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Validation of the hepatic portal vein cannulation technique using Atlantic salmon *Salmo salar* L.

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This study assessed the hepatic portal vein cannulation technique and surgical recovery in Atlantic salmon *Salmo salar*. Haematocrit levels were maintained and blood variables, including cortisol, returned to baseline levels within 1–3 days post-surgery, indicating that this technique is a viable, useful method to study the digestive physiology of fishes.

Key words: acid–base balance; Atlantic salmon; cortisol; glucose; ion regulation; surgical techniques.

The digestive physiology of fishes has received little attention to date. Most of the knowledge about fish digestion has come from an extensive literature on growth and digestibility trials (Higgs *et al.*, 1995) or metabolic studies measuring specific dynamic action (Jobling, 1981; Halver & Hardy, 2002) and a handful of studies on blood flow to the gastrointestinal tract (Farrell *et al.*, 2001). Qualitative studies of nutrient uptake across the gut of fishes, however, are lacking. This is because this type of study requires blood sampling from the hepatic portal vein. While techniques are well established for cannulating arteries in fishes, *e.g.* the ventral aorta (Farrell *et al.*, 1979; Axelsson *et al.*, 1994) and the dorsal aorta (Smith & Bell, 1964; Soivio *et al.*, 1975; Kiessling *et al.*, 2003), only recently has a reliable method emerged for cannulating major venous vessels such as the *ductus Cuvier* (Farrell & Clutterham, 2003; Sandblom *et al.*, 2005). Blood sampled from arteries and the *ductus Cuvier*, however, is unlikely to exactly reflect the composition of blood leaving the intestinal mucosa *via* the hepatic portal system since modification during passage

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through the liver is highly likely. While McLean & Ash (1989) have provided the only report, as far as is known, that demonstrates the feasibility of chronically cannulating the hepatic portal vein in rainbow trout *Oncorhynchus mykiss* (Walbaum), nobody has successfully measured blood variables using this technique. Thus, fish nutritionists who are looking at new methods of assessing nutrient uptake need a validation of this hepatic portal vein cannulation technique, which if combined with the well established dorsal aorta cannulation techniques would provide a methodology to examine both nutrient uptake and hepatic metabolic transformation. This would allow much more precise evaluation of the benefits of different feed sources or feed blends. Additionally, this technique will help in the examination of systemic physiological changes associated with many more aspects of gut function, not just digestion (*e.g.* acid–base balance and ion and osmotic regulation), in greater detail than previously possible. These are all areas that are now ready for further investigation in fishes and this technique should prove very valuable towards that end.

Since the surgery required for the hepatic portal vein cannulation is fairly invasive, the objective of this study was to assess the recovery of Atlantic salmon *Salmo salar* L. from the hepatic portal vein cannulation for 7 days post-surgery. This study therefore provides the first report of variables measured on blood sampled repeatedly from the hepatic portal vein in the same individual fish.

Atlantic salmon (body mass 0·95–1·71 kg, $n = 13$) were held at the Norwegian Institute for Water Research, Solbergstrand, Norway, in 4000 l tanks under a 12L:12D photoperiod. Tanks contained filtered and aerated flow-through sea water (salinity = 33·7 ± 0·4, mean ± S.D.) at a temperature of 9·2 ± 0·8° C. Fish were fed a 1% body mass ration of commercial feed 5 days a week. Prior to cannulation, individual fish were transferred to an experimental tank and starved for 24–48 h. Eight 800 l (1·0 × 1·0 × 0·3 m with water) experimental tanks were equipped with an individual light source and a shelter consisting of a 0·3 × 0·5 m shelf attached to the tank wall 0·05 m above the water surface. The experimental tanks received sea water at 2 l min^{-1} and each tank was equipped with a water pump to create current. This arrangement in combination with a hole in the tank wall just above the water allowed blood samples to be drawn with minimal disturbance to the fish.

For the cannulation procedure, fish were individually sedated and anaesthetized as described by Kiessling *et al.* (2003). In brief, each fish was exposed to 0·5 mg l^{-1} metomidate (Syndell, Inc., Vancouver, BC, Canada) in the experimental tank water for 15 min and then transferred to a separate bath containing 100 mg l^{-1} metacanium (MS-222, tricaine methane sulphonate; Norwegian Medical Depot, Bergen, Norway). This procedure reduced the initial stress normally associated with netting and submerging the fish in a full strength anaesthesia bath with metacanium. In addition, exposure to a pre-anaesthesia sedation reduces the dose of metacanium needed, thereby reducing the post-surgery recovery time. The water was irrigated over the gills by a submergible pump and when the coughing reflex ceased, the fish was placed ventral side up, left side exposed, on a surgical table and covered with a wet cloth. The gills were continually irrigated with a re-circulating, chilled anaesthetic solution (50 mg l^{-1} MS-222). Lidocaine (Xylocain®, 20 mg ml^{-1}; Astra, Indal, Sweden)

was injected (4 × 0·1 ml) along the incision line, just posterior to the pectoral fin, extending from a few cm below the ventral midline to a few cm above the lateral line. The use of local anaesthetics at the incision site kept the fish at stage III, plane 1–2 anaesthesia rather than stage III, plane 4 to stage IV anaesthesia (based on Stoskopf, 1991), which is normal for more invasive surgery in fishes using MS-222. This together with pre-anaesthesia sedation significantly reduces post-surgery recovery time. The skin was then cut using a scalpel and Mayo scissors were used to extend the incision. Retractors were used to keep the incision site open.

In Atlantic salmon, several larger vessels merge into the hepatic portal vein (Fig. 1) and although venous vasculature varies considerably between fish, a branch off either the ventral or dorsal intestinal vein was selected for cannulation. The desired vessel was isolated using sterile cotton swabs and fine curved forceps were used to lift a 0·75 cm section of the vessel just enough to prevent blood flow (Fig. 1). A piece of tubing was slipped around the forceps to prevent them from spreading too much and stretching the vessel. Two pieces of 2-0 silk were placed around the vessel and the posterior thread was tightened to occlude the vessel, while the other thread was loosely knotted but not tightened. Using a dissecting microscope and microscissors, a V-shaped cut was made at a 45° angle into the vessel between the two pieces of silk without transecting the vein. A pair of fine forceps was used to direct the silastic tip of the cannula into the vessel and advance it into the hepatic portal vein. The

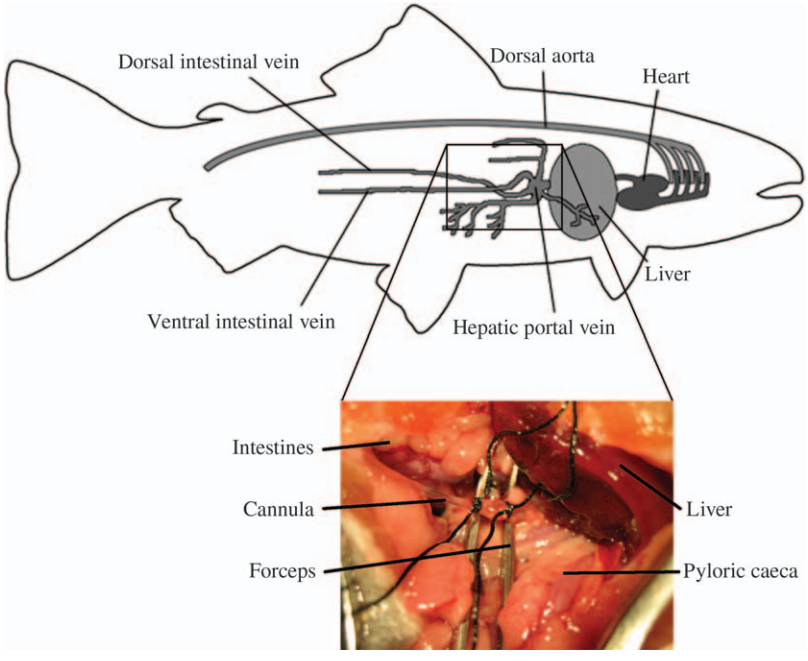

FIG. 1. Schematic of the salmonid hepatic portal system (adapted from Thorarensen *et al.*, 1991) and a photograph of a cannula inserted into a side vessel off the dorsal intestinal vein.

two silk threads were tightened around the cannula on either side of a bubble to secure the cannula in place. Blood was withdrawn using a syringe to ensure that the silk threads were not tied too tightly around the silastic portion of the cannula. In order to prevent any bacterial contamination of the body cavity, 0·6 ml of Aquacycline antibiotic (oxytetracycline; Ceva Sante Animal, Libourne, France; 100 mg ml^{-1}) was injected into the body cavity before the incision site was closed using interrupted 2–0 silk sutures. The cannula was secured to the skin of the fish by the second and third bubbles using 2–0 silk sutures. The PE 50 cannula was filled with 150 IU ml^{-1} heparin (heparin aqua solution, injection quality; Nycomed Pharma Ltd, Oslo, Norway) in isotonic sodium chloride solution (injection quality; Fresenius Kabi, Uppsala, Sweden) and then resealed by heat. All equipment was disinfected with 70% ethanol. All cleaned skin areas were re-covered with mucous by gently covering the skin with mucus from an untreated area with a wet hand before the fish was returned to the tank. After its return to the tank, the fish's recovery was monitored until normal breathing and swimming behaviour reassumed, normally within 0·5–2·0 min and 10–15 min, respectively.

The cannula was a 50 cm length of polyethylene tubing (PE 50, Intramedic; Clay Adams, New Jersey, NJ, U.S.A.), fashioned with three bubbles 2 mm, 5 cm and 7 cm from the end. A short length (*c.* 4 cm) of silastic tubing (0·5 mm internal diameter × 0·94 mm external diameter, Silastic; Dow Corning, Midland, MI, U.S.A.) was stretched over the end of PE 50 tubing (flush with the first bubble) using fine watchmaker's forceps and trimmed to the desired length (2–3 cm, depending on the size of the fish) at a 45° angle for easier insertion into the vessel. Several back cuts and small holes were made in the end of the silastic tip. The cannula was rinsed and filled with heparinized saline (150 IU ml^{-1}) and stored in a fridge for >24 h prior to surgery in order to prevent clotting and make the silastic tubing slightly more rigid to aid insertion into the vessel.

Blood (0·5 ml) was sampled using a 1 ml syringe from the hepatic portal vein immediately and again 1 h post-surgery, and then after 1, 2, 3, 4, 5, 6 and 7 days post-surgery. The saline and first 50–100 μl of blood was discarded before collecting a 0·5 ml sample for analysis. The blood was replaced with an equivalent volume of injection quality heparinized saline (150 IU ml^{-1}). Haematocrit was measured in duplicate using 20 μl microhaematocrit tubes (Compur microspin; Bayer, Leverkusen, Germany). Plasma was obtained immediately by centrifugation at 7500 *g* for 5 min and initially stored at −20° C and then transferred to −80° C within hours. Plasma levels of cortisol were measured using a radioimmunoassay kit (Orion Diagnostica, Espoo, Finland, Spectria Cortisol Ria test). Plasma glucose, Na^+, K^+, total CO_2 (TCO_2), pH, partial pressure of CO_2 (pCO_2) and HCO_3 were measured using an i-STAT portable clinical analyser (Jacobs *et al.*, 1993; Harrenstien *et al.*, 2005). Values for TCO_2, pH, pCO_2 and HCO_3 were temperature-corrected to 9·2° C.

Statistical comparisons were made using ANOVA and P values of <0·05 were considered statistically significant. If significance was found using ANOVA, a Tukey–Kramer multiple comparisons was used to compare differences. Means ± S.E. are presented.

Plasma cortisol levels were significantly higher at 1 h post-surgery, but had returned to a baseline level by 1 day post-surgery and remained at the baseline

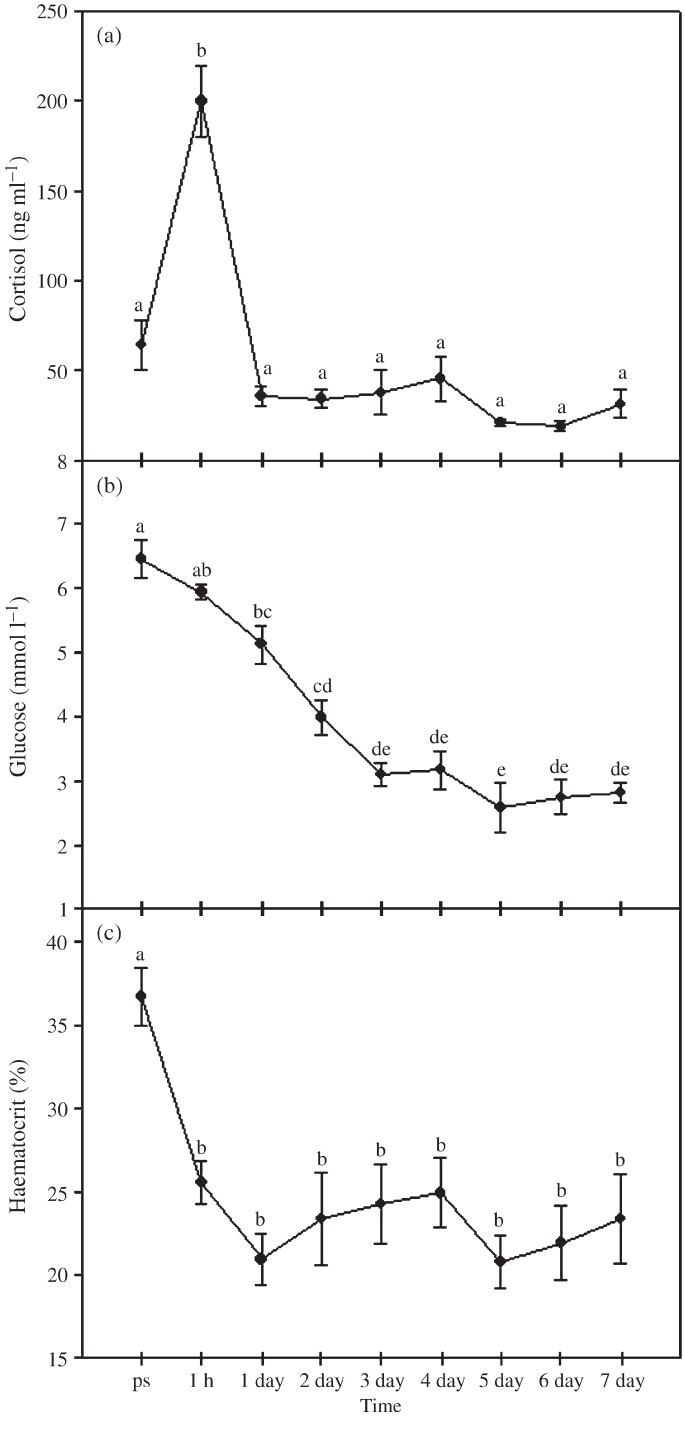

FIG. 2. Mean ± S.E. values of (a) cortisol, (b) glucose and (c) haematocrit levels in Atlantic salmon with time. Differing superscript lower case letters denote statistically significant differences ($P < 0{\cdot}05$). ps, pre-surgery.

level for the remaining 7 days post-surgery (Fig. 2). This baseline level was within the normal range for plasma cortisol found in healthy, unstressed Atlantic salmon (Olsen *et al.*, 1995; Einarsdottir & Nilssen, 1996).

Plasma glucose concentrations were significantly elevated after surgery but declined steadily to the baseline level by day 3 (Fig. 2). Glucose levels were stable thereafter (2·6–3·2 mmol l^{-1}). This baseline level was well within normal glucose levels for healthy, unstressed Atlantic salmon (Einarsdottir & Nilssen, 1996).

Haematocrit was highest (36·7 ± 1·8%) immediately after surgery (Fig. 2), but quickly declined to a stable baseline level (25·6 ± 1·3%) 1 h post-surgery. This baseline level is within normal haematocrit levels for unstressed, free swimming fishes (Gallaugher *et al.*, 1995). A potential drawback of repeated blood sampling is haemodilution. Haematocrit varied between 21 and 26% for the remainder of the recovery period, which suggests internal haemorrhaging, stress and haemodilution were not serious problems. Previous studies have shown that 10% of the total blood volume can be withdrawn without adverse effects on the fish, particularly when the blood is replaced with an equivalent volume of saline solution (Ash *et al.*, 1989; McLean & Ash, 1989; Gallaugher *et al.*, 1995). Consistent with earlier experience from dorsal aorta cannulation (Kiessling *et al.*, 2003), the present results confirm that haematocrit can be maintained even after sampling blood eight times over 7 days.

Plasma pH was significantly lower immediately post-surgery (7·68 ± 0·02), but recovered by 1 h and was maintained for the duration of the study (7·87–7·96). Plasma HCO_3, TCO_2, pCO_2, Na^+, and K^+ displayed small, sometimes significant variations over the recover period, but with no particular pattern beyond the first day post-surgery (Table I). All values were within expected ranges for unstressed, healthy fishes (Eddy *et al.*, 1977; Larsen *et al.*, 1997; Brauner *et al.*, 2000; Powell & Nowak, 2003).

This study clearly shows that there is great potential for the hepatic portal vein cannulation technique in fishes despite the apparent lack of interest in

TABLE I. Plasma pH, Na^+, K^+, pCO_2, TCO_2 and HCO_3 levels (T, total; p, partial pressure). Data are presented as means ± S.E. Means with differing superscript lower case letters are significantly different ($P < 0·05$)

	Blood variables					
	pH	Na^+ (mmol l^{-1})	K^+ (mmol l^{-1})	pCO_2 (mm Hg)	TCO_2 (mmol l^{-1})	HCO_3 (mmol l^{-1})
ps	$7·68 \pm 0·02^{a}$	$157·8 \pm 0·7^{c}$	$2·8 \pm 0·1^{ab}$	$4·8 \pm 0·2^{a}$	$5·8 \pm 0·2^{b}$	$5·6 \pm 0·2^{b}$
1 h	$7·92 \pm 0·02^{b}$	$162·3 \pm 0·9^{ab}$	$3·0 \pm 0·1^{a}$	$3·6 \pm 0·1^{b}$	$7·5 \pm 0·4^{a}$	$7·4 \pm 0·4^{a}$
1 day	$7·90 \pm 0·01^{b}$	$165·8 \pm 1·4^{a}$	$2·5 \pm 0·1^{b}$	$3·2 \pm 0·1^{bc}$	$6·4 \pm 0·2^{ab}$	$6·3 \pm 0·2^{ab}$
2 day	$7·96 \pm 0·04^{b}$	$162·7 \pm 1·3^{ab}$	$2·6 \pm 0·1^{ab}$	$2·9 \pm 0·2^{c}$	$6·4 \pm 0·1^{ab}$	$6·3 \pm 0·1^{ab}$
3 day	$7·95 \pm 0·02^{b}$	$159·1 \pm 1·1^{bc}$	$2·6 \pm 0·0^{ab}$	$3·1 \pm 0·1^{bc}$	$7·0 \pm 0·4^{ab}$	$6·9 \pm 0·4^{ab}$
4 day	$7·88 \pm 0·02^{b}$	$161·3 \pm 1·9^{abc}$	$2·7 \pm 0·1^{ab}$	$3·3 \pm 0·2^{bc}$	$6·2 \pm 0·3^{ab}$	$6·1 \pm 0·3^{ab}$
5 day	$7·87 \pm 0·02^{b}$	$159·2 \pm 0·9^{bc}$	$2·5 \pm 0·0^{ab}$	$3·0 \pm 0·2^{bc}$	$5·6 \pm 0·5^{b}$	$5·5 \pm 0·5^{b}$
6 day	$7·93 \pm 0·03^{b}$	$157·2 \pm 1·1^{c}$	$2·5 \pm 0·1^{ab}$	$2·8 \pm 0·3^{bc}$	$5·9 \pm 0·4^{ab}$	$5·8 \pm 0·3^{ab}$
7 day	$7·90 \pm 0·01^{b}$	$158·5 \pm 0·6^{bc}$	$2·5 \pm 0·0^{b}$	$3·3 \pm 0·1^{bc}$	$6·6 \pm 0·2^{ab}$	$6·5 \pm 0·2^{ab}$

ps, pre-surgery.

the technique from the scientific community since the feasibility of the technique was established 17 years ago. The results clearly show that the majority of the post-surgery effects had worn off after 1 day and all measured effects by the third day post-surgery and blood sampling could be repeated over a 7 day period, which is more than sufficient for a fish to fully digest a single meal. Moreover, since the invasive surgical techniques are quite similar to those used previously by researchers who have studied gastrointestinal blood flow in fishes, these results provide indirect support of their methodologies being sound. It is concluded that this technique may benefit fish physiologists to gain in basic knowledge of the digestive physiology of salmonids and more generally the aquaculture industry who are interested in new diet development. Additionally, this technique will help with the study of gut physiology in relation to acid–base balance, ion and osmotic regulation in much more detail than previously possible.

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