. All fish used in the present study were confirmed by dissection to be female, and the high frequency peak observed by PSA for *M. scorpius* of an oscillatory frequency every 7.1 heart beats was close to that of the female Atlantic salmon. Both peaks fall in the range that it has been suggested is linked to blood pressure control (Altimiras et al., 1996). However, visual observations of ventilation rates in sculpin gave mean values of 2.1±0.08 s immediately after surgery, and 3.33±0.2 s from resting fish, values that are 7 times less than the rate of the high frequency peak in the HRV signal. This peak in the PSA therefore probably does not represent centrally generated respiration related activity, analogous to the respiratory sinus arrhythmia described in recordings of HRV in mammals (Taylor et al., 1999), and sculpin are more like reptiles, which also do not show cardiorespiratory synchrony (Gonzalez and Porcell, 1988).

The low-frequency peak in the HRVS may correspond to the adrenergic effects described by DeVera and Priede (1991). In intact fish, adrenaline increases systemic resistance and decreases gill resistance. The heart may show reflex responses to these changes, or direct responses of the myocardium to catecholamines, generating the low frequency peak in the HRVS. In the sculpin both peaks are abolished by vagotomy so that control seems to be largely cholinergic. However, the vagus may contain mixed cholinergic and adrenergic fibres in sculpin, similar to the vagosympathetic trunk observed in other species of teleost fishes (Gibbons, 1994).

This study highlights the importance of cholinergic innervation of the heart in *M. scorpius.* Stimulation of the vagus caused cardiac inhibition similar to that observed in plaice (Cobb and Santer, 1972) and carp (Saito, 1973), suggesting that cholinergic innervation has a major influence on the cardiac pacemaker. In addition, sectioning of the vagus nerve led to an increase in f_H in resting fish and was previously shown to abolish the approach reflex in fish, i.e. the period of bradycardia observed in intact fish when disturbed (Priede, 1974). Furthermore, the beat-to-beat variability observable in the instantaneous f_H of intact fish was abolished after vagotomy. Calculation of relative cholinergic and adrenergic tonus on the heart suggested that cholinergic tonus exerts a greater influence on f_H than adrenergic control. This differs from the results of Axelsson et al. (1987), which put a relative adrenergic and cholinergic tonus for *M. scorpius* of 25.9 and 11.9%, respectively. However, those observations were made with an intrinsic heart rate (~43 beats min^{-1}) that was below the resting HR (~48 beats min^{-1}), in contrast to the present study where a resting f_H as low as 18 beats min^{-1} was obtainable in *M. scorpius* if left for >72 h, while the degree of cholinergic tonus as measured by PSA did not reach its maximum until ~120 h post surgery. As *M. scorpius* was only left for 18 h in the pharmacological study by Axelsson et al. (1987), they presumably would not have measured resting f_H.

The degree of cholinergic tonus calculated in the present study assumed that at rest adrenergic tonus was minimal and cholinergic control maximum, whilst immediately after handling cholinergic control was minimal and adrenergic tone maximum. Previous estimates of relative tonus using pharmacological blockade make assumptions that the f_H of a resting fish also is exhibiting maximum cholinergic tonus and minimal adrenergic tonus. However, methods used in this study allow the measurement of f_H from animals undisturbed for a much longer time span than pharmacological studies allow, while the use of PSA to estimate HRV is crucial in determining a true resting f_H and consequently relative tonus. Although we cannot assess adrenergic tonus directly, the reduction in f_H 144 h after vagotomy, whilst HRV (generated by parasympathetic, vagal input) did not recover, suggests this reduction in f_H is solely due to withdrawal of adrenergic tonus. We accept that the level of adrenergic tonus after surgery may not be the maximum possible for the fish and therefore our estimates of relative cholinergic and adrenergic tone must be regarded as maximum and minimum values, respectively.

In summary, the stress of anaesthesia, handling, minor surgery and placement of sculpin into respirometers, caused an elevation in both f_H and $\dot{M}_{O_2}$ values. The increase in f_H was accompanied by a loss of HRV and paralleled the effects of cardiac vagotomy, indicating that both are the consequence of a reduction in an inhibitory cholinergic influence on the cardiac pacemaker. Mean f_H was correlated with mean $\dot{M}_{O_2}$ in resting sculpin, and was fine-tuned on a beat-to-beat basis by vagal control, although the low frequency rate of oscillations in *M. scorpius* f_H were not correlated with ventilation rate.

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References

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C. and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuations: a quantitative probe of beat-to-beat cardiovascular control. *Nature* **213**, 220-222.

Altimiras, J. (1999). Understanding autonomic sympathovagal balance from short-term heart rate variations. Are we analyzing noise? *Comp. Biochem. Physiol.* **124A**, 447-460.

Altimiras, J., Feliu, M., Aissaoui, A. and Tort, L. (1994). Computing heart rate variability using spectral analysis techniques: HRVUAB, a ready-to-use program. *CABIOS* **10**, 559-562.

Altimiras, J., Aissaoui, A. and Tort, L. (1995). Is the sort-term modulation of heart rate in teleost fish physiologically significant? Assessment by spectral analysis techniques. *Braz. J. Med. Biol. Res.* **28**, 1197-1206.

Altimiras, J., Johnstone, A. D. F., Lucas, M. C. and Priede, I. G. (1996). Sex differences in the heart rate variability spectrum of free-swimming Atlantic salmon (*Salmo salar* L.) during spawning season. *Physiol. Zool.* **69**, 770-784.

Altimiras, J., Aissaoui, A., Tort, L. and Axelsson, M. (1997). Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? *Comp. Biochem. Physiol.* **118A**, 131-139.

Armstrong, J. D. (1986). Heart rate as an indicator of activity, metabolic rate, food intake and digestion in pike, *Esox Lucius*. *J. Fish. Biol.* **29**, 207-221.

Armstrong, J. D., DeVera, L. and Priede, I. G. (1988). Short-term

oscillations in heart rate of teleost fishes: *Esox lucius* L. and *Salmo trutta* L. *J. Physiol. Lond.* **409**, 41.

Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. Exp. Biol.* **137**, 287-303.

Axelsson, M., Ehrenstrom, F. and Nilsson, S. (1987). Cholinergic and adrenergic influence on the teleost heart *in vivo*. *Exp Biol.* **46**, 179-186.

Axelsson, M., Farrell, A. P. and Nilsson, S. (1990). Effects of hypoxia and drugs on the cardiovascular dynamics of the Atlantic hagfish (*Myxine glutinosa*). *J. Exp. Biol.* **151**, 297-316.

Axelsson, M. and Fritsche, R. (1994). Cannulation techniques. In *Biochemistry and Molecular Biology of Fishes*, vol. 3 (ed. P. W. Hochachka and T. P. Mommsen), pp. 17-35. Oxford: Elsevier Science.

Bootsma, M., Swenne, C., Van Bolhuis, H., Chang, P. C., Cats, V. M. and Bruschke, A. V. G. (1994). Heart rate and heart rate variability as indexes of sympathovagal balance. *Am. J. Physiol.* **266**, H1565-H1571.

Cobb, J. L. and Santer, R. M. (1972). Excitory and inhibitory innervation of the heart of plaice (*Pleuronectes platessa*); anatomical and electrophysiological studies. *J. Physiol. Lond.* **222**, 42-43.

DeVera, L. and Priede, I. G. (1991). The heart rate variability signal in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **156**, 611-617.

Farrell, A. P. (1981). Cardiovascular changes in the Lingcod (*Ophiodon elongatus*) following adrenergic and cholinergic drug infusions. *J. Exp. Biol.* **91**, 293-305.

Farrell, A. P. and Jones, D. R. (1992). The heart. In *Fish Physiology*, vol. XIIA (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1-88. San Diego, Academic Press.

Farrell, A. P., Sobin, S. S., Randall, D. J. and Crosby, S. (1980). Intralamellar blood flow patterns in fish gills. *Am. J. Physiol.* **239**, R428-R436.

Gibbons, I. (1994). Comparative anatomy and evolution of the autonomic nervous system. In *Comparative Physiology and Evolution of the Autonomic Nervous System* (ed. S. Nilsson and S. Holmgren), pp. 1-68. London: Howard Academic Publishers.

Gonzalez, J. G. and Porcell, L. V. (1988). Spectral analysis of heart rate variability of lizard, *Gallotia gallotia*. *Am. J. Physiol.* **254**, R242-R248.

Johnston, I. A. and Battram, J. C. (1993). Feeding energetics and metabolism in demersal fish species from Antarctica, temperate and tropical environments. *Mar. Biol.* **115**, 7-14.

Laurent, P., Holmgren, S. and Nilsson, S. (1983). Nervous and humoral control of the fish heart: structure and function. *Comp. Biochem. Physiol.* **76A**, 525-542.

Lillywhite, H. B., Zippel, K. C. and Farrell, A. P. (1999). Resting and maximal heart rates in ectothermic vertebrates. *Comp. Biochem. Physiol.* **124A**, 369-382.

Lucas, M. C. (1994). Heart rate as an indicator of metabolic rate and activity in adult Atlantic salmon, *Salmo salar*. *J. Fish Biol.* **44**, 889-903.

Malik, M. (1996). Heart rate variability. Standards of measurement, physiological interpretation and clinical use. *Eur. Heart J.* **17**, 354-381.

Priede, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. Exp. Biol.* **60**, 305-319.

Saito, T. (1973). Effects of vagal stimulation on the pacemaker action potentials of carp heart. *Comp. Biochem. Physiol.* **44A**, 191-199.

Steffenson, J. F., Bushnell, P. G. and Schurmann, H. (1994). Oxygen consumption in four species of teleosts from Greenland: no evidence of metabolic cold adaptation. *Polar Biol.* **14**, 49-54.

Stevens, E. D. and Randall, D. J. (1967). Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *J. Exp. Biol.* **46**, 307-315.

Taylor, E. W. (1992). Nervous control of the heart and cardiorespiratory interactions. In *Fish Physiology*, vol. XIIb (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 343-387. San Diego, Academic Press.

Taylor, E. W., Jordan, D. and Coote, J. H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiol. Rev.* **79**, 855-916.

Taylor, E. W., Short, S. and Butler, P. J. (1977). The role of the cardiac vagus in the response of the dogfish, *Scyliorhinus canicula* to hypoxia. *J. Exp. Biol.* **70**, 57-75.

Thorarensen, H., Gallaugher, P. E. and Farrell, A. P. (1996). The limitations of heart rate as a predictor of metabolic rate in fish. *J. Fish Biol.* **49**, 226-236.