Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (Oncorhynchus tshawytscha) and snapper (Pagrus auratus)

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Received 24 September 2004; received in revised form 20 December 2004; accepted 2 January 2005

Abstract

We investigated recovery from anaesthesia in Chinook salmon (Oncorhynchus tshawytscha) with and without surgery. Fish either underwent light sedation on exposure to 60 ppm AQUI-S\textsuperscript{m} or surgical depth anaesthesia with 120 ppm AQUI-S. Surgical depth anaesthesia experiments were replicated using New Zealand snapper (Pagrus auratus). During light sedation, there was no evidence of catecholamine release in salmon despite changes in heart rate and blood pressure. Following surgical anaesthesia both salmon and snapper released high concentrations of catecholamines into the circulation. Plasma half-life of adrenaline in salmon was 9.3+/–0.7 min (n=7) and in snapper was 4.4+/–3.3 min (n=7). There was no further release of catecholamines, despite attempts by both species to escape their enclosures. Though clearance of the catecholamines was rapid, the cardiovascular effects of anaesthesia were prolonged. Dorsal aortic blood pressure (P\textsubscript{DA}) and heart rate (HR) were high following anaesthesia, falling by 60 min in the 60 ppm exposed salmon but remaining high in the 120 ppm group. Following anaesthesia ventral aorta blood pressure (P\textsubscript{VA}) in snapper was positively correlated with HR, as was P\textsubscript{DA} and haematocrit in salmon. Recovery of cardiovascular control processes is prolonged in recovery from anaesthesia if the fish become hypoxic.

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Keywords: Anaesthesia; Blood pressure; Catecholamines; Haematocrit; Heart rate; Recovery; Salmon; Snapper

1. Introduction

Cardiovascular function is changed when teleost fish are anaesthetised (Houston et al., 1971; Houston and Woods, 1976; Fredricks et al., 1993; Hill and Forster, 2004) as is the case in humans (Campagna et al., 2003). Changes include direct effects of the anaesthetics on tissues such as heart muscle, the smooth muscle in vascular walls, and autonomic nerves (Hill et al., 2001; Hill and Forster, 2004) as well as secondary effects of the associated hypoxia and acid–base disturbances (Farrell, 1981; Randall, 1982; Fritsche and Nilsson, 1989; Iwama et al., 1989).

Teleost fish release catecholamines in response to a variety of stressors, which can range from exhaustive exercise to anaemia to acid infusion (Reid et al., 1998; Perry et al., 1999b). Historically, anaesthesia during either experimental or commercial (e.g. aquaculture) manipulation has widely been accepted as a strong potentiator of catecholamine release in fish, particularly salmonids (Randall and Perry, 1992; Wendelaar Bonga, 1997; Reid et al., 1998; Perry and Bernier, 1999). More recently however, it has become apparent that catecholamine release in fish varies greatly between species and between stressors. Indeed, several investigations have indicated that catecholamine release is most likely due to hypoxaemia rather than blood acidosis or direct anaesthetic effects (Aota et al., 1990; Fievet and Motais, 1991; Perry et al., 1991; Iwama et al., 1999; Perry and Bernier, 1999). In salmonids, eels and...
some tropical fish catecholamine release occurs at a PaO₂ value corresponding to the P₅₀ (50% Hb–O₂ saturation) (Randall and Perry, 1992; Perry and Reid, 1994; Hill and Forster, 2004; Perry et al., 2004).

Clearance rates of adrenaline and noradrenaline from the circulation have been reported for the rainbow trout (Oncorhynchus mykiss) and plasma half-lives are short (Nekvasil and Olson, 1986; Gamperl and Boutliier, 1994). Given that the effects of cardiovascular disturbances produced by anaesthesia in fish can last for hours if not days (Soivio et al., 1977), it is surprising that more studies have not tried to correlate catecholamine concentrations with cardiovascular and haematological changes over several hours following a hypoxic event. The purpose of this study was to determine whether anaesthesia per se stimulates catecholamine release in Chinook salmon or whether release is associated with a prolonged period without gill ventilation. Measurement of cardiovascular variables such as heart rate and dorsal aortic blood pressure as well as mean cell haemoglobin concentration and haematocrit allowed the effects of anaesthesia and/or catecholamines on the cardiovascular physiology of the whole animal to be monitored. We were also able to calculate the half-lives of adrenaline and noradrenaline in the circulation and so add to the small number of such determinations recorded in the literature.

2. Materials and methods

Two series of experiments were performed. In the first series Chinook salmon were cannulated under anaesthesia and then allowed to recover from the effects of surgery for 48 h. The fish were then subjected to a second anaesthetic induction lasting 5 min. Cardiovascular and haematological parameters were measured prior to and following this second induction. In the second series, changes in cardiovascular function and blood parameters were measured following anaesthesia and surgery, and a marine species, snapper, was used as a comparison.

2.1. Series 1. Cardiovascular changes and haematology of Chinook salmon during and after light anaesthesia

Chinook salmon (Oncorhynchus tshawytscha) used in Series 1 were supplied by Issac Salmon Farm, McLeans Island, Christchurch. After transport to the University of Canterbury in chilled oxygenated water, they were stored in an outdoor tank (1.5 × 1.5 × 1.5 m) with constantly flowing fresh artesian water. The tank water came from the same source as that used in the experiments and had a mean temperature of 12.5 ± 2.5 °C and was >95% air saturated with respect to oxygen.

When required, salmon (1047 ± 227 g, range 844–1562 g, n=7) were removed from the holding tank with a net and placed in a large bucket of MS222 (100 ppm MS222 + 300 ppm NaHCO₃) until surgical anaesthesia was reached (gill ventilation had stopped—stage 3; Iwama and Ackerman, 1994). Fish were then placed ventral side up on an operating sling and a polyethylene cannula (1 mm OD, 0.5 mm ID, approximately 1 m long) was inserted into the dorsal aorta via the roof of the mouth. The procedure, originated by Smith and Bell (1964), is described in Hill et al. (2004). On recovery the fish were placed in clear Perspex experimental containers (0.95 × 0.15 × 0.15 m) supplied with fresh running water for 48 h. The containers were covered with black polythene to shield the fish from external stimuli that might have disturbed them.

Several cardiovascular variables were monitored before and after induction. Dorsal aortic blood pressure (Pₐₐ, cm H₂O) and heart rate (HR, beats per minute, bpm) were measured by connecting the cannula to a disposable pressure transducer (PVB 6003, Surgicare, Victoria, Australia) linked to a digital amplifier (Power Lab 400 Quad bridge, ADInstruments, Melbourne). Data were collected on a computer using appropriate software (Power Lab ‘Chart’, version 3.4 ADInstruments). The cannula was also used to extract whole blood samples (300 μl) allowing determination of haematocrit, mean cell haemoglobin concentration (MCHC) and plasma catecholamine concentrations. After 48 h the fish were exposed to a 5-min anaesthetic induction with 60 ppm AQUI-S™. The active agent in this anaesthetic is isoeugenol and to achieve rapid induction we used it at three times the concentration recommended for rested harvesting of salmon. Surgery had been performed using a different anaesthetic, MS222, to avoid accumulation of one anaesthetic in the tissues. Ventilatory movements persisted and this was judged to be stage 2 anaesthesia (Iwama and Ackerman, 1994). The anaesthetic solution was introduced via the same inflow valve as the normal water supply. Snap connectors on the hose from the anaesthetic bucket and the experimental tank allowed rapid connection to the valve with minimal disturbance to the fish. Prior to induction, Pₐₐ and HR were recorded for 2 min as a control and were then continuously recorded throughout the induction period. A pre-induction blood sample was also taken via the cannula. Immediately after the induction period, a second blood sample was taken and Pₐₐ and HR were then recorded. Recordings were made and blood samples taken over 6 h following induction.

For haemoglobin analysis the blood was diluted 100 fold with Drabkin’s solution and stored at 4 °C until required. Samples were analysed by measuring UV light adsorption at λ=540 nm against a haemoglobin standard (Sigma kit, Sigma Chemical Company, St Louis, MO, USA) using a spectrophotometer (UV Kontron Spectrophotometer 860). Haematocrit was determined by spinning 60 μl whole blood in heparinized microcapillary tubes (Clay Adams, New Jersey) in a Heraeus Haemofuge for 5 min. The remaining blood was spun and the plasma collected and stored with
EDTA/reduced glutathione (25 μL, 0.2 M/0.2 M) at −70 °C for catecholamine determination analysis. The method for plasma catecholamine determination using high performance liquid chromatography has been previously described (Forster et al., 1998).

2.2. Series 2. Effects of deep anaesthesia and determination of plasma catecholamine half-life in Chinook salmon and snapper

2.2.1. Experiment 2.1–Chinook salmon

We used surgical anaesthesia (stage 3; Iwama and Ackerman, 1994) as the induction event in this series as in initial trials the large fish used (mean body mass of experimental animals 2412±105 g) did not maintain patent cannulae for the required 48 h recovery period. To achieve deep anaesthesia a higher concentration of 120 ppm AQUI-S was used. This achieved surgical anaesthesia within 10 min and prevented movement during surgery. The fish were stored in a large round indoor tank (1.5 m diameter × 1.5 m depth), at Isaacs Salmon Farm, with constantly flowing fresh water. Both the tank water and that used in the experiments came from the same source and had a constant temperature of 12±0.2 °C and was >90% air saturated with respect to oxygen. The surgical technique was as described for Series 1 and surgery was completed in 7–8 min. Immediately after surgery, a 300 μL blood sample was taken and replaced with an equal volume of Cortland freshwater teleost saline. Gills were then irrigated with fresh water.

Dorsal aortic blood pressure and heart rate were recorded at 10, 20 and 40 min post-recovery. Recovery was deemed to begin when the fish first resumed active (unassisted) ventilation. Further blood pressure and heart rate recordings were taken over the next 4-h post-recovery. Blood samples were also taken at these times to record haematocrit, MCHC and plasma catecholamine levels. There was a delay of 20 min from taking the first blood sample to taking the second as the fish required much attention on recovery and we could not be sure of collecting it earlier. The effective half-life of plasma catecholamines present in salmon and snapper blood was calculated using a one-phase exponential decay model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model. The determination of half-life for both adrenaline and noradrenaline using this method was calculated in the model.

2.2.2. Experiment 2.2–Snapper

These experiments were carried out in Nelson, New Zealand. New Zealand snapper (Pagrus auratus (mean mass 861.3±64.6 g, n=7)) were obtained from and held at the Seafoods and Marine Extracts unit in large (6.1 m²) darkened tanks supplied with filtered seawater and maintained at a constant temperature of 11±0.2 °C. To anaesthetise the fish, 120 ppm AQUI-S was used. The time to reach surgical anaesthesia in snapper was 20–25 min.

Once anaesthetised, snapper were then placed upright, angled on their right hand side and oxygenated 60 ppm AQUI-S was flushed over the gills continuously. The left operculum was pulled forward to expose the gill arches and the third gill arch was isolated from the others. A small hole was made in the afferent branchial artery of the third gill arch with an 18-gauge needle. The hole was on the left hand side, immediately ventral to the gill filaments and in the mid-section of the arch. The vessel was then occlusively cannulated with polyethylene cannula (0.86 mmID, 1.27 mmOD) and 1 mL of heparinized marine teleost saline slowly injected into the fish (composition in mmol L⁻¹: NaCl 125, KCl 5.1, NaH₂PO₄ 1, MgSO₄ 0.9, NaHCO₃ 30, CaCl₂ 1, and glucose 5.6). To further secure the cannula, a ligature was tied around the gill arch. A blood sample (300 μL) was taken at the time when the cannula was secured.

Fish recovered in darkened experimental boxes supplied with filtered seawater, with a PO₂ exceeding 145 mmHg. Ventral aortic pressure (P̅VA) and HR were measured continuously throughout recovery. Blood sampling regimes and processing were identical to that for Chinook salmon in experiment 2.1, with the exception that plasma samples were frozen in liquid nitrogen for storage.

Raw data was analysed using a one-way repeated measures analysis of variance (ANOVA) with Newman–Keuls post-test analysis. This allowed comparison of data points within a group and also comparison between groups. In all cases P≤0.05 was used to indicate a significant difference. Linear regression analysis was performed to investigate changes in dorsal aortic pressure relating to heart rate and haematocrit.

3. Results

3.1. Series 1. Light anaesthetic induction in salmon

Following anaesthesia with 60 ppm AQUI-S™, fish recovered their righting reflex and responded to external stimuli within 5 min. Pre-anaesthesia mean P̅DA was 41.1±3.4 cm H₂O. Through induction there was a non-significant 4.5% decrease in P̅DA, stabilising around 35 cm H₂O in the last 90 s of anaesthesia (Fig. 1a). Prior to induction the mean resting heart rate for all fish was 43.7±3.5 beats per minute. Throughout induction there was a steady increase in heart rate, which became significantly different from pre-induction values 3 min into anaesthesia (Fig. 1b).

Following anaesthesia, there was an immediate rise in P̅DA with values in the first 5 min all significantly higher than during induction. By 60-min post-induction blood pressure had returned to levels just above pre-induction values and stayed this way over the next 6 h (Fig. 1a). There was a gradual but steady drop in heart rate throughout the initial 5-minute recovery period, plateauing in the final 90
sec to a mean value of 40.7 bpm. By 60 min into recovery, heart rate was significantly lower than during induction. Over the following 6 h, HR rose to just above pre-induction values. Over most of the experiment there was no correlation between heart rate and $P_{DA}$ (Fig. 2). However, at 6 h post induction there was a significant negative correlation between heart rate and $P_{DA}$ ($r^2=0.63, P<0.05$) (Fig. 2, inset).

Haematocrit increased significantly following anaesthetic induction from $21.9\pm2.7\%$ to a post-induction value of $25.9\pm2.4\%$ (Fig. 3a). Throughout recovery there was a weak correlation between $P_{DA}$ and haematocrit ($r^2=0.22, P<0.01, n=7$) suggesting that the increased haematocrit was at least partly responsible for the rise in $P_{DA}$ following anaesthesia. By 6 h, $P_{DA}$ and haematocrit had fallen, returning to pre-induction values. MCHC did not change significantly, from a pre-induction value of $30.3\pm1.8$ g dL$^{-1}$ to a post-induction value of $26.0\pm1.5$ g dL$^{-1}$ (Fig. 3b).

Although there was some variability, plasma catecholamine levels did not rise significantly post-induction. Both adrenaline and noradrenaline levels remained below 5

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**Fig. 1.** Graphs of dorsal aortic blood pressure, $P_{DA}$, (a) and heart rate, HR, (b) of Chinook salmon before (■), during (▲) and after (▼) 5-min anaesthetic induction with 60 ppm AQUI-S™. Values are means±1 SEM. *Indicates a significant difference ($P<0.05, n=7$) from pre-induction values. + Represents a significant difference ($P<0.05, n=7$) from induction values. Shaded area represents time of induction.

**Fig. 2.** Graphs showing dorsal aortic blood pressure and heart rate in Chinook salmon during recovery from 5-min anaesthetic induction with 60 ppm AQUI-S™. If data for all fish ($n=7$) at all times during recovery are considered, there is no correlation between $P_{DA}$ and heart rate. Inset: For 6 h values there is a significant correlation ($r^2=0.63, P<0.05, n=7$) between $P_{DA}$ and heart rate indicating return of a functional baroreflex.

**Fig. 3.** Haematocrit, Hct, %, (a) and mean cell haemoglobin concentration, MCHC, g dL$^{-1}$, (b) of Chinook salmon blood before and after 5-min induction with 60 ppm AQUI-S™. Values are means±1 SEM. *Denotes a significant difference ($P<0.05, n=7$) from pre-induction values. + Denotes a significant difference from values at 3 and 6 h recovery ($P<0.05, n=7$). Shading indicates time of induction.
nmol L$^{-1}$ (Fig. 4) and were not significantly different from resting levels. Only one fish released detectable levels of noradrenaline.

3.2. Series 2. Recovery from surgical anaesthesia

3.2.1. Chinook salmon

Following anaesthesia with 120 ppm AQUI-S and surgery, all fish recovered their righting reflex and responded to external stimuli within 5–8 min following restoration of water flow. During recovery, several fish reacted to enclosure within the box by struggling vigorously to escape.

$P_{DA}$ rose initially over the first 15 min of recovery before stabilising at a mean of 65 cm H$_2$O for the next 45 min (Fig. 5a). By 120 min post-surgery, pressure was significantly lower ($P<0.01$) than during the first hour of recovery. $P_{DA}$ fell to 37 cm H$_2$O for the final 2 h of recovery, a value similar to the 41 cm H$_2$O pre-anaesthesia values of the Series 1 salmon. Throughout the first hour of recovery from deep induction, HR rose gradually from an initial mean value of 55 bpm in the first 15 min to a maximum of 68 bpm (Fig 5b). However, by 2-h post-induction, HR had returned to initial values, remaining at approximately 54 bpm over the next 2 h. There was no significant correlation between heart rate and $P_{DA}$.

![Graphs of dorsal aortic blood pressure, $P_{DA}$, and heart rate, HR, of Chinook salmon following surgical anaesthesia with 120 ppm AQUI-S.$^{TM}$](image1)

![Fig. 5.](image2)

![Fig. 6.](image3)
Haematocrit dropped significantly over the first hour of recovery while MCHC increased (Fig. 6).

In all fish, we noticed a monoexponential decline in circulating plasma adrenaline and noradrenaline levels throughout recovery (Fig. 7a) with plasma half lives calculated at 9.3$\pm$0.7 min (Fig. 7a) and 11.3$\pm$4.4 min (Fig. 7b), respectively. Despite the high plasma levels of both catecholamines at surgery, the catecholamines were cleared rapidly. There was no correlation between half-life and plasma catecholamine concentrations at the end of surgery, suggesting that the clearance mechanism was not saturated.

A strong positive correlation ($r^2=0.67$, $P<0.001$) existed between plasma concentrations of adrenaline and noradrenaline released during surgery suggesting a concomitant release of the two catecholamines. Despite several fish struggling vigorously in the experimental boxes during the first hour of recovery, there was no evidence of further significant release of either catecholamine.

### 3.2.2. Snapper

Survival of snapper, post-surgery, was poor. In common with gill artery cannulation in some other species (e.g. *Salmo salar*; Powell et al., 2002) there was a rapid attenuation of the pulse pressure. This meant that reliable recordings of $P_{VA}$ in all seven fish could only be made over the first 2 h. However, blood samples were able to be collected via the cannula despite difficulties with pressure recordings. Therefore values for any given variable at 2 h recovery represents $n=5$. $P_{VA}$ and heart rates are given only up to 2 h recovery, whereas MCHC and haematocrit data are given up to 4 h recovery.

Following surgery, $P_{VA}$ rose steadily and significantly for the first 30 min of recovery, as did HR (Fig. 8). There was a significant positive regression between $P_{VA}$ and HR throughout the first 70 min of recovery ($r^2=0.29$, $P<0.001$). Haematocrit declined significantly throughout the first 70 min of recovery (Fig. 9a). MCHC rose at 4 h into recovery and although this represents only $n=4$ fish, all individuals showed an average increase in red cell haemoglobin concentration (Fig. 9b).

Half-lives for plasma catecholamines in snapper were calculated over the first 50 min of recovery from surgery. The mean half-life for circulating plasma adrenaline in snapper was calculated as 4.35$\pm$3.26 min ($n=7$) (Fig. 10). As with Chinook salmon, plasma catecholamines decreased exponentially throughout recovery, despite many fish struggling to escape confinement. Plasma adrenaline values in snapper were considerably lower than those seen in
Chinook salmon. The average plasma adrenaline concentration measured at the end of surgery in snapper was $149 \pm 62$ nM. Only one fish released detectable levels of noradrenaline throughout recovery.

4. Discussion

Anaesthetics act by widespread depression of the central nervous system and, in mammals, have been shown to have direct effects on a variety of ion channels, including potassium and calcium channels (both voltage and ligand sensitive), glutamate, serotonin and GABA$_A$ channels (Trudell and Bertaccini, 2002). Depression of the CNS inhibits ventilation and can induce hypoxaemia. In most teleostean species hypoxia elicits a significant increase in both $P_{VA}$ and $P_{DA}$ (Holeton and Randall, 1967; Fritsche, 1990; Fritsche and Nilsson, 1990). This increase in blood pressure continues during recovery and is often coupled with a significant cardio-acceleration following the hypoxic period. Holeton and Randall (1967) found that in *O. mykiss* changes in $P_{VA}$ were more marked than in the $P_{DA}$ post-hypoxia and the introduction of oxygenated water tripled HR within 2–3 beats.

Pre-induction values for $P_{DA}$ and HR in our salmon fall within the range reported for the congeneric species *O. mykiss* (Randall and Perry, 1992; Fredricks et al., 1993; Olson, 1998) and values for *O. tsawyszschua* reported by Hill and Forster (2004). In the Series 1 experiment great care was taken to minimise disturbance of the fish when the anaesthetic was introduced and we did not observe the transient fall in HR seen in the first 2 min of exposure of this species by Hill et al. (2004) to the same concentration of AQUI-S. Characteristically teleost fish exhibit a vagally mediated bradycardia when startled (Nilsson and Axelsson, 1987). Hill and Forster (2004) found that nerve conduction in the cardiac branch of the vagus was not blocked on local exposure to 60 ppm AQUI-S in an in situ preparation, which suggests that the rise in HR on recovery from anaesthetic exposure was not due to an inhibition of acetylcholine release (Shipton, 1999). However, following uptake across the gill, anaesthetic concentrations can accumulate in the animal’s tissues to values greater than that in the water (Kildea et al., 2004). The rise in HR could not be due to blood borne catecholamines, as these concentrations stayed low. Intrinsic HR might also be modified by sympathetic nerves, which are present in *O. mykiss* (Nilsson and Axelsson, 1987). That HR rose following deep anaesthesia in both Chinook salmon and snapper suggested that there was an inhibition of HR during surgery, due to either a direct effect of the anaesthetic or systemic hypoxia.

$P_{DA}$ was elevated in recovery in Chinook salmon, as has been previously reported. All three anaesthetics in the study of Hill and Forster (2004) induced such a response. In the current study elevation of $P_{DA}$ in recovery of Series 1 fish occurred in the absence of a rise in plasma catecholamines. Some of the initial rise in $P_{DA}$ may have been due to a rise in vascular resistance associated with increased haematocrit (Hct), as $P_{DA}$ was correlated with Hct in the Series 1 fish.

![Fig. 9. Haematocrit, Hct, (a) and mean cell haemoglobin concentration (b) of snapper blood following surgical anaesthesia with 120 ppm AQUI-S™. Values are means±1 SEM. *Denotes a significant difference ($P<0.001$, $n=7$) from time zero (surgery). Values for the first 70 min represent $n=7$. Values for 2 and 4 h represent $n=5$.](image_a)

![Fig. 10. Mean half-life of plasma adrenaline in New Zealand snapper following surgical anaesthesia with 120 ppm AQUI-S™. Values are means±1 SEM. *Denotes a significant difference ($P<0.001$, $n=7$) from time zero (surgery). Mean half-life over the first 50 min of recovery was calculated as 4.4±3.3 min, $n=7$.](image_b)
However, $P_{DA}$ remained high once Hct started falling (Series 2 Chinook) which must be due to other factors. The systemic vasculature also receives significant adrenergic innervation (Morris and Nilsson, 1994) and, in rainbow trout, resting systemic resistance is set by neurally mediated adrenergic tonic (Bushnell et al., 1992). Increased sympathetic activity acts on $\alpha$-adrenergic receptors increasing systemic vascular resistance and hence $P_{DA}$ (Perry and Bernier, 1999). In the Atlantic cod, Gadus morhua, a significant increase in both $P_{VA}$ and $P_{DA}$ in response to hypoxia was attributed to increased activity of adrenergic nerves, with circulating plasma catecholamines augmenting the adrenergic tone of the systemic vasculature (Fritsche and Nilsson, 1989). It is possible that anaesthetic blockade of nicotinic acetylcholine receptors (Shipton, 1999) caused a fall in aortic pressure during deep anaesthesia in our Chinook salmon and snapper. As the anaesthetic was removed from neuromuscular binding sites during recovery, the effect of increased adrenergic tone may have contributed to the rise in aortic pressures. The rise in systemic resistance may also have been offset by the direct vasodilatory effects of AQUI-S (Hill et al., 2004). In vivo, sympathetic activity and direct anaesthetic effects on the vasculature may be acting antagonistically. However, anaesthetic exposure does not abolish or attenuate the vasoconstrictory action of adrenaline (S.E. Rothwell and M.E. Forster, unpublished). Both species would also be paying a significant oxygen debt in recovery, with its magnitude likely to be greater in snapper due to the longer time taken in surgery.

In Chinook salmon there was no correlation between heart rate and $P_{DA}$ during recovery from deep anaesthesia, although they rose simultaneously. The immediate rise in heart rate following anaesthesia and surgery may well be due to high plasma catecholamine concentrations. In the present study, salmon exhibited a significant negative correlation between heart rate and $P_{DA}$ six h after a 5-min induction with 60 ppm AQUI-S. This might indicate that a functional baroreflex was operating. This relationship was not seen in fish that had undergone deep anaesthesia with 120 ppm AQUI-S. Heart rate remained relatively high (when compared to similar sized fish used in previous experiments) in the group 2.2 fish for at least 4 h following induction. This suggests a long-term effect on the heart of exposure to hypoxia. Campbell et al. (2004) concluded that parasympathetic cholinergic control was lost in short-horned sculpin post-surgery, evidenced by a lack of beat to beat variability and short R–R interval of the heart, which was not fully restored by 24 h. A similar situation might have prevailed in our fish.

In snapper there was a positive correlation between $P_{VA}$ and HR. In this species it is possible that HR is more influenced than stroke volume in determining cardiac output, which contrasts with the situation in most teleosts (Farrell, 1991). Salmon and snapper could also differ in their response to hypoxia. Snapper, as a marine species are unlikely to encounter hypoxia, whereas it is possible in the freshwater phase of the salmon life-cycle.

In all three experiments there was a rise in haematocrit whether compared to pre-anaesthesia values (where measured) or published normal values for either salmon or snapper. Hct in Chinook salmon has been previously measured at 25–30% (Hill and Forster, 2004) while values for snapper have been published to range anywhere from 26–54% (Canfield et al., 1994). Haematocrit can rise due to red cell swelling or recruitment of stored red cells from the spleen (Nilsson and Grove, 1974) and can also be changed by transcapillary fluid movements (Olson et al., 2003). In teleost fish, splenic contraction is the primary cause of arterial blood oxygen increase during hypoxaemia induced elevation of circulating catecholamines (Randall and Perry, 1992). In salmon, the decrease in Hct during recovery was coupled with an increase in mean cell haemoglobin concentration (MCHC) in both Series 1 and 2. Although snapper showed an elevated Hct at the start of recovery, MCHC remained relatively stable throughout the first 50 min of recovery, before rising gradually over the next 4 h. In a number of teleost species, including tench and carp, the Na+/H+ antiporter is not influenced by catecholamines (Koldkjaer Knudsen and Jensen, 1998) and snapper may belong to this group. Chinook are likely to have a number of $\beta_1$ adrenergic receptors for catecholamines coupled to Na+/H+ antiporter activity, as occurs in O. mykiss (Reid and Perry, 1991). Also red cell swelling can occur by non-adrenergic mechanisms (Nikinmaa and Salama, 1998). Given the low concentrations of plasma catecholamines measured in Series 1 it is possible that erythrocyte swelling was caused by a degree of respiratory hypoxia and CO2 accumulation in the blood. However, in Series 2 salmon, MCHC registered even lower values post-surgery and this occurred in the presence of high concentrations of both adrenaline and noradrenaline. In this case, it is probable that blood cells were released from the spleen and then underwent adrenergically mediated cell swelling (Thomas and Egée, 1998).

Our plasma catecholamine concentrations are similar to those reported for O. mykiss by Gingerich and Drottar (1989), both low concentrations in chronically cannulated animals and high concentrations following surgery in their fish. They too reported a rise in the ratio of adrenaline to noradrenaline in plasma after surgery. Our half-life data for the catecholamines support the concept that hypoxaemia is the primary stimulus for their release in salmonids (Randall and Perry, 1992; Perry and Bernier, 1999; Perry et al., 2004). In series 2, both species found themselves on recovery in a novel and constrained environment and struggled violently to escape, and a neurogenic stress response could have been expected to result in a stimulus to the chromaffin tissue to release catecholamines, but no further release occurred. If there had been a further release, it is doubtful that we could have fitted a single exponential curve to the data. It is possible that we might have missed...
small “peaks” associated with minor release due to our sampling regime, but there were no major releases comparable to that on surgery.

The plasma half-life for circulating catecholamines reported in salmon in this study is similar to that reported for the congeneric species *O. mykiss* by Nekvasil and Olson (1986) and Gamperl and Boutilier (1994) of less than 10 min for both adrenaline and noradrenaline. Ungell and Nilsson (1979) also reported a rapid disappearance of plasma catecholamines (under 10 min for adrenaline) in the Atlantic cod *G. morhua*. The half-life of circulating adrenaline in snapper was even shorter in our study. Our data confirm that extremely high concentrations of adrenaline are still cleared rapidly, with the post-surgery concentrations in our study being seven times higher than in adrenaline loaded *O. mykiss* (Gamperl and Boutilier, 1994).

Given the rapidity of plasma clearance, our experimental design, in which it took some time to return the fish to their recovery containers, irrigate the gills and reattach cannulae for pressure measurements, precluded blood sampling at shorter intervals and consequently a more accurate determination of plasma half-lives. In previous studies, half-life was determined by intra-arterial injection of radiolabelled catecholamines. Catecholamines released into the circulation are rapidly cleared by a combination of tissue accumulation/bindng and metabolic degradation. In *Carassius auratus*, *G. morhua*, and *O. mykiss* there was a bi-phasic disappearance of radiolabelled catecholamines from the plasma, with an initial fall followed by a slow decline (Busacker and Chavin, 1977; Ungell and Nilsson, 1979; Gamperl and Boutilier, 1994; Olson et al., 1997). The initial rapid phase predominantly reflects uptake into the tissues, while the slow phase indicates subsequent release, catabolism and excretion (Olson et al., 1997). In our experimental design plasma titres reflect endogenous release rather than exogenous additions.

Catecholamines are important circulating hormones that during times of acute stress and hypoxia are important in maintaining adequate tissue oxygenation in teleost fish. However, the high plasma concentrations that can be released need to be rapidly cleared before their advantageous actions are offset by their detrimental actions. This study, and those on *O. mykiss* and *G. morhua*, has shown that these species are highly efficient at lowering plasma catecholamine levels, even in the face of micromolar concentrations.

Our data confirm that stage 2 anaesthesia, where ventilation continues, does not provoke significant catecholamine release in Chinook salmon. We did find that stage 3 anaesthesia, presumed to be linked with hypoxaemia, caused a large release of catecholamines into the blood stream in both Chinook salmon and snapper. The time courses of the interactions, and sometimes opposing actions of anaesthetics and catecholamines, as affecting vasomotion for example, complicate analysis of cardiovascular changes on anaesthesia. Altered sensory and motor nerve functions following anaesthesia are additional factors with potential effects on cardiovascular regulation. The cardiovascular effects, measured as increased HRs and raised vascular pressures, persisted long after the concentrations of adrenaline had fallen. Olson et al. (1997) have shown that the cardiovascular responses to bolus injections of catecholamines attenuate rapidly, with a half-time of 3–4 min. If there are no prolonged metabolic effects of catecholamines, this suggests that other factors induced by hypoxaemia or as a secondary consequence of the transient rise in plasma catecholamine concentrations maintain these longer lasting effects.

**Acknowledgements**

The authors thank Isaac Salmon Farm for the donation of fish. We gratefully acknowledge financial assistance from the Foundation for Research, Science and Technology. Helpful comments from two referees improved the manuscript.

**References**

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