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Cardiac responses to anoxia in the Pacific hagfish, *Eptatretus stoutii*

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SUMMARY

In the absence of any previous study of the cardiac status of hagfishes during prolonged anoxia and because of their propensity for oxygen-depleted environments, the present study tested the hypothesis that the Pacific hagfish *Eptatretus stoutii* maintains cardiac performance during prolonged anoxia. Heart rate was halved from the routine value of 10.4±1.3 beats min^{-1} by the sixth hour of an anoxic period and then remained stable for a further 30 h. Cardiac stroke volume increased from routine (1.3±0.1 ml kg^{-1}) to partially compensate the anoxic bradycardia, such that cardiac output decreased by only 33% from the routine value of 12.3±0.9 ml min^{-1} kg^{-1}. Cardiac power output decreased by only 25% from the routine value of 0.26±0.02 mW g^{-1}. During recovery from prolonged anoxia, cardiac output and heart rate increased to peak values within 1.5 h. Thus, the Pacific hagfish should be acknowledged as hypoxic tolerant in terms of its ability to maintain around 70% of their normoxic cardiac performance during prolonged anoxia. This is only the second fish species to be so classified.

Key words: anoxia, *Eptatretus stoutii*, cardiovascular function, cardiac output, cardiac power output, heart rate, hypoxia, metabolic suppression.

INTRODUCTION

Only a few of the more than 25,000 species of fishes are known to be tolerant of severe hypoxia, and even anoxia. Even so, there are representatives among cyclostomes, elasmobranchs and teleosts. Among teleosts, the carp family has several hypoxia-tolerant species, e.g. the common carp, *Cyprinus carpio* (Stecyk and Farrell, 2002; Stecyk et al., 2004) and the tilapia, *Oreochromis* hybrid sp. (Speers-Roesch et al., 2010), but the crucian carp, *Carassius carassius*, stands out as being tolerant of anoxia for days to weeks at temperatures of 5–8°C (Hyvärinen et al., 1985; Nilsson, 1990; Nilsson, 2001; Shoubridge and Hochachka, 1980; Stecyk et al., 2004; Vornanen and Tuomennoro, 1999). The most hypoxia-tolerant elasmobranch appears to be the epaulette shark, *Hemiscyllium ocellatum*, which can withstand hours of severe hypoxia at 25°C (Nilsson and Renshaw, 2004; Stenslokken et al., 2004; Wise et al., 1998). Among cyclostomes, hagfishes routinely inhabit oxygen-depleted environments, such as hypoxic sediments. In addition, their feeding strategy of burrowing into the coelomic cavities of dead or moribund animals exposes them to short periods of severe hypoxia or anoxia (Axelsson et al., 1990; Forster et al., 1992; Perry et al., 1993; Perry et al., 2009). However, whether or not hagfishes are anoxia tolerant is unclear, although there are certainly suggestions that the heart can function under anoxic conditions (Forster, 1991; Hansen and Sidell, 1983).

Given the possibility that hagfishes might be anoxia tolerant, the objective of the present study was to characterize the cardiac responses in the Pacific hagfish, *Eptatretus stoutii* (Lockington 1878), to prolonged anoxia because most vertebrate hearts do not tolerate even severe hypoxia. It has been suggested that the ability to maintain cardiac function without sufficient oxygen requires a low routine cardiac power output and ATP demand, a high cardiac glycolytic potential and a means of dealing with anaerobic wastes (Farrell, 1991b; Farrell and Stecyk, 2007). In this regard, hagfishes are recognized as having the lowest cardiac power output among all fishes (Farrell, 2007a), an *in vitro* or *in situ* cardiac glycolytic potential that can serve these needs (Forster, 1991; Hansen and Sidell, 1983) and a blood volume larger than any vertebrate (Forster et al., 2001).

Although the cardiovascular features of hagfish suggest that they could maintain cardiac performance during prolonged periods of anoxia, the requisite measurements of cardiac power output, the product of ventral aortic blood flow and pressure, are lacking. Instead, such measurements have been limited to severely hypoxic hagfishes for a period of 30 min or less. Under these conditions, cardiac output was maintained or slightly increased in the Atlantic hagfish, *Myxine glutinosa*, and the New Zealand hagfish, *Eptatretus cirrhatus* (Axelsson et al., 1990; Forster et al., 1992). Furthermore, Hansen and Sidell (Hansen and Sidell, 1983) had earlier shown that the gross mechanical activity (frequency and force of heart beat) of an *in situ* heart was maintained in anaesthetized *M. glutinosa* for 3 h after cardiac poisoning with either cyanide or azide to stop mitochondrial respiration, as well as during severe hypoxia (gills perfused with nitrogen-equilibrated water). However, given that the majority of hypoxia-tolerant vertebrates reduce cardiac power output by 50–95% during severe hypoxia, the only exception being crucian carp (Stecyk et al., 2004), there is the possibility that hagfish, extant representatives of fish with the most primitive chambered heart, also depress cardiac power output during prolonged anoxia. Thus, the purpose of this study was to test the hypothesis that the Pacific hagfish, like the crucian carp, maintain cardiac performance during prolonged anoxia.

MATERIALS AND METHODS

Animals

Hagfish (*Eptatretus stoutii*) were captured at ~100 m depth in Barkley Sound, British Columbia, Canada (48°50′N, 125°08′W), and transported to the DFO-UBC Centre for Aquaculture and Environmental Research, West Vancouver, British Columbia,

Canada. They were housed year round in 1100 l tanks with aerated, flow-through seawater (~30 p.p.t. at 10±1°C). Fish were fed squid once every 3 weeks. Ten fish (0.16±0.04 kg) were used in this study and food was withheld for a minimum of two weeks prior to experimentation. All of the following procedures were approved by the University of British Columbia Animal Care Committee (A08-0312) and conducted in accordance with their guidelines.

Surgical procedure

Fish were anaesthetized by immersion in 10°C seawater and tricaine methanesulfonate (MS-222; 0.4 g l^{-1}; Sigma, St Louis, MO, USA) for approximately 45 min. Fish were weighed, transferred to a surgery table and placed ventral side up on water-soaked foam. In order to measure ventral aortic pressure (P_{va}), cardiac output ($\dot{Q}$) and heart rate (f_H), a ventral midline incision was made to gain access to the ventral aorta. A cannula (PE 50, with a PE 10 tip) was occlusively implanted in the second or third afferent gill artery to measure P_{va}, as described by Axelsson et al. (Axelsson et al., 1990). The cannula, filled with heparinized saline (200 i.u. ml^{-1}), was advanced into the ventral aorta. Directly following cannula insertion, a Transonic transit-time blood flow probe (2.5 mm SB, Transonic Systems, Ithaca, NY, USA) was positioned around the ventral aorta immediately anterior to the heart to measure $\dot{Q}$. The cannula and the lead of the flow probe were secured to the body wall with silk sutures.

Following surgery, fish were revived in a 2.5 liter Plexiglas Loligo respirometer chamber (Loligo Systems, Tjele, Denmark). The chamber was flushed continuously with fresh aerated seawater at a flow rate of 1 l min^{-1}. The relatively large volume of the respirometer allowed fish to adopt their relaxed curled position during normoxia and their uncurled position during anoxia. Fish were allowed to recover overnight for at least 12 h. Owing to the propensity of hagfish to tie themselves and any attached leads in knots, attaining sustained blood pressure readings was challenging.

Data acquisition

The day after surgery, the cannula was connected to a pressure transducer (model DPT-6100, pvbMedizintechnik, Kirchseeon, Germany) calibrated against a static column of seawater every 10 h during the anoxic exposures. A 4chAmp amplifier (Somedic, Hörby, Sweden) was used to amplify signals from the transducer. The flow probe was connected to a T206 Transonic flow meter (Transonic Systems, Ithaca, NY, USA). Data were recorded for subsequent analysis using a Power Lab unit (ADInstruments, Castle Hill, Australia) connected to a laptop computer running LabChart Pro software (v.6.0; ADInstruments). Routine $\dot{Q}$, f_H and P_{va} were recorded for several hours prior to and during the anoxic exposure.

Anoxic exposure and recovery

The chamber was made progressively hypoxic by flushing (1 l min^{-1}) nitrogen-saturated seawater directly from a gas exchange column into the chamber until anoxia was achieved (0.0 kPa, typically after ~1 h). Cardiovascular variables ($\dot{Q}$, f_H and P_{va}) were recorded continuously during anoxia and recovery. During the initial trials f_H steadily decreased from the anoxic steady state following 24 h of anoxia and following 36 h of complete anoxia the fish were returned to normoxia. All fish survived and recovered fully from the 36 h anoxic exposures. Water P_{O_2} in the chamber was measured every second using a MINI-DO probe (Loligo Systems, Tjele, Denmark). The oxygen probes were calibrated using fully aerated seawater and sodium sulphite-derived oxygen-free distilled water prior to each trial. The chamber remained anoxic for 36 h. Following the anoxic period, normoxia was restored within 5 min by introducing normoxic seawater at a flow rate of 1 l min^{-1}. Fish were monitored for a further 36 h under normoxic conditions, after which they were sacrificed and the ventricular mass (M_v) determined.

Calculations and statistics

Cardiac output through the ventral aorta was measured by the Transonic flow probe and f_H was determined by counting the number of systolic peaks over a 4 min period. Cardiac stroke volume (V_s) was calculated by dividing $\dot{Q}$ by f_H. Reported values are mean values (±s.e.m.), typically for 10 fish unless stated otherwise. Cardiac power output (PO) was calculated as the product of $\dot{Q}$ and P_{va} and was expressed per gram ventricular mass. With many hagfish tying knots in the pressure cannula, the number of fish with viable P_{va} measurements was reduced. Only data for fish in which P_{va} and $\dot{Q}$ were measured simultaneously during the 36-h anoxic exposure are reported. Consequently, PO is reported for three fish during anoxia and only one cannula remained functional during anoxic recovery. In order to test for statistical differences, comparisons among control (routine), anoxic and recovery values were tested using a one-way, repeated measures ANOVA followed by a Holm–Sidak post-hoc test. Statistical significance was set at $P \leq 0.05$.

RESULTS

Examples of the raw cardiovascular trace from a single hagfish at selected time periods during an experiment are presented in Fig. 1. As can be seen from these traces, the beat-to-beat cardiovascular status was extremely stable and any changes that took place did so gradually. In both normoxia and anoxia, there was a period of zero ventral aortic flow during diastole. Cardiovascular responses to the 36 h period of anoxia and during the subsequent period of recovery in normoxia are summarized in Figs 2, 3 and 4.

Anoxia

Hagfish often became active as water P_{O_2} fell below 3 kPa, which was during the first 30 min of the anoxic period. They swam around the respirometer and explored the edges, presumably trying to find an escape route from the respirometer. Some of these swimming movements were forceful. A minor tachycardia accompanied this activity. Subsequently, f_H decreased progressively and by 1 h into the anoxic period it had decreased significantly from the normoxic routine value of 10.4±1.3 beats min^{-1} to 8.1±0.8 beats min^{-1}. A new steady state for f_H was reached after 2 h of anoxia, when f_H remained at about 50% of the normoxic rate for the remainder of the 36 h anoxic exposure (Fig. 2).

Routine $\dot{Q}$ during normoxia was 12.3±0.9 ml min^{-1} kg^{-1} (Fig. 2) and $\dot{Q}$ remained unchanged during the first 3 h of anoxia, despite the significant bradycardia that had developed by 1 h of anoxia. However, after 6 h of anoxia, $\dot{Q}$ was significantly reduced by 33% (Fig. 2) and remained at this value for the remaining 30 h of the anoxic period.

Routine V_s during normoxia was 1.3±0.1 ml kg^{-1} and doubled to 2.7 ml kg^{-1} during the first 3 h of anoxia to offset the bradycardia. This elevated V_s was maintained for the remainder of the anoxic period (Figs 2, 3).

Routine P_{va} during normoxia was 0.89±0.04 kPa. The initial period of tachycardia during the first 1 h of anoxia was associated with a 60% increase in P_{va} (Fig. 3). By 3 h into the anoxic period, routine P_{va} was restored and remained stable for the remaining 33 h of anoxia. In fish in which P_{va} and $\dot{Q}$ were measured simultaneously (N=3 fish), the pattern of change for f_H, $\dot{Q}$ and V_s was similar to that shown in Fig. 2, where only $\dot{Q}$ was recorded (Fig. 3).

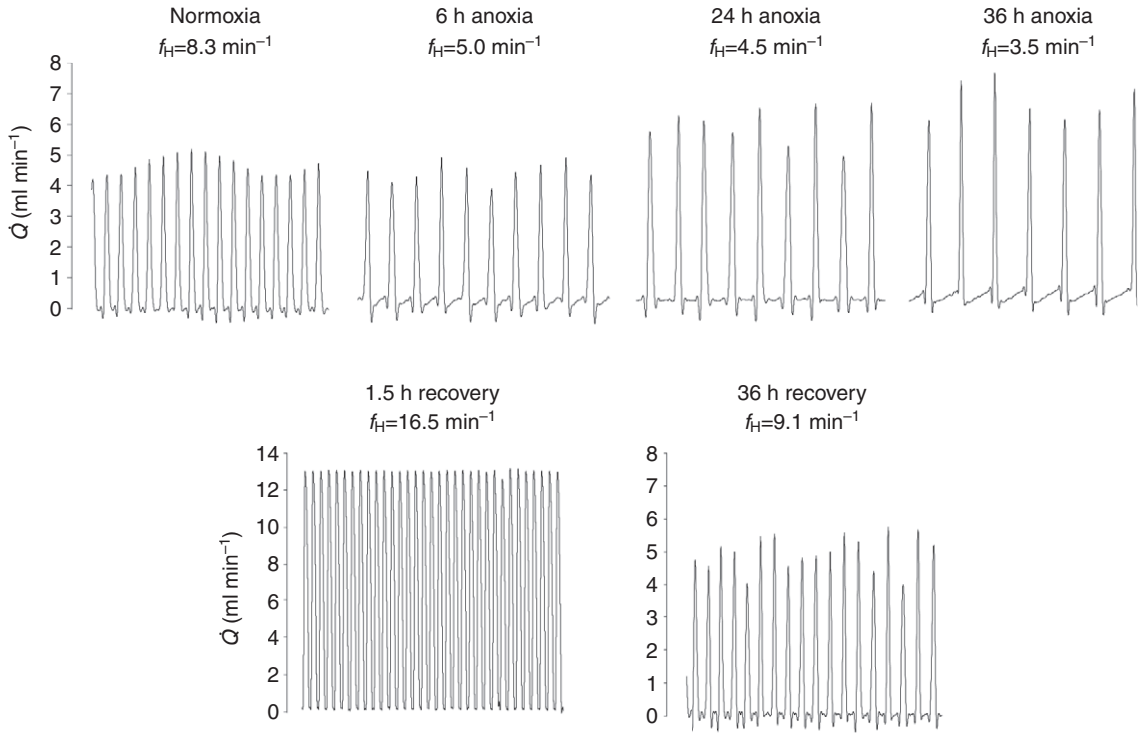

Fig. 1. Representative ventral aortic blood flow recordings taken from one hagfish after 6, 24 and 36 h of anoxia, and during 1.5 and 36 h of normoxic recovery from anoxia. Each trace is for 2 min and heart rates (f_H) are reported as beats per min. Note the different *y*-axis scale for 1.5 h recovery. $\dot{Q}$, cardiac output.

Routine cardiac PO was estimated at 0.26±0.02 mW g^{-1} from simultaneous recordings of P_{va} and $\dot{Q}$ (N=3 fish). During the 36 h anoxic period, cardiac PO mirrored the changes observed for P_{va}, becoming stable at ~0.2 mW g^{-1} (Fig. 3).

Normoxic recovery

Within the first 10 min of the return to normoxic conditions, f_H had increased significantly when compared with the final 36 h anoxic value. By 1 h into recovery, f_H was significantly 50% higher than the initial routine f_H during normoxia (Fig. 4). A maximum f_H was reached after 1.5 h and routine normoxic f_H was restored after 6 h of recovery (Fig. 4).

Similarly, within the first 10 min $\dot{Q}$ had increased significantly compared with the final 36 h anoxic value and had almost doubled (24.6±3.6 ml min^{-1} kg^{-1}) after 1 h of recovery. By 3 h after the return to normoxic conditions, routine $\dot{Q}$ was restored (Fig. 4). V_s briefly remained significantly higher (P<0.05) than routine V_s during recovery, but by 1 h into recovery V_s was significantly lower than the 36 h anoxic value and was similar to routine V_s (Fig. 4).

Only one fish had a functioning cannula during recovery and P_{va} increased to 1.2 kPa following 10 min of normoxia, decreasing back to 1.0 kPa by 20 min and remaining constant until 6 h of recovery. By 24 h of recovery, P_{va} had returned to a routine value of 0.9 kPa.

DISCUSSION

This is the first *in vivo* characterization of the cardiac performance of hagfish exposed to prolonged anoxia. The results clearly illustrate that hagfish tolerate and recover from 1.5 days of anoxia at 10°C. Although this anoxia tolerance does not surpass that of the crucian carp and related goldfish, it does surpass that of all other fishes. Furthermore, like crucian carp, after a period of cardiovascular adjustment to anoxia (in the case of hagfish just the first 3–6 h), the heart adopts a new anoxic steady state that is maintained until the end of the experimental period. However, unlike crucian carp, hagfish display a profound anoxic bradycardia that halves f_H, and although V_s increases to compensate, there is still a 33% reduction in $\dot{Q}$. This reduction had very little effect on P_{va} because of compensatory changes in vascular resistance. Hence PO and likely cardiac ATP demand were reduced by no more than 25% during 30 h of anoxia. Although these results show a decrease in cardiac performance during anoxia, such a small change for over a long duration remains extremely impressive.

Routine and maximum cardiovascular variables in normoxia, hypoxia and anoxia

Table 1 compares the routine and maximum cardiovascular variables for *E. stoutii* with previous literature values. Routine $\dot{Q}$ for *E. stoutii* was between those reported for *M. glutinosa* and *E. cirrhatus* (Table 1) (Axelsson et al., 1990; Forster et al., 1992). The challenge with any work on hagfishes is their remarkable ability to tangle and knot cannulae and electrical leads secured to their body, which could lead to various levels of stress and correspondingly elevated cardiorespiratory parameters. In the present study, the routine measurements were made when the hagfish were quietly resting in the coiled position that they adopted in the larger holding tanks. V_s in *E. stoutii* was twice that of other hagfish species, and routine f_H was correspondingly 50% lower than routine f_H for *M. glutinosa* and *E. cirrhatus* (Axelsson et al., 1990; Forster et al., 1992; Foster and Forster, 2007) (Table 1). Johnsson and Axelsson (Johnsson and Axelsson, 1996) observed a V_s of 1.3 ml kg^{-1} for the perfused heart of *M. glutinosa* at a routine physiological preload (0.1 kPa) for that species. This value is similar to the *in vivo* measurement made here. A period of zero ventral flow was observed during diastole, as observed earlier in other hagfish species (Axelsson et al., 1990;

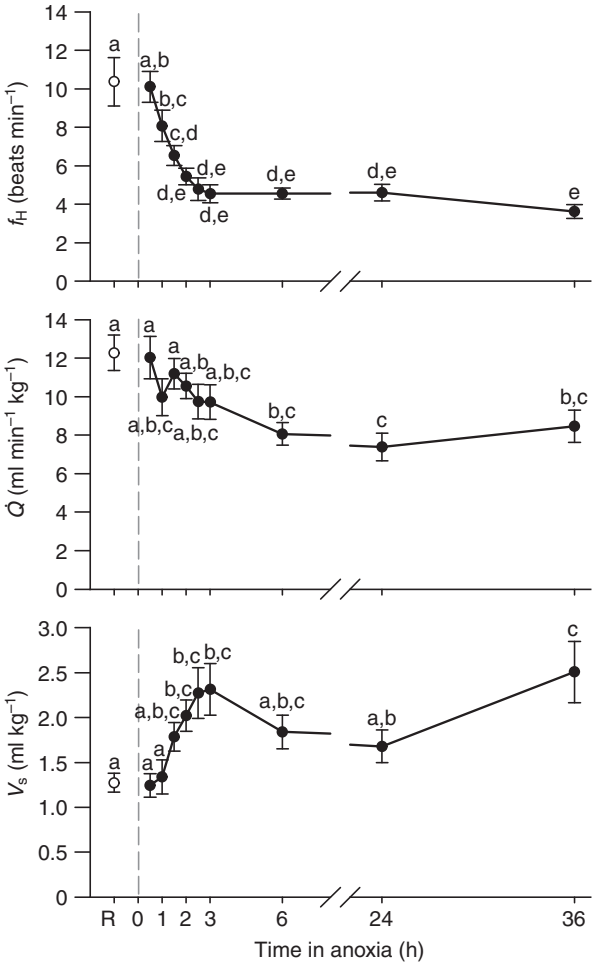

Fig. 2. Simultaneously recorded cardiovascular variables from hagfish during a 36 h anoxic exposure. Time 0 indicates the start of anoxia. R indicates routine values. N=10 for all time points with the exception of the 36 h time point, for which N=9; s.e.m. is indicated by vertical bars. Statistical differences (P<0.05) are indicated by dissimilar letters. f_H, heart rate; $\dot{Q}$, cardiac output; V_s, stroke volume.

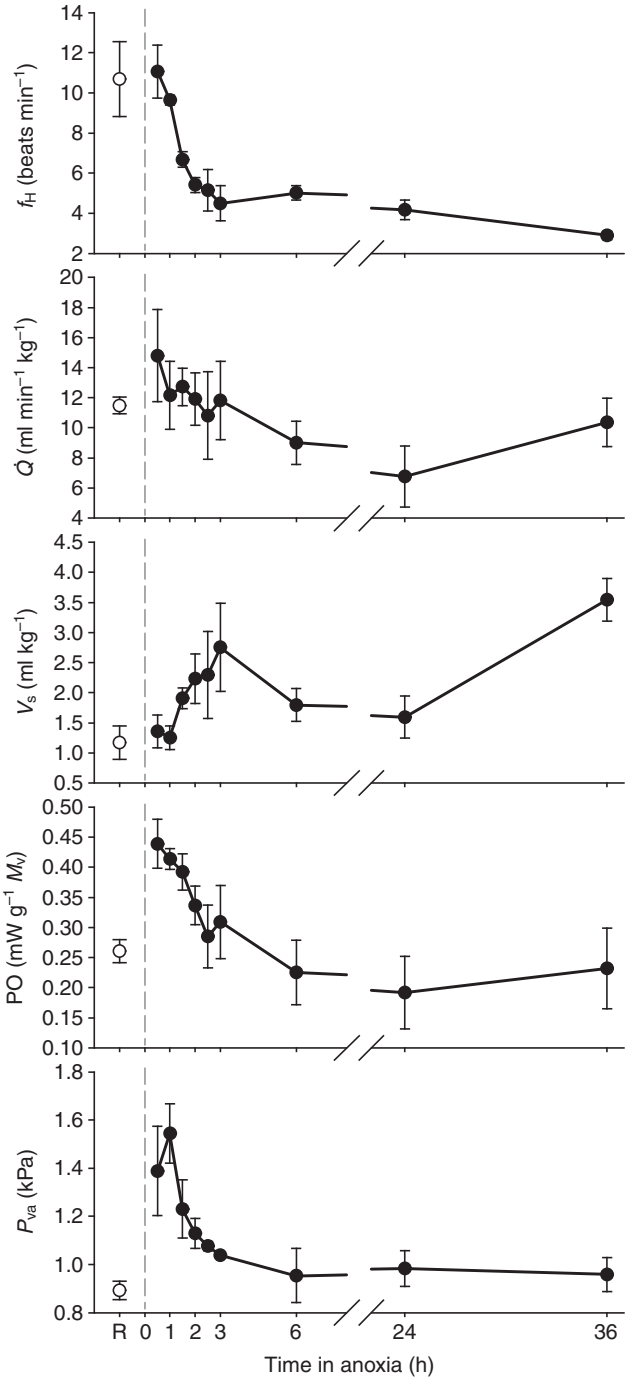

Fig. 3. Simultaneously recorded cardiovascular variables for cannulated fish during a 36 h anoxic exposure. Time 0 indicates the start of anoxia. R indicates routine values. N=3; s.e.m. is indicated by vertical bars. f_H, heart rate; $\dot{Q}$, cardiac output; V_s, stroke volume; PO, power output; P_{va}, ventral aortic pressure.

Davie et al., 1987; Satchell, 1986). Zero flow in the ventral aorta results from a relatively inelastic outflow tract from the ventricle and contrasts with the continuous diastolic flow seen in both elasmobranchs and teleosts (Farrell and Jones, 1992).

The maximum $\dot{Q}$ of 26 ml min^{-1} kg^{-1} compares well with the observed maxima for *M. glutinosa* and *E. cirrhatus*, and might approach the maximum $\dot{Q}$ for this species at this temperature. Hagfish can triple V_s (Axelsson et al., 1990; Forster et al., 1992), as can other fish species. Here, V_s doubled (Fig. 2, Table 1). Anaesthetized *E. stoutii* increased f_H by 3-fold (15–42 beats min^{-1}) at 8–10°C (Chapman et al., 1963). A similar 3-fold increase in f_H occurred here, but to a maximal f_H lower than those observed previously for other hagfish species (Table 1).

Blood pressure measurements are difficult to maintain over prolonged periods because of the ease with which hagfish can tie themselves in knots. Regardless of this, the hagfish heart is well known for generating the lowest ventral aortic blood pressure among fishes based on *in vivo* measurements and with perfused heart preparations (Table 1) (Farrell, 1991b; Forster et al., 1988; Johnsson and Axelsson, 1996). The measurements of routine P_{va} during normoxia in the current study were found to be the lowest among hagfish values (Table 1). One previous study reported P_{va} for *E. stoutii* to be 0.5 kPa higher than the values attained here (Reite, 1969).

Estimating cardiac PO from $\dot{Q}$ and P_{va} yielded a value intermediate between those for *M. glutinosa* and *E. cirrhatus* (Table 1). Maximal cardiac PO (0.4 mW g^{-1}) attained during this study was upon entry into anoxia (Fig. 3, Table 1). To date, no study has reported a maximum cardiac PO of more than 0.8 mW g^{-1} for

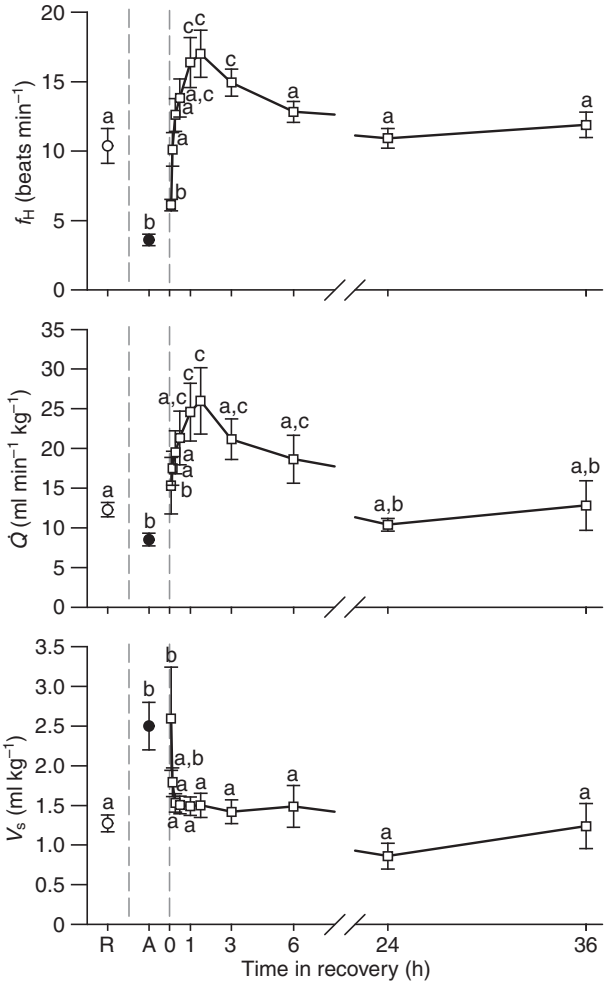

Fig. 4. Simultaneously recorded cardiovascular variables during a 36 h normoxic recovery from a 36 h anoxic exposure. Time 0 indicates the start of the normoxic flush period. R indicates routine values and A indicates anoxic values at 36 h. N=7; s.e.m. is indicated by vertical bars. Statistical differences (P<0.05) are indicated by dissimilar letters. f_H, heart rate; $\dot{Q}$, cardiac output; V_s, stroke volume.

any hagfish species. This is an important finding because it has been suggested that the maximum glycolytic potential of the hagfish heart can support a cardiac PO of 0.8 mW g^{-1}, provided sufficient glucose was available and glycolytic waste products did not accumulate to damaging levels (Farrell, 1991a; Farrell, 2007a; Forster et al., 1992; Hansen and Sidell, 1983).

Cardiovascular responses to severe hypoxia and anoxia

The previous hagfish studies that report a slight elevation in $\dot{Q}$ during short-term (maximum 35 min) severe hypoxia (Table 1) are consistent with the present study in which there was a modest tachycardia during the transition to the anoxic state. In *E. cirrhatus*, venous P_{O_2} was below the P_{50}, but venous blood oxygen content was not completely depleted (Forster et al., 1992). In the present study, this transition state likely reflected a run down of tissue oxygen stores, which might maintain routine metabolic rate (RMR) for ~29 min if we assume *E. cirrhatus* has a RMR of 12 mg O_2 kg^{-1} h^{-1} (Forster, 1990), a blood oxygen carrying capacity of 0.022 ml O_2 per ml of blood (Wells et al., 1986), a 15% blood volume (Forster et al., 2001), a myoglobin concentration of 0.0078 g per g of tissue and that both haemoglobin and myoglobin bind 1.34 ml of O_2 per g of tissue (Davison et al., 1990; Wells and Forster, 1989). In fact, the low P_{50} of hagfish blood [~1 kPa (Forster et al., 1992) to 1.64 kPa (Wells et al., 1986)] and a constant blood pH (Forster et al., 1992) following hypoxic exposures could mean that the length of hypoxia exposures in previous studies might have been insufficient to elicit an anoxic response. The activity observed within the first 30 min of anoxia in the present study, as well as in earlier studies (Forster, 1990; Forster et al., 1992; Perry et al., 1993), also might have contributed to tachycardia. *In situ* heart preparations remove the influence of activity, and Hansen and Sidell observed no change in relative cardiac performance for 3 h of anoxia (Hansen and Sidell, 1983). However, *E. stoutii* in the current study did not decrease $\dot{Q}$ significantly until after 3 h of anoxia.

The decrease in $\dot{Q}$ during anoxia was relatively small and could be explained by a reduction in blood flow to the gut, which normally accounts for 30–40% of total $\dot{Q}$ in unfed fish (Thorarensen et al., 1993). Gut blood flow is one of the fish vascular beds that should be sacrificed during hypoxia (Farrell et al., 2001), as shown for Atlantic cod, *Gadus morhua* (Axelsson and Fritsche, 1991).

The similarities and dissimilarities of the cardiac response to anoxia have already been noted. Turtles are also anoxia tolerant, but their cardiac strategy during anoxia involves a switch to a radical hypometabolic state rather than maintenance or near maintenance of cardiac performance. During anoxia, cold-acclimated freshwater turtles reduced cardiac power output and metabolic rate in parallel to about 5% of routine (Arthur et al., 1997; Farrell and Stecyk, 2007; Herbert and Jackson, 1985; Hicks and Farrell, 2000a; Hicks and Farrell, 2000b; Jackson and Ultsch, 1982), even though the initial response (1–2 h) was to increase the glycolytic rate (Daw et al., 1967; Lutz et al., 2005; Wasser et al., 1991). Even warm-acclimated turtles (22°C) reduced cardiac PO by 5-fold during anoxia (Hicks and Farrell, 2000a). The hypoxia-tolerant common carp reduced cardiac PO by 3.2- to 7.7-fold during severe hypoxia at 5 to 15°C, respectively (Stecyk and Farrell, 2002). Thus, compared with other known cardiac responses to anoxia and severe hypoxia, the 25% decrease in cardiac PO is rather small. Additionally, Olson et al. (Olson et al., 2001) have observed that vasoconstriction in dorsal aortic rings of the hagfish *E. cirrhatus* could be maintained at 70% of maximum for 8 h in anoxia (Olson et al., 2001). This indicates that the tolerance of the hagfish to anoxia extends to the vascular system.

Others have shown that the cardiac ATP requirement of hagfish lies within its glycolytic ATP generating capacity (Forster, 1991; Hansen and Sidell, 1983), but this is primarily because of its low pressure generating ability (Farrell, 1991a; Farrell, 1991b; Farrell, 2007a). The present study emphasizes the long-term glycolytic ability of the heart. The cardiac ATP turnover rate [calculated from the depletion of cardiac glycogen stores during a 20-h exposure to hypoxia; P_{O_2}<0.3 kPa (Hansen and Sidell, 1983)] can be sustained only with either a 100-fold depression in routine cardiac PO or an increase in the extracellular glucose supply (Farrell and Stecyk, 2007). The present study suggests that the second scenario is the more likely.

Cardiac control in hagfish

Hagfish lack autonomic cardiac regulation (Farrell, 2007a; Nilsson, 1983). Although one study has reported nerves and ganglion cells adjacent to and within the epicardium of *E. stoutii* (Hirsch et al., 1964), the hagfish heart is considered functionally aneural (Augustinsson et al., 1956; Greene, 1902; Jensen, 1961; Jensen,

Table 1. Comparison of routine and maximal cardiovascular variables *in vivo* for hagfish and species-specific responses to either hypoxia or anoxia

Species	Cardiac output (ml min^{-1} kg^{-1})			Stroke volume (ml kg^{-1})			Heart rate (beats min^{-1})			Ventral aortic pressure (kPa)			Power output (mW g^{-1} M_v)		
	Routine	Max.	Hypoxic/ anoxic response	Routine	Max.	Hypoxic/ anoxic response	Routine	Max.	Hypoxic/ anoxic response	Routine	Max.	Hypoxic/ anoxic response	Routine	Max.	Hypoxic/ anoxic response
Myxine glutinosa‡	8	24*	=	0.4	0.7*	=	22	24*	=	1.04	1.6*	↑15%	0.15	0.62*	=
Eptatretus stoutii§	12.3±0.9	26.0±4.2	↓ 26%	1.3±0.1	2.6±0.7	↑ 75%	10.4±1.3	17.0±1.7	↓ 55%	0.89±0.04	1.2±0.01	=	0.26±0.02	0.44±0.04	=
Eptatretus cirrhatus¶	16	25*	↑ 40%	0.7	1.3† 1.0*	↑ 40%	25	29*	=	1.60	2.3*	↑ 30%	0.42	0.88*	↑ 52%

*Maximal values following injection of adrenaline.
†Maximal values following injection of propranolol.
'–' indicates that the hypoxic/anoxic response was to maintain routine values.
‡*Myxine glutinosa* data taken from Axelsson et al., 1990) and Farrell (Farrell, 2007). Test condition for *M. glutinosa*: temperature of 8–10°C with a hypoxic exposure of 15–35 min at P_{O_2} of 1.5–2.2 kPa.
§*Eptatretus stoutii* data taken from current study.
¶*Eptatretus cirrhatus* data taken from Forster (Forster, 1992) and Farrell (Farrell, 2007). Test conditions for *E. cirrhatus*: temperature of 17°C with a hypoxic exposure of 15 min at P_{O_2} 5.3 kPa.

1965; Nilsson, 1983). Yet, hagfish halved f_H during anoxia and nearly doubled f_H during recovery. Although the anoxic bradycardia took nearly 6 h to fully develop, the tachycardia during recovery was much faster, resulting in an overall increase in f_H of nearly 4-fold within 2 h. Clearly, the vagal-mediated reflex bradycardia typically observed in most fish species in response to environmental hypoxia (Farrell, 2007b) cannot explain these changes in f_H.

However, there are some other possibilities. Adrenergic control of f_H and contractility exists in hagfishes, and the heart has its own intrinsic stores of catecholamines (Augustinsson et al., 1956; Bloom et al., 1961; Johnels and Palmgren, 1960; Ostlund et al., 1960; Perry et al., 1993; von Euler and Fänge, 1961). The depletion of these cardiac catecholamine stores by reserpine inhibited contractility and produced bradycardia in both *E. stoutii* and *M. glutinosa* (Chapman et al., 1963; Bloom et al., 1961). Furthermore, β-adrenoreceptor antagonists decreased f_H, whereas injection of catecholamines increased f_H (Axelsson et al., 1990; Chapman et al., 1963; Forster et al., 1992; Johnsson and Axelsson, 1996). These results have led to the suggestion of humoral cardiac control via catecholamine release into the circulation (Axelsson et al., 1990; Forster et al., 1992; Perry et al., 1993), or of paracrine cardiac control via cardiac catecholamine stores. Indeed, blood catecholamine levels rise in response to acute hypoxia, anoxia and air exposure, but only to relatively low levels compared with those of teleosts (Perry et al., 1993). Thus, to elicit a response the heart must either have higher affinity receptors or the paracrine signaling mechanism dominates (Farrell, 2007a). Constant release of catecholamines in an *in situ* heart preparation could indicate a paracrine effect (Johnsson and Axelsson, 1996). Although catecholamines from one source or another could elicit tonic control of f_H in normoxia and elicit tachycardia during recovery in the present experiment, catecholamines cannot be synthesized in the absence of oxygen and so the anoxic bradycardia could be a progressive depletion of catecholamine stores until the intrinsic cardiac pacemaker rate was reached. Once oxygen becomes available, catecholamine synthesis can resume, restoring and even increasing f_H. The rate of change of f_H during anoxia and on recovery could then be related to the respective rates of catecholamine depletion and re-synthesis. This possibility could be easily examined in future studies.

In summary, we reject the hypothesis that this species of hagfish maintains its cardiac performance during prolonged anoxia. Even so, such a small change in cardiac performance (~30%) over a long duration (over 30 h) remains extremely impressive. This level of cardiac anoxia tolerance has only been superseded by crucian carp and related goldfish to date. How hagfish alter f_H by up to 4-fold, including a halving of f_H during anoxia, remains an enigma.

LIST OF ABBREVIATIONS

f_H	heart rate
M_v	ventricular mass
P_{50}	P_{O_2} at which hemoglobin is 50% saturated with oxygen
P_{O_2}	partial pressure of oxygen
PO	power output
P_{va}	ventral aortic pressure
$\dot{Q}$	cardiac output
RMR	routine metabolic rate
V_s	stroke volume

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