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The efficacy of field techniques for obtaining and storing blood samples from fishes

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Prompted by the dramatic increase in the use of blood analyses in fisheries research and monitoring, this study investigated the efficacy of common field techniques for sampling and storing blood from fishes. Three questions were addressed: (1) Do blood samples taken *via* rapid caudal puncture (the 'grab-and-stab' technique) yield similar results for live *v*. sacrificed groups of fishes? (2) Do rapidly obtained caudal blood samples accurately represent blood properties of fishes prior to capture? (3) Does storage of whole blood in an ice slurry for a working day (8·5 h) modify the properties of the plasma? It was shown that haematocrit, plasma ions, metabolites, stress hormones and sex hormones of caudal blood samples were statistically similar when taken from live *v*. recently sacrificed groups of adult coho salmon *Oncorhynchus kisutch*. Moreover, this study confirmed by using paired blood samples from cannulated *O. kisutch* that blood acquired through the caudal puncture technique (mean ± s.e. 142 ± 26 s after capture) was representative of fish prior to capture. Long-term (8·5 h) cold storage of sockeye salmon *Oncorhynchus nerka* whole blood caused significant decreases in plasma potassium and chloride, and a significant increase in plasma glucose. Previous research has suggested that these changes largely result from net movements of ions and molecules between the plasma and erythrocytes, movements that can occur within minutes of storage. Thus, blood samples from fishes should be centrifuged as quickly as practicable in the field for separation of plasma and erythrocytes to prevent potentially misleading data. © 2011 The Authors
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Key words: field physiology; ions; plasma; red blood cells; salmon; stress response.

INTRODUCTION

Studies of wild animals in their natural environment offer insights generally unattainable with laboratory-based studies due to the inherent difficulties of replicating the complex biotic and abiotic factors influencing free-roaming individuals (Costa & Sinervo, 2004). Indeed, rapid sampling of blood from wild animals has become a

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popular technique to provide a snapshot of their physiological status in a variety of situations (Cooke *et al*., 2005; Clark *et al*., 2009; Romero & Wikelski, 2010; Voss *et al*., 2010). The trade off, however, is obtaining a reliable blood sample that represents the true status of the animal, ensuring the quality of the data and the correct interpretation of results.

Perhaps, the most dramatic increase in the use of rapid blood sampling techniques in recent years has occurred in the field of fisheries research, where scientists have investigated the effects of various natural and anthropogenic perturbations on the physiology and biochemistry of fishes. Colloquially referred to as the grab-and-stab technique, fishes are typically caught by angling or netting and then the live or sacrificed fish is sampled for blood (most commonly *via* caudal or cardiac puncture) as soon as possible after capture and without anaesthetic (Wells *et al*., 1986; Pankhurst, 1990; Slater *et al*., 1994; Di Marco *et al*., 1999; Marino *et al*., 2001; Killen *et al*., 2003; Suski *et al*., 2003; Cooke *et al*., 2005; Donaldson *et al*., 2011). While efforts are typically made to minimize handling time before blood sampling, a number of minutes usually lapse during which time physiological changes associated with the capture stress can begin to modify the properties of the blood. Nonetheless, surprisingly little research has examined this issue, and as far as is known, no previous study has quantified the efficacy of caudal or cardiac puncture techniques in direct comparison with paired blood samples taken from resting, cannulated fishes. As tissue trauma has the capacity to influence the properties of circulating blood (*e.g.* through the release of potassium ions from cells), another important question concerns whether or not caudal blood samples from sacrificed fishes have similar properties as those from live-sampled fishes.

A third issue is the speed with which blood can be processed in the field to prevent changes in the variables of interest. Fortunately for field biologists, some blood variables can be measured immediately using portable analysers appropriately calibrated for the blood of fishes (*e.g.* glucose, lactate and haemoglobin concentration) (Iwama *et al*., 1995; Wells & Pankhurst, 1999; Clark *et al*., 2008). Nevertheless, in instances where blood variables must be measured using laboratory-based analysers, or where centrifugation to separate blood constituents (*e.g.* plasma and erythrocytes) must be conducted under laboratory conditions prior to freezer storage, samples are often stored on ice while in the field and subsequently transported to the laboratory after a number of hours with the expectation that the blood properties do not change significantly in the interim. Although some studies exist on the effects of storage on the blood properties of fishes and other vertebrates (Lumeij, 1985; Korcock *et al*., 1988; Nielsen & Lykkeboe, 1992; Ito *et al*., 1998; Reece *et al*., 2006), inconsistent findings emphasize the need for further research on new species.

In light of the challenges encountered by field biologists, this study investigates the reliability of some common field techniques for obtaining and storing blood samples from fishes. With a focus on adult salmonids caught during their spawning migration, this study specifically addresses the following three questions: (1) Do blood samples taken *via* rapid caudal puncture yield similar results for live *v*. sacrificed groups of fishes? (2) Do rapidly obtained caudal blood samples accurately represent blood properties of fishes prior to capture? (3) Does storage of whole blood in an ice slurry for a working day (8·5 h) modify the properties of the plasma? By investigating these questions, this study helps to highlight some best practices for use in fisheries research and monitoring.

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MATERIALS AND METHODS

Anadromous adult Pacific salmonids *Oncorhynchus* spp. from British Columbia, Canada, were used in this study throughout 2009 (studies 1 and 2; fish from fresh water) and 2010 (study 3; fish from seawater). All fishes were caught during their spawning migration from the Pacific Ocean to freshwater spawning locations in the Fraser River watershed. All procedures were conducted with the approval of the Animal Ethics Committee of the University of British Columbia, in accordance with the Canadian Council on Animal Care.

STUDY 1: LIVE *v.* SACRIFICED FISH

Adult male coho salmon *Oncorhynchus kisutch* (Walbaum 1792) [$n = 20$; mean ± S.E. fork length (L_F) = 58 ± 1 cm; mean ± S.E. body mass (M_b) = 2·5 ± 0·2 kg] were dip-netted in November 2009 as they completed their 140 km upriver migration from the ocean to the Chehalis River Hatchery, where they had been released as juveniles *c*. 2–3 years prior. Males were selected in order to remove the influence of sex-specific differences in blood properties that are common in mature salmonids (Sandblom *et al*., 2009; Clark *et al*., 2010; Donaldson *et al*., 2010). To minimize the influence of interindividual variation in baseline levels of blood variables, all *O. kisutch* were exposed to a standard treatment before blood sampling. All *O. kisutch* individually underwent a 3 min manual chase protocol around a circular tank (diameter 150 cm, water depth 40 cm) followed by 1 min of air exposure in a dip-net above the water surface, and then they were placed in individual holding boxes (L × W × D = 100 × 60 × 60 cm, water depth 30 cm) supplied with ambient fresh water at 50 l min^{-1} (8° C). At 1 h post-treatment, when many of the blood stress indices measured here were presumed to be at a maximum plateau (Donaldson *et al*., 2010), *O. kisutch* were individually removed from the holding boxes and a 2 ml blood sample was taken from the caudal vasculature using a 38 mm, 21 gauge needle and a 4 ml lithium-heparinized vacutainer (Becton-Dickinson; www.bd.com). While half of the fish were held supine in a V-shaped, water-filled padded sampling trough to obtain the blood sample before they were sacrificed by cerebral concussion, the other half were sacrificed immediately prior to sampling blood from the fish in air. The duration between removal from the holding box and the blood sample was kept consistent between the two sampling methods and was <1 min in all cases. Haematocrit (Hct) was measured in the whole blood of each fish, and then the plasma was collected and stored in liquid nitrogen for subsequent analyses.

STUDY 2: CANNULATION *v.* CAUDAL PUNCTURE

Adult male *O. kisutch* ($n = 7$; mean ± S.E. $L_F = 67 \pm 2$ cm; mean ± S.E. $M_b = 3{\cdot}3 \pm 0{\cdot}3$ kg) were individually collected from the Chehalis River Hatchery in November 2009, as indicated above, and placed in an anaesthetic bath containing tricaine methanesulphonate (MS-222; 100 mg l^{-1}; Sigma; www.sigma.com) buffered with sodium bicarbonate ($NaHCO_3$; 200 mg l^{-1}). Once anaesthetized, the fish were positioned supine on a padded surgery bench where the gills were continuously irrigated with aerated water containing a maintenance dose of MS-222 (75 mg l^{-1} with 150 mg l^{-1} $NaHCO_3$). The dorsal aorta was cannulated with polyethylene (PE)-50 catheter posteriorly through the roof of the mouth using the method described by Soivio *et al*. (1975*a*), and the catheter was filled with sodium-heparinized (200 IU ml^{-1}) saline and sealed with a metal pin.

Following surgery, each *O. kisutch* was placed in an opaque holding tube (length 120 cm, diameter 30 cm; wire mesh at each end, with a slit in the top to externalize the catheter) submerged in a large channel (L × W × D = 10 × 5 × 2 m, water depth 60 cm) supplied with flow-through river water (8° C). *Oncorhynchus kisutch* were given 36 h to recover before two consecutive blood samples were taken from each individual. The first blood sample was taken into a heparinized syringe from the dorsal aorta *via* the catheter, with great care being taken not to disturb the fish. Then, the fish was removed from the holding tube, sacrificed and sampled for blood from the caudal vasculature using a vacutainer as detailed above. The time of each blood sample was considered to be the point at which the sample was finalized. The mean ± S.E. time lapse between the two blood samples was 142 ± 26 s (range

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51–219 s). Haematocrit was measured in whole blood from all samples, and then the plasma was collected and stored in liquid nitrogen for subsequent analyses. This protocol allowed a direct comparison of the blood properties of each individual before and after handling.

STUDY 3: STORAGE OF WHOLE BLOOD

Wild adult sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) ($n = 11$; seven males, four females; mean ± S.E. $L_F = 58 \pm 1$ cm) were troll-caught in August 2010 aboard F.V. *El Rayo* during their migration through Discovery Passage, BC, Canada, *en route* to freshwater spawning grounds. Water temperature was 11° C during the period of capture. Once brought aboard the vessel, *O. nerka* were placed in a holding tank for 2–15 min before being individually netted and placed supine in a V-shaped, water-filled padded sampling trough. A 5 ml blood sample was taken from the caudal vessels using a lithium-heparinized vacutainer and was well mixed before being separated equally between two vacutainers. One vacutainer containing whole blood was placed immediately into a thermally insulated storage container filled with an ice slurry (0–1° C), while the other vacutainer was immediately centrifuged and the plasma was removed and stored in liquid nitrogen for subsequent analyses. Following mean ± S.E. 8·5 ± 0·4 h of storage in the ice slurry, the remaining blood sample was centrifuged and the plasma was stored in liquid nitrogen. The 8·5 h storage duration was chosen to represent a situation where blood samples are taken at a field site in the morning and stored until arrival at the laboratory at the end of the day.

BLOOD ANALYSES

Haematocrit of whole blood was measured using microcapillary tubes spun at 10 000 *g* for 7 min. Remaining whole blood was spun at 7000 *g* for 7 min and then the plasma was collected in Eppendorf tubes and stored in liquid nitrogen prior to being transferred to a −80° C freezer for subsequent analyses. Single plasma measurements were made of lactate and glucose, with an internal calibration run every five samples (YSI 2300 stat plus analyser; www.ysilifesciences.com). Plasma measurements were made in duplicate of cortisol (Neogen ELISA with Molecular Devices Spectramax 240pc plate reader; www.labconco.com; www.neogen.com), osmolality (Advanced Instruments 3320 freezing point osmometer; www.aicompanies.com), chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Parmer, model 410 single channel flame photometer; www.coleparmer.com) (further details in Farrell *et al*., 2001). The hormones, testosterone and 17β-oestradiol, were assayed in duplicate (each study on a single assay plate to avoid inter-plate variability) after appropriate dilution and ether extraction (Neogen ELISA; www.neogen.com).

DATA ANALYSES AND STATISTICS

All statistical tests were performed using SigmaStat (Build 3.01.0, Systat Software Inc.; www.systat.com) after processing all data in Microsoft Excel (Microsoft Corporation; www.microsoft.com). Non-paired *t*-tests were used to compare blood samples from live *v*. sacrificed *O. kisutch* in study 1. Paired *t*-tests were used to compare caudal and dorsal aortic blood samples in study 2, and to compare initial and post-storage blood samples in study 3. Significance was considered as $P < 0{\cdot}05$. Values are given as means ± S.E. unless otherwise indicated.

RESULTS

STUDY 1: LIVE *v*. SACRIFICED FISH

All adult *O. kisutch* were visibly exhausted following the exercise and air exposure treatment. While the blood variables of all *O. kisutch* showed signs of physiological stress at 1 h post-treatment, there were no significant differences in any blood variable

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TABLE I. Comparison of blood samples taken from adult male *Oncorhynchus kisutch* (8° C), where blood was sampled by caudal puncture from either live or sacrificed fish 1 h following an exhaustive exercise treatment (study 1). Data are means ± S.E. with ranges in parentheses, and $n = 10$ in each group. *P*-values shown are the outcomes of *t*-tests (no significant differences between groups for any variable)

Variable	Alive	Dead	*P*-value
Haematocrit (%)	45·9 ± 2·0 (37·0–60·9)	46·6 ± 1·7 (38·9–56·5)	>0·05
Potassium (mmol l^{-1})	1·0 ± 0·2 (0·1–2·0)	1·1 ± 0·2 (0·1–2·0)	>0·05
Sodium (mmol l^{-1})	158·4 ± 3·3 (148·0–176·4)	165·9 ± 3·7 (144·3–191·9)	>0·05
Chloride (mmol l^{-1})	130·9 ± 1·5 (119·8–136·3)	126·7 ± 3·0 (102·4–134·7)	>0·05
Osmolality (mOsm kg^{-1})	342·0 ± 4·1 (311·3–354·0)	336·8 ± 4·6 (302·3–350·0)	>0·05
Glucose (mmol l^{-1})	6·9 ± 0·4 (5·7–8·7)	6·4 ± 0·2 (5·3–7·7)	>0·05
Lactate (mmol l^{-1})	17·9 ± 0·9 (13·1–21·6)	19·9 ± 0·6 (16·6–22·8)	>0·05
Cortisol (ng ml^{-1})	132·5 ± 16·6 (77·3–250·9)	189·1 ± 34·8 (85·5–465·9)	>0·05
Testosterone (ng ml^{-1})	47·9 ± 5·0 (25·9–75·0)	63·9 ± 10·3 (25·3–132·0)	>0·05

between the live and sacrificed groups (Table I). Thus, the tissue trauma associated with cerebral concussion did not compromise the quality of the blood data obtained from *O. kisutch* at a group level.

STUDY 2: CANNULATION *v.* CAUDAL PUNCTURE

Blood obtained rapidly from adult *O. kisutch* using the caudal puncture technique provided statistically similar data to blood obtained *via* the dorsal aortic cannula (Table II), with the only exception being that plasma osmolality was significantly higher in the former. This finding for osmolality must be treated with caution because of a low sample size ($n = 3$) due to insufficient quantities of blood plasma from the dorsal aortic cannula of four *O. kisutch*. Nevertheless, most variables tended to be slightly elevated in the caudal blood samples (Table II), perhaps suggesting that the period of time between fish handling and caudal blood sampling in this study (142 ± 26 s) approached a maximum for obtaining a blood sample uninfluenced by capture and handling (Table II). Even so, linear regressions of time between blood samples (ranging from 51 to 219 s) *v.* change in blood variables (both relative and absolute changes for each blood variable) yielded non-significant relationships in all cases.

STUDY 3: STORAGE OF WHOLE BLOOD

Storage of whole blood from adult *O. nerka* for 8·5 ± 0·4 h in an ice slurry significantly altered plasma potassium, chloride and glucose concentrations (Fig. 1). While plasma glucose increased by 0·34 ± 0·13 mmol l^{-1} (5·6 ± 2·0%) during whole blood storage, plasma chloride decreased by 2·7 ± 0·9 mmol l^{-1} (1·8 ± 0·6%) and plasma potassium decreased dramatically by 2·3 ± 0·3 mmol l^{-1} (65·7 ± 6·8%; Fig. 1). Although other plasma variables did not change significantly during storage with

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TABLE II. Comparison of paired blood samples from individual male *Oncorhynchus kisutch* (8° C), where the first sample was from a cannula in the dorsal aorta and the second sample was taken 142 ± 26 s (mean ± S.E.) later by caudal puncture (study 2). Data are means ± S.E. with ranges in parentheses. Data for individual fish were used to calculate difference as tail − catheter and % change as $100(xy^{-1} - 1)$, where x = tail and y = catheter. P-values shown are the outcomes of paired t-tests (significant values $P < 0{\cdot}05$)

Variable	n	Cannula	Caudal	Difference	% change	P-value
Haematocrit (%)	7	28·8 ± 1·2 (24·1 to 32·0)	30·5 ± 2·2 (23·9 to 35·9)	1·5 ± 1·1 (−3·2 to 6·3)	4·7 ± 3·9 (−11·7 to 21·2)	>0·05
Potassium (mmol l^{-1})	5	2·3 ± 0·5 (0·5 to 3·5)	1·9 ± 0·4 (0·6 to 3·1)	−0·5 ± 0·6 (−1·6 to 1·3)	24·5 ± 58·8 (−71·1 to 250·0)	>0·05
Sodium (mmol l^{-1})	5	142·2 ± 2·0 (137·2 to 146·2)	148·7 ± 1·8 (143·7 to 154·1)	6·5 ± 2·6 (2·0 to 16·6)	4·6 ± 1·9 (1·4 to 12·0)	>0·05
Chloride (mmol l^{-1})	5	124·2 ± 1·7 (120·3 to 129·1)	126·5 ± 2·0 (119·7 to 131·7)	2·3 ± 1·4 (−1·2 to 6·0)	1·9 ± 1·2 (−1·0 to 5·0)	>0·05
Osmolality (mOsm kg^{-1})	3	297·3 ± 3·1 (292·3 to 303·0)	304·1 ± 3·8 (299·3 to 311·5)	6·8 ± 1·1 (4·8 to 8·5)	2·3 ± 0·4 (1·6 to 2·8)	<0·05
Glucose (mmol l^{-1})	7	8·0 ± 1·1 (5·3 to 13·1)	7·8 ± 1·2 (4·5 to 14·0)	−0·2 ± 1·1 (−7·0 to 1·7)	2·0 ± 10·9 (−61·0 to 26·0)	>0·05
Lactate (mmol l^{-1})	7	0·9 ± 0·4 (0·2 to 3·2)	1·0 ± 0·4 (0·2 to 3·4)	0·0 ± 0·1 (−0·2 to 0·2)	4·4 ± 11·1 (−52·0 to 30·8)	>0·05
Cortisol (ng ml^{-1})	7	113·1 ± 11·2 (87·3 to 169·3)	122·1 ± 13·8 (71·4 to 183·2)	9·0 ± 6·2 (−22·4 to 24·7)	7·7 ± 6·2 (−23·9 to 23·4)	>0·05
Testosterone (ng ml^{-1})	6	44·7 ± 9·8 (8·7 to 71·0)	54·9 ± 13·3 (16·2 to 107·2)	10·2 ± 5·3 (2·5 to 36·2)	29·3 ± 13·3 (4·8 to 86·2)	>0·05

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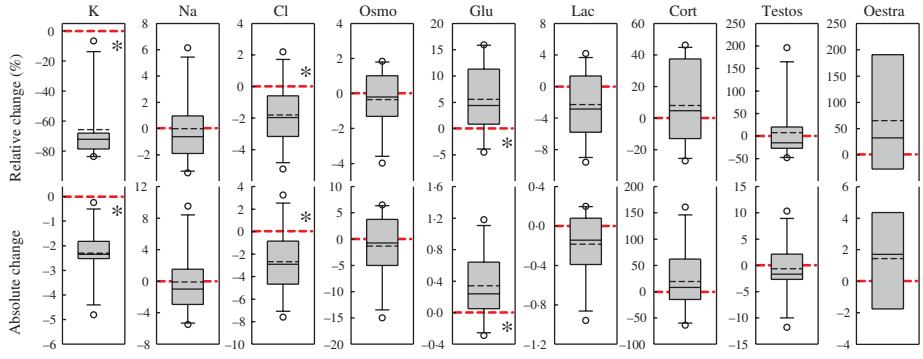

FIG. 1. Relative change (top row) and absolute change (bottom row) in blood plasma properties of adult *Oncorhynchus nerka* following storage of whole blood in an ice slurry for 8·5 ± 0·4 h (mean ± S.E.) (study 3). Initial, pre-storage values for each variable were K (potassium) = 3·5 ± 0·3 mmol l^{-1}, Na (sodium) = 159·1 ± 1·4 mmol l^{-1}, Cl (chloride) = 148·8 ± 1·4 mmol l^{-1}, Osmo (osmolality) = 362·9 ± 3·5 mOsm kg^{-1}, Glu (glucose) = 6·3 ± 0·3 mmol l^{-1}, Lac (lactate) = 7·0 ± 0·7 mmol l^{-1}, Cort (cortisol) = 288·6 ± 64·6 ng ml^{-1}, Testos (testosterone) = 11·2 ± 1·9 ng ml^{-1} and Oestra (17β-oestradiol) = 4·8 ± 1·0 ng ml^{-1}. The bottom and top boundaries of each shaded box represent the 25th and 75th percentiles; ___, within each box represents the median; - - -, represents the mean; vertical whiskers on each box represent the 10th and 90th percentiles; ○, represent outliers. A significant effect of storage (paired *t*-tests) existed for plasma potassium ($P < 0·001$), chloride ($P < 0·05$) and glucose ($P < 0·05$), as denoted by ∗. Where storage did not have a significant effect on a plasma variable, the 25th and 75th percentiles encompass the (- - -) representing zero change. $n = 11$ (seven males and four females) for all variables except 17β-oestradiol, where $n = 4$ because no traces of this hormone were found in male plasma samples.

regard to mean levels, the response of individual samples sometimes showed substantial variation. For example, for two of the 11 paired plasma testosterone samples, one increased by 10·3 ng ml^{-1} (196·1%) and the other decreased by 11·8 ng ml^{-1} (48·1%) during whole blood storage. As traces of 17β-oestradiol were not found in plasma samples from male *O. nerka*, the dataset for this variable was limited to four female fish. The response in 17β-oestradiol varied greatly between samples, exemplified by an increase of 4·5 ng ml^{-1} (229·3%) in one sample during whole blood storage and a decrease of 2·1 ng ml^{-1} (32·6%) in another sample (Fig. 1).

DISCUSSION

BLOOD SAMPLING

The results of this study confirm that a snapshot of the blood physiology of a population of fish can be reliably obtained from live or sacrificed individuals using rapid caudal puncture (Table I). Thus, fishes that are not going to be released can be sacrificed prior to sampling to remove the difficulties of obtaining blood from live and struggling fishes. This study also confirms that blood sampled rapidly (142 ± 26 s) using the caudal puncture technique provides an accurate representation of the properties of the circulating blood prior to capture (Table II). This new knowledge is particularly important as the caudal puncture technique is increasing

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in use throughout fisheries research but with little proof of its efficacy. Indeed, as far as is known, this is the first study to investigate the properties of blood sampled using the caudal puncture technique in direct comparison with paired samples taken from cannulated fish prior to handling.

Despite the present findings for adult salmonids at 8° C, further work is required to determine the general efficacy of the caudal puncture technique under different circumstances and for different species. For example, fish size and water temperature may influence the rate of blood circulation and the speed of alterations in the blood properties, thus modifying the time available to obtain an accurate blood sample following fish capture (Di Marco *et al*., 1999). Indeed, a study of the common carp *Cyprinus carpio* L. 1758 (479 g, 20° C) reported an increase in plasma glucose and a decrease in cortisol when blood was sampled by cardiac puncture 2 and 5 min following capture in comparison with blood sampled immediately following capture (Svobodova *et al*., 1999). A study of sea bass *Dicentrarchus labrax* (L. 1758) (372 g, 14° C) reported increases in most of the measured plasma variables when blood was sampled 5 min following capture in comparison with blood sampled rapidly, while plasma potassium was the only variable that was significantly different in blood obtained through cardiac *v*. caudal puncture (Marino *et al*., 2001).

In addition to investigating the efficacy of blood sampling techniques, this study provides insight into the baseline levels of blood physiology of adult *O. kisutch* as well as the changes in blood properties induced by an exhaustive bout of exercise. Indeed, the blood obtained from cannulated and rapidly caudal sampled *O. kisutch* (Table II) is generally more representative of resting individuals than samples taken in previous studies of adult *O. kisutch* from freshwater and marine environments (Farrell *et al*., 2001; Donaldson *et al*., 2010). The measurements from blood taken 1 h after an exhaustive stressor (Table I) are within the ranges reported for adult *O. kisutch* following the same period of recovery from exhaustive exercise, with the exception that plasma ions and osmolality were higher in *O. kisutch* sampled in the marine environment (Farrell *et al*., 2001; Donaldson *et al*., 2010). Thus, this study helps to define the scope in blood variables attainable for *O. kisutch*, and provides a baseline with which future studies can compare.

BLOOD STORAGE

Storage of *O. nerka* whole blood for 8·5 h at 0–1° C caused significant decreases in plasma potassium and chloride, and a significant increase in plasma glucose (Fig. 1). It is probable that the plasma concentrations of these variables changed during storage largely as a result of net movement between the plasma and the cytosol of the erythrocytes. While it is not possible to confirm this suggestion as the measurements of this study were restricted to the plasma, the putative underlying mechanisms can be discussed.

The stress of capture by trolling probably (1) decreased whole blood pH by increasing lactate (plasma lactate = $7{\cdot}0 \pm 0{\cdot}7$ mmol l^{-1}; Fig. 1) and carbon dioxide tension, (2) activated the erythrocyte sodium-hydrogen ATPase through an increase in circulating catecholamines, (3) decreased oxygen tension in the caudal vasculature and (4) induced erythrocyte swelling (Wood, 1991; Brauner & Randall, 1996). While the initial plasma sample was probably influenced by these processes, large changes in the plasma properties occurred throughout storage presumably because

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these and other processes continued to influence the transmembrane movement of ions and molecules. Indeed, erythrocyte volume is known to increase with a concomitant decrease in plasma chloride and potassium when salmonid blood is stored at low oxygen tensions (Soivio *et al*., 1974, 1975*b*; Borgese *et al*., 1991), and the effects can be exacerbated at low pH (Nielsen & Lykkeboe, 1992). Conversely, exposing salmonid blood to high oxygen tensions and warm temperatures elicits an increase in plasma chloride and potassium due to a net loss from erythrocytes (Korcock *et al*., 1988; Borgese *et al*., 1991; Nielsen & Lykkeboe, 1992), although this pattern does not seem to hold for avian blood (Lumeij, 1985; Reece *et al*., 2006). Such ion transport has been shown to occur within minutes in stored fish blood (Borgese *et al*., 1991).

Activity of the sodium–hydrogen ATPase in stored blood should promote transport of sodium ions into, and hydrogen ions out of erythrocytes at the expense of ATP, thus elevating intracellular pH as well as causing swelling of the erythrocytes due to osmotic uptake of water. While transmembrane glucose transport in stored *O. nerka* blood was probably through volume-activated diffusion (Kirk *et al*., 1992), a significant decline in intracellular ATP observed in chilled and stored salmonid blood (Korcock *et al*., 1988) provides support for the occurrence of active transmembrane transport of ions and molecules throughout storage. As intracellular pH increases through the activity of the sodium-hydrogen ATPase, thus increasing haemoglobin affinity, intracellular bicarbonate increases and is subsequently exported out of the erythrocyte in exchange for chloride ions (Caldwell *et al*., 2006). Such transmembrane bicarbonate–chloride exchange is enhanced at high carbon dioxide tensions (Brauner *et al*., 2000), conditions which were probably present in *O. nerka* blood during storage in this study. Despite this putative chain of events, plasma sodium remained unchanged throughout storage in this study (Fig. 1) and in a previous study of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) blood (Nielsen & Lykkeboe, 1992). The highly variable responses in sex hormones to storage in this study highlight the need for further investigation. For example, while subsequent studies should further investigate the effects of blood storage, they should also investigate variability stemming from sample preparation, subtle variations in laboratory techniques, and inconsistency between assay plates and kits.

RECOMMENDATIONS AND FUTURE DIRECTIONS

This study tested the efficacy of some common blood sampling and storage approaches in fisheries research and monitoring. The data confirm that rapid (<3 min) caudal puncture can provide a useful tool for obtaining blood samples that are representative of unhandled fishes, although it is cautioned that similar studies should be conducted on species of different body mass, at different temperatures, and across a broad range of capture-to-sampling durations before researchers adopt this technique more generally.

The caudal puncture technique provides a means to acquire physiological and biochemical data from wild fishes and return the fishes to their natural environment. This technique may be desirable, for example, when working on rare or imperilled taxa, when blood samples are to be used to predict post-release behaviour or fate, or when there is a need to serially blood sample fishes (*e.g.* in stress responsiveness studies). With this approach, it is imperative to understand the long-term consequences of the

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sampling procedure on the physiology, behaviour and survival of the fish (Voss *et al*., 2010 provide a review on blood sampling of birds). Where appropriate, researchers may choose to sacrifice fishes prior to using the caudal puncture technique, as this does not compromise blood properties, it may be favourable from an animal ethics standpoint, it reduces the difficulty involved with collecting blood samples and it may help to attenuate changes in blood properties after capture in comparison with live and struggling fishes. Once blood samples are obtained, they should be centrifuged as quickly as practicable for separation of the plasma and erythrocytes to prevent transmembrane transport of ions and molecules that can occur within minutes and result in misleading data (Borgese *et al*., 1991). Identifying species-specific threshold blood storage durations would be useful for those instances where it is impractical or impossible to centrifuge blood immediately.

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