

**Physiological Indicators of Stress of Capture and Mortality Risk in Commercial
Non-Retention Salmon Fisheries**

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Abstract

Over 300 coho salmon (*Oncorhynchus kisutch*) were examined for their physical and metabolic condition following capture with three different commercial salmon fishing gear types (seine, troll and gillnet). Regardless of the gear type, all fish arrived on board in a severe state of metabolic exhaustion and physiological stress as based on the eleven physiological measurements we performed. In our opinion, exhaustion and stress of this severity would only permit aerobic swimming at a very low velocity or a few seconds of burst (anaerobic) activity if fish were released immediately after capture. It appears that the metabolic status of the muscle was such that it would severely limit further muscular contractions of any magnitude or duration. Therefore, it is unlikely that these fish could avoid predators if released immediately after capture.

The visual assessments of physical condition that were made (a 5 point scale) correlated well with some of our physiological measures of stress (plasma osmolality, plasma lactate and sodium ion concentrations). The hematocrit measurements suggest that anemia, due to poor handling practices and external damage to the skin and gills, was not a significant factor (<1%). Differences in the level of exhaustion between gear types did exist, but they were small. This is also true for differences observed between the different methods of fishing within gear types (i.e., gillnet, 30 and 60 minute soak times; seine, ramp, brail, or brail with modified bunt). In each case, only one or two of the variables measured were different, rather than a whole suite of variables. Some of these observed differences are likely explained by differences in the normal time between landing the fish and the actual exhaustion at capture and degree of air exposure. The data is sufficient for us to say that seine fishing using brailing techniques compared with ramping, and gillnet fishing with 30 minute compared with 60 minute soak times, are less stressful to coho salmon.

We have made the first comprehensive assessment of the metabolic status of fish held in recovery (blue) boxes for 30- and 60-minute periods following capture. Our conclusions regarding the effectiveness of the blue boxes have caveats regarding (a) the ineffective design of the blue box in promoting the best possible level of recovery, and (b) the possibility that the method of tissue sampling introduced a sampling error. Although the values for the physiological variables measured immediately following capture are very similar to those reported in other field and laboratory studies with exhaustive exercise, the recovery data differs. Overall, we did not observe evidence of the type of metabolic recovery observed under optimum conditions within the same period of recovery in these other studies. Two exceptions to this generalization are the significant improvements in potassium ion concentrations after 30 minutes and increases in muscle phosphocreatine levels in gillnet samples after 60 minutes recovery. These results are encouraging and perhaps a better-designed recovery box would be effective. Since restoration to pre-exercise levels for a number of these variables (e.g., plasma lactate, osmolality, ion concentrations) is more apparent after a longer holding period, extending the length of time held in blue boxes before release should also be tested. We conclude that the blue boxes did not make matters worse for the fish, and may have promoted more effective gill ventilation and restored blood oxygen status to normal.

Our recommendations for future experiments are:

Test recovery in a better designed box with compartments and adequate flow of water to allow fish to swim slowly into a water current;

Use a muscle biopsy sampling method to eliminate sampling error and get a more accurate picture of the immediate recovery of muscle metabolites;

Correlate the physiological data with the ability of the fish to swim post-capture; and

Test longer recovery periods in the blue boxes in real-life fishing situations.

In our opinion, we cannot proceed with the current selective fisheries policy until we know whether recovery boxes are valuable in facilitating recovery and know more about the long term survival and spawning success of fish released after capture. Having a better designed blue box is critical to this assessment.

Introduction

Perspective – the development of a science-fisheries industry partnership to assess the effectiveness of the policy of selective fisheries.

For salmon in British Columbia, "catch and release" (Petit, 1977) has long been the policy in the sports fishing industry but the concept of non-retention of non-target species of salmon after capture is relatively new to the commercial fishing industry. In recent years, with the growing concern about declines in some stocks of coho salmon (*Oncorhynchus kisutch*) (see Speaking for the Salmon, June 1998a), various members of the commercial sector have taken the initiative to reduce the catch or retention of weaker stocks. In addition, they have experimented with changes to both gear and fishing technique to reduce the mortality of non-target species and increase the likelihood of survival following live release. For example, in 1997 Area D Gillnetters signed a joint project agreement with Fisheries and Oceans Canada (FOC) to test the effectiveness of the new Alaska twist web compared with the standard gillnet web as a conservation measure to protect the coho. In addition, they also all carried revival (also called blue) boxes on board to hold fish for a period of recovery before live release, with the expectation that this would increase the chances of survival and successful reproduction (see Speaking for the Salmon, 1998b).

The seine fleet has also experimented with modifications to gear and fishing techniques. In response to the concerns arising from the 1994 salmon fishing season, seine boat operators Weigold and Cook (1995) proposed a pilot study to test "various release methods including the use of knotless bunts, tow-off skiffs and large knotless dipnets for removing fish from the net". In the summers of 1995-1997, special seine fishery experiments were conducted in the Skeena River to test the effects of brailing and the use of recovery boxes on non-target species (J.O. Thomas and Associates, 1997). A number of seine modifications thought to reduce subsequent mortality after live release of non-target species were described at a workshop in early May 1998, including the practice of brailing and direct release from the net using dipnets as well as the mandatory use of a revival box. At the same workshop, members of the troll fleet made a number of suggestions for more successful live release of weaker stocks including: continuous monitoring of gear, release of catch in the water and use of barbless hooks (see Speaking for the Salmon, 1998b).

In May 1998, in response to the grave concerns about the health of some coho salmon stocks, the Minister of Fisheries and Oceans Canada announced a coast-wide policy of selective fisheries for the 1998 commercial salmon fishing season. A call for proposals to minimize bycatch of the endangered coho was issued to the industry and revival boxes were made mandatory for all gear types.

Physiological indicators of the stress of capture

That fish show delayed mortality following severe exertion was first shown over 60 years ago and has been repeatedly demonstrated in studies aimed at understanding the causative mechanism (see below). Postcapture mortality rates have been reported as high as 70%. Though we do not yet understand the proximate cause of delayed mortality, it is clear that the complex set of changes associated with physiological stress play a central role in the sequel of events that can precede mortality. Thus, by focusing on markers of physiological stress, we can make predictions about the likelihood of survival for non-retention fish. The premise is that a lower level of physiological stress during capture and handling will promote better survival upon release.

It has long been known that capture, confinement, air exposure and handling cause physiological stress in fish (see below). The effects observed in laboratory studies include:

- muscle fatigue as the result of exhaustive exercise leading up to capture;
- marked disturbances to acid base, osmotic and electrolyte balance due to the accumulation of metabolic wastes and fluid shifts;
- significant reductions in high energy fuels such as phosphocreatine (PCr), adenosine triphosphate (ATP) and muscle glycogen;

- elevated levels of anaerobic metabolites including plasma and muscle lactate;
- elevated hematocrit owing to catecholamine-induced red blood cell swelling, spleen transfusion, and fluid shifts; and
- gill collapse resulting from air exposure.

In addition, numerous hormones are released as part of the involvement of the pituitary adrenal axis.

In the short-term these stress-induced effects could impede swimming capacity after release. This could increase the mortality risk from predation and possibly reduce the potential for successful migration depending on where the release took place. In the long-term it would be expected that the energy required to restore homeostasis would be diverted from investment activities such as gonadal development (Jonsson *et al.*, 1991), potentially affecting the ability of live released salmon to get to the spawning grounds and reproduce successfully. There is growing evidence that stress negatively affects reproductive processes in fish (Pankhurst and Dedual, 1994).

A number of studies have examined the mortality rates associated with non-retention or capture and release techniques in both the salmon sports fishery (e.g., Gjernes *et al.*, 1993; Vincent-Lang *et al.*, 1993; Cox-Rogers, 1998) and commercial salmon fishery (e.g., Parker *et al.*, 1959; Parker and Black, 1959; Candy *et al.*, 1996; J. O. Thomas and Associates, 1997; see also Ricker, 1976, Chopin and Arimoto, 1995). However, relatively few studies have examined the physiological basis of capture and live release techniques in wild salmon. Booth *et al.* (1995) and Wilkie *et al.* (1996, 1997) demonstrated significant changes in white muscle metabolites and blood disturbances in wild Atlantic salmon (*Salmo salar*) following angling and induced exhaustive exercise and the degree of change has been linked to water temperature (Wilkie *et al.*, 1997) and air exposure (Ferguson *et al.*, 1992). Parker *et al.* (1959) and Parker and Black (1959) demonstrated evidence of muscle fatigue in troll-caught wild Pacific coho salmon in seawater and freshwater and in chinook salmon in seawater, and Thompson and Hunter (1973) linked mortality rates to both the loss of scales and stress in adult sockeye salmon caught in gillnets.

Physiological changes associated with exhaustive exercise and during ensuing recovery have been demonstrated in laboratories with hatchery-raised salmonids. Here exhaustive exercise is simulated either by inducing periods of swimming by touching the tail of the fish in a holding tank until the fish can no longer swim, or by forcing the fish to swim against a current of increasing velocity in a swim tunnel (tread mill exercise). Effects associated with exhaustive swimming in these types of studies include: increased levels of blood lactate, muscle lactate, and plasma ions, osmotic disturbances, increased hematocrit, increased levels of stress hormones (e.g., catecholamines and cortisol) and decreased levels of muscle PCr, ATP and glycogen (see Figure 1, from Milligan, 1996). Osmotic changes have also been described following exhaustive swimming (see Gallagher, 1994) and there is evidence of blood flow redistribution away from the viscera towards the musculature during exhaustive swimming (Thorarensen *et al.*, 1993). Results obtained in laboratory experiments using exhaustive chasing protocols for a number of the variables described above are comparable to those observed in a series of experiments with Atlantic salmon angled in the wild (Booth *et al.*, 1995; Brobbel *et al.*, 1996; Wilkie *et al.*, 1996, 1997).

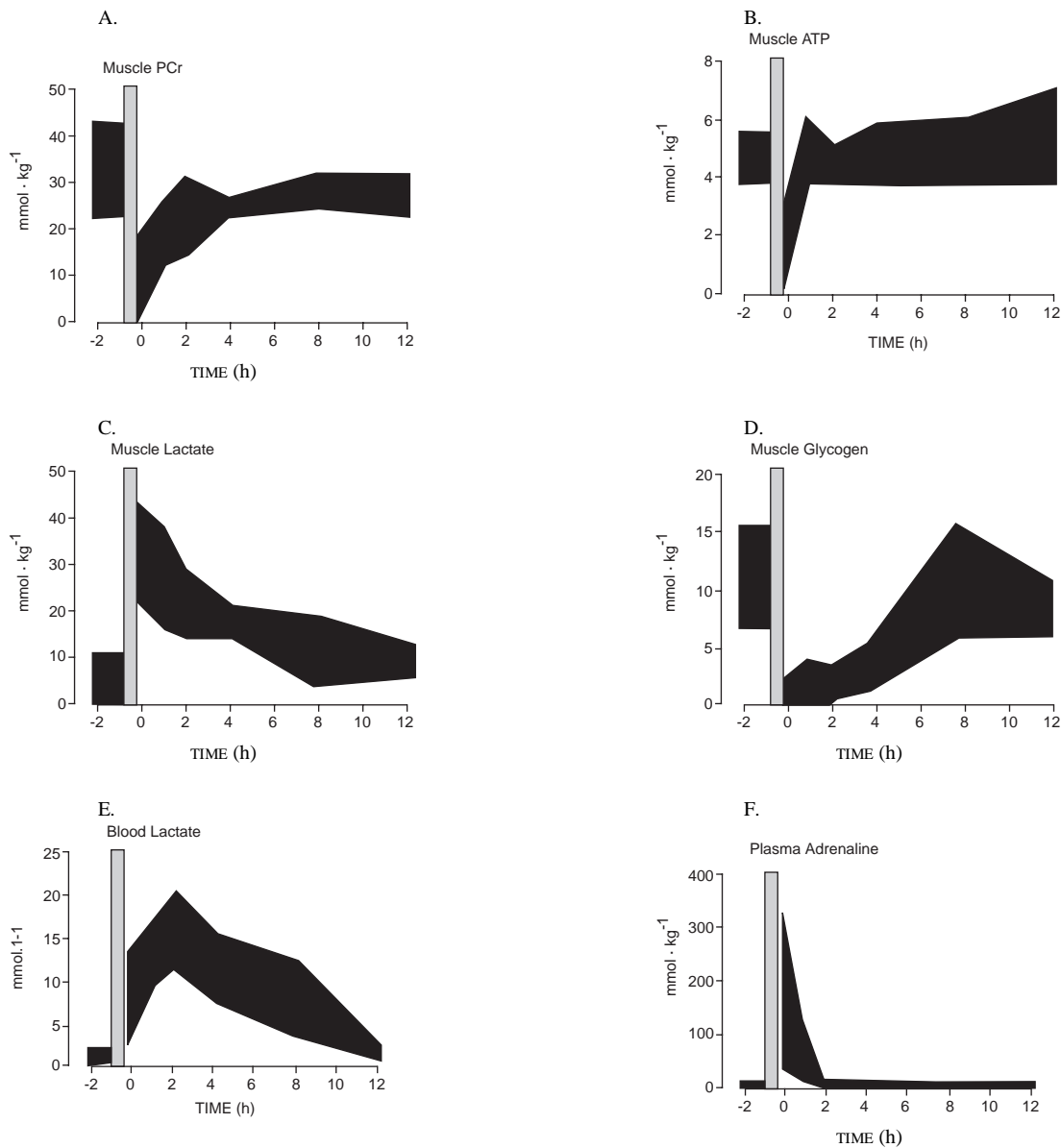


Figure 1. Typical changes in muscle phosphocreatine (PCr, A), muscle ATP (B) lactate (C), glycogen (D), blood lactate (E) and plasma adrenaline (F) following laboratory-induced exhaustive swimming in rainbow trout and during recovery. Bar represents period of exercise (5 -7 min of manual chasing). Shaded area indicates range of values. After Milligan, 1996.

Several studies have monitored changes in a number of these variables during the recovery period providing some indication of the time required for a return to homeostatic levels (see Figure 1, Milligan, 1996). However, what is not well understood is which changes during recovery are critical to (or predictive of) future swimming capacity and whether there are threshold levels for these variables. Farrell *et al.* (1998) have shown that mature wild sockeye can perform a treadmill exercise test to exhaustion and, after only a 45 min recovery period, swim equally well in a second swim. By measuring metabolic variables it was discovered that the fish that did not swim as well a second time were those that had a higher plasma lactate level at exhaustion during the first swim. It would appear that fish that are forced or choose to produce high levels of muscle lactate pay the price subsequently by having a longer recovery time before they can swim normally.

Summer 1998 joint DFO-fishing industry-SFU Barkley Sound study

Given the severity of the methods of capture used by all commercial gear types we predicted that the combined effects of exhaustive swimming, handling, air exposure and physiological stress would be at least as great if not greater than those observed under laboratory conditions. We also predicted that some of these effects would vary between gear types and gear modifications.

While it was mandatory for all commercial gear types to carry recovery boxes on board in 1998, and in spite of a description of recovery boxes being "very effective in increasing survival of non-target species in some cases" (Blewett and Taylor, 1999), to our knowledge the use of recovery boxes has never been tested in an appropriately designed scientific study. A much earlier study involving tagging of troll-caught coho and chinook (Parker *et al.*, 1959) cast doubt on whether 'recovery' did indeed occur in 'live' boxes. Further, J. O. Thomas and Associates (1997) observed greater mortality rates for seine caught salmon held in recovery boxes compared with salmon released immediately after capture.

Moreover, given the evidence of the factors that cause physiological stress in fish we felt it was important to determine whether live-released fish survive to successfully escape predators in the short term and in the long term to reproduce on the spawning grounds. Without this information, it is impossible to assess whether or not the policy of "live release" of non-target species of salmon is an effective conservation measure.

With this in mind, we joined the DFO and members of the commercial salmon industry in a project designed to measure physiological variables which may be used as indicators of mortality risk for "live release" following capture. The experiments were conducted in Barkley Sound in September 1998. Representatives of the seine, gillnet and troll fleets targeted a healthy stock of Alberni Inlet coho salmon for these experiments. Our goal was "to assess the effectiveness of the new measures which were implemented or proposed by DFO and the salmon fishing industry in 1998, to reduce the incidental catches, and improve the survival of coho that were live-released from commercial seine, gillnet and troll fishing gear" (DFO background information document).

The overall experiment measured mortality as a function of a number of gear and fishing modifications. For all gear types, some fish were tagged immediately upon capture and transported to net pens where they were held for a 24-hour recovery before release. All boats carried recovery boxes on board. Fish were held in these boxes for either 30 or 60 minutes and compared to fish sampled directly upon landing to test the effectiveness of the blue box in facilitating recovery.

For the gillnet gear, we tested:

- the condition of fish after soak times of 30 or 60 minutes and
- the condition of each group of fish after 30 or 60 minutes recovery in blue boxes

For the seine gear, we tested:

- the condition of fish captured by: traditional ramping, standard brailer, or standard brailer with modified bunt (fine mesh knotless and selectivity grids), and
- the condition of each group of fish after 30 or 60 minutes recovery in blue boxes

For the troll gear, we tested:

- the condition of fish released directly at the water line or immediately after being released from the hook and brought on board, and
- the condition of each group of fish after 30 minutes recovery in blue boxes

The purpose of the physiological study was to address the following three questions:

- 1) How stressed/fatigued/damaged were the fish upon landing?
- 2) What is the extent of recovery after 30 to 60 minutes in blue (recovery) boxes or 24 hours in a net pen? and
- 3) Are there any differences between or within the gear types for 1 and 2 above?

The variables that were measured included: muscle glycogen, muscle lactate, muscle glucose, muscle phosphocreatine, plasma lactate and glucose (indicators of fatigue); hematocrit (indicator of bleeding, air exposure and stress); and plasma osmolality and ion (sodium, potassium and chloride) concentrations (indicators of physiological stress and osmotic shock)

Material and Methods

Experimental protocol

Directly upon landing, an evaluation of the fish condition was made by a DFO observer using 5- levels of visual criteria: level 1, vigorous, not bleeding; level 2, vigorous, some bleeding; level 3, lethargic, not bleeding; level 4, lethargic, bleeding; level 5, dead. Only fish rated 1 – 3 were used in the experiment. Fork length of the fish ranged from 53.5 to 80.0 cm. The fish were randomly assigned to 0 (no recovery), 30, or 60 minute recovery periods in the blue boxes. Recovery boxes were supplied with a continuous supply of 12 - 13° C fresh seawater via mechanical pump at the rate of > 30 l per minute.

It was necessary to sacrifice the fish in this experiment so as to obtain muscle samples. For any given protocol the fish was stunned by a sharp blow to the skull at the exact time that the fish would normally be "live released". The fish was then immediately placed in V-shaped trough, and within 45 seconds or less a muscle sample (approximately a 0.5 x 1.0 x 1.0 cm cube) was excised from below and slightly to the anterior of the dorsal fin, and was instantly frozen between tongs pre-cooled on dry ice. The frozen tissue was placed in tin foil and stored in dry ice. Directly following removal of the muscle tissue a 3 ml sample of blood was drawn by puncturing the caudal vessels with a hypodermic syringe and drawing blood into a vacutainer. Two heparinized hematocrit tubes were immediately filled from the 3 ml blood sample. Both the blood and hematocrit samples were then centrifuged within 20 minutes of sampling. Hematocrit values were recorded and plasma was aliquoted between three 1ml eppendorf tubes which were then stored on dry ice. The muscle and plasma samples were subsequently transferred to and stored at -80 °C until analysis.

Analytical Techniques

Blood and Muscle Tissues: Plasma lactate and glucose concentrations were measured using a YSI 2300 lactate/glucose analyzer. Samples were thawed immediately before use, vortexed to remove any stratification, spun in a centrifuge for 2 minutes at 2000 rpm and then aspirated for analysis. The analyzer was set to automatically calibrate after every five measurements. Duplicate samples were within 2% of each other.

Muscle tissue samples were powdered under liquid nitrogen by the use of a pre-cooled mortar and pestle. Approximately 500 mg of powdered tissue was added to a pre-cooled, pre-weighed vial containing 1 ml of ice-cold 0.6N perchloric acid (PCA). Each vial was re-weighed and made to a final dilution of 7 volumes (vol/wt). The PCA extracts were homogenized on ice for 2 x 15 seconds at maximum speed with a tissue homogenizer (Ultraturex). An aliquot was immediately frozen in liquid nitrogen for determination of glycogen. The remaining PCA extract was then centrifuged for 2 minutes at 13,000 rpm in a microcentrifuge. A known volume of supernatant was removed and immediately transferred to another eppendorf tube and neutralized with tris (hydroxymethyl) aminomethane. The neutralized extracts were stored at -80 °C until analysis. Glycogen was digested with amyloglucosidase (Bergmeyer, 1983) and glucosyl units determined on the YSI 2300 Stat Plus lactate/glucose analyzer. Final glycogen values were determined after subtracting muscle glucose values. Muscle glucose and lactate values were determined on the analyzer following the same procedure as for the blood lactate and glucose analysis. PCr was determined enzymatically following the production of NADPH at 340 nm (Bergmeyer, 1983).

Osmolality and Ions: Plasma samples were thawed, vortexed to eliminate stratification, and centrifuged for 5 minutes immediately before analysis. Plasma chloride concentrations were measured in duplicate using a Haake Buchler digital chloridometer, model 4425000. The measurements were repeated if there was disagreement between duplicates greater than 2.5 mEqL⁻¹. The chloridometer was checked against a standard

chloride solution (100 mEqCl^{-1}) before and during the process (approximately every 10 duplicates). Sodium and potassium ions were measured using a Turner flame photometer, Model 510. $5 \mu\text{l}$ of plasma were diluted 1:200 with a prepared 15 mEqL^{-1} Lithium diluent for analysis. The machine was calibrated prior to use, and checked against a standard approximately every 6 samples. If there was disagreement between duplicates beyond 2% of absolute value, the measurement was repeated.

Osmolality was measured in duplicate on $10 \mu\text{l}$ samples using one of two calibrated Wescor Vapour Pressure Osmometers, Model 5500 (Wescor, Logan, Utah). If there was disagreement between duplicates beyond 3% of absolute value, the measurement was repeated. The thermocouple heads were periodically cleaned in order to maintain consistency.

Statistical Analysis

The primary analysis tested for (i) differences between the different fishing methods in stress indicators at the time the fish were brought on board, and (ii) evidence of changes over time in the recovery boxes. These tests were done through a two-factor analysis of variance with fixed effects, interactions, and a 5% significance level. The analysis of variance model was also specially coded so as to direct the test for differences between fishing methods to the time when the fish were first brought on board. Results were also tested for differences between visually assigned fish condition using a single-factor analysis of variance, again with a 5% significance level.

All analyses were followed by multiple comparison tests (with the experiment-wise error rate fixed at 5%) to determine which means were significantly different.

The conclusions are subject to two reservations. First, vessel-to-vessel differences could not be thoroughly assessed and accounted for in the analysis. It was impossible within the seine component, for example, to sort out the effects of differences between boats (including crews) from differences between fishing methods. However, it was possible within the gillnet component to test for vessel-to-vessel differences, and no statistically significant evidence of differences was found. This suggests that boat-to-boat differences may not have been large, but because (i) the test may not have been sufficiently powerful and (ii) it applied to only one gear type, the possibility of important boat-to-boat differences could not be definitively discarded.

Second, observations were taken only on fish that appeared to be in relatively good condition. Therefore, all conclusions apply only to that portion of the catch.

For all variables measured the 'n' was based on approximately 20 fish and the mean value for this number of fish and the standard error of the mean are presented, unless otherwise stated.

Results

The mean hematocrit value for 303 fish was $49.92 \% \pm 0.45 \text{ sem}$. There were no significant differences between gear types and Hct values did not change significantly during either the 30- or 60- minute recovery periods (Figure 2, Tables 1 and 2).

Plasma sodium ion concentrations ($[\text{Na}^+]$) were significantly higher for gillnet compared with troll and for gillnet compared with seine. Differences between seine and troll were not significant. No differences were observed within gear types. For all gear types $[\text{Na}^+]$ increased significantly after the 30-minute recovery period and again after the 60-minute recovery period (Figure 3, Tables 1 and 2).

Plasma chloride ion concentrations ($[\text{Cl}^-]$) were significantly higher in gillnet compared with seine, but did not differ between either gillnet and troll, or seine and troll. $[\text{Cl}^-]$ was significantly higher in seiners that ramped compared other seine methods. $[\text{Cl}^-]$ increased significantly after the 30-minute recovery period but did not increase further after the 60-minute recovery period (Figure 4, Tables 1 and 2).

Plasma potassium ion concentrations ($[K^+]$) were not significantly different at 0 time between gear types or within gear types. $[K^+]$ decreased significantly after the 30 minute recovery period, but there was no further decrease at 60 minutes (Figure 5, Tables 1 and 2).

Plasma osmolality was significantly higher in gillnet compared with seine and in gillnet compared with troll, but the lower values observed in troll compared with seine were not statistically significant. Osmolality increased significantly after the 30-minute recovery period and again after the 60-minute recovery period (Figure 6, Tables 1 and 2).

Plasma lactate levels were significantly higher in gillnet compared with seine and in gillnet compared with troll, but not between the seine and the troll. No significant differences were observed within gear types. Plasma lactate concentrations were significantly increased after the 30-minute recovery period and again after the 60-minute recovery period (Figure 7, Tables 1 and 2).

Plasma glucose levels were not significantly different between or within gear types. Plasma glucose increased significantly after the 30-minute recovery period, but increased no further with the 60-minute recovery period (Figure 8, Tables 1 and 2).

Muscle lactate or muscle glucose concentrations did not change significantly between or within gear types. There were no significant changes observed during the 30- and 60-minute recovery times (Figures 9 and 10, Tables 1 and 2).

Muscle phosphocreatine levels at 0 time immediately following landing were extremely low for all gear types and there were no significant differences between gear types. Levels showed no significant increases after the 30- or 60-minute recovery periods although they were higher after the 60-minute recovery compared with 0 time for the gillnet, suggesting some recovery of this important variable (Table 4). For these samples n was only 8 and because there was considerable variability among the individual values, the statistical power was not great. Further processing of tissues would be needed to increase the statistical power.

Muscle glycogen levels were also extremely low at 0 time and showed no differences between or within gear types and levels did not increase after the 30- and 60-minute recovery periods (Figure 11, Tables 1 and 2).

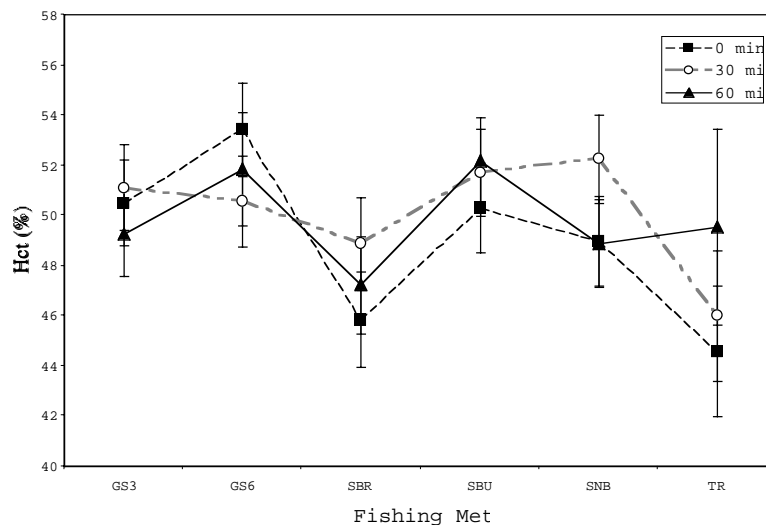


Figure 2. Hematocrit values as a function of fishing method (Gillnet, 30 minute soak, GS3, 60 minute soak, GS6; Seine, ramping without brail, SBR, brail, SBU, brail with modified bunt, SBU; troll, TR) and recovery time (0, 30 or 60 minutes). Values are means \pm se.

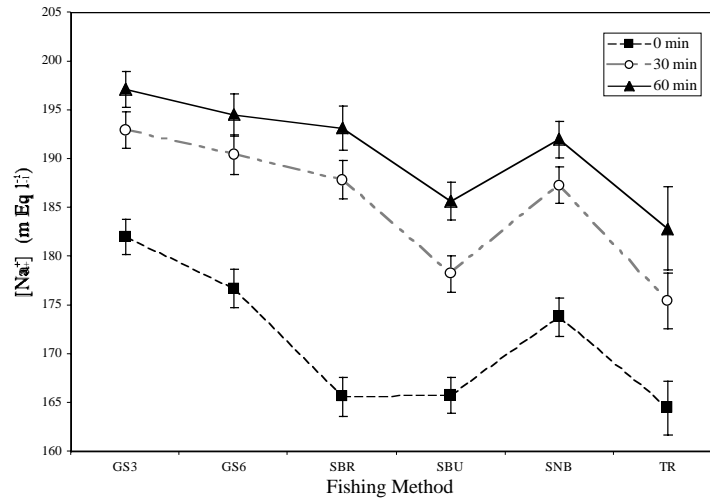


Figure 3. Sodium ion concentrations ($[Na^+]$) as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

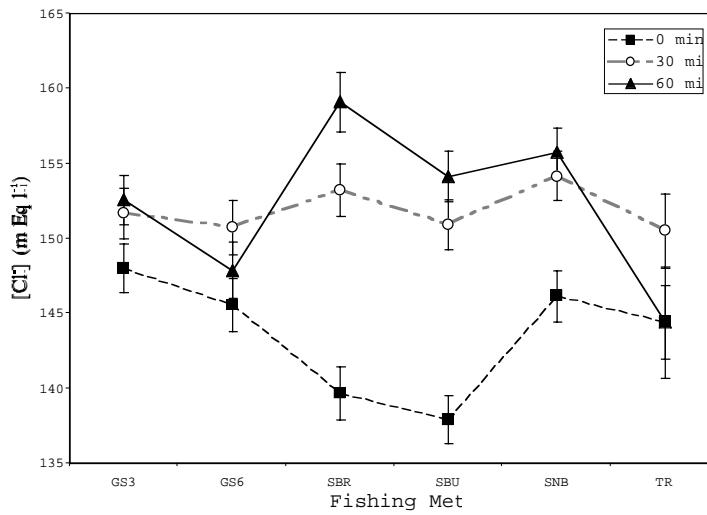


Figure 4. Chloride ion concentrations ($[Cl^-]$) as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

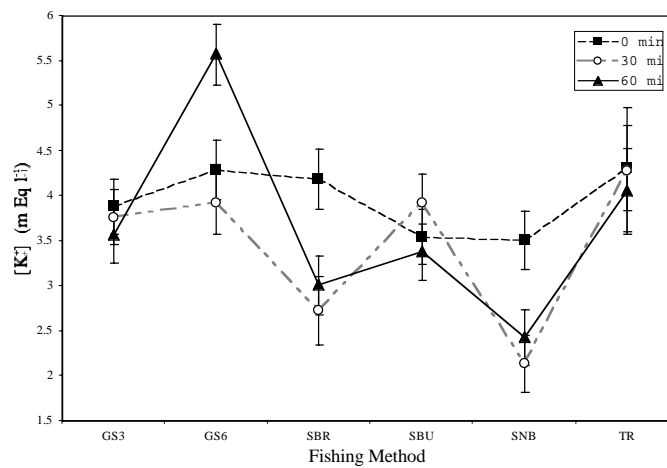


Figure 5. Potassium ion concentrations ($[K^+]$) as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

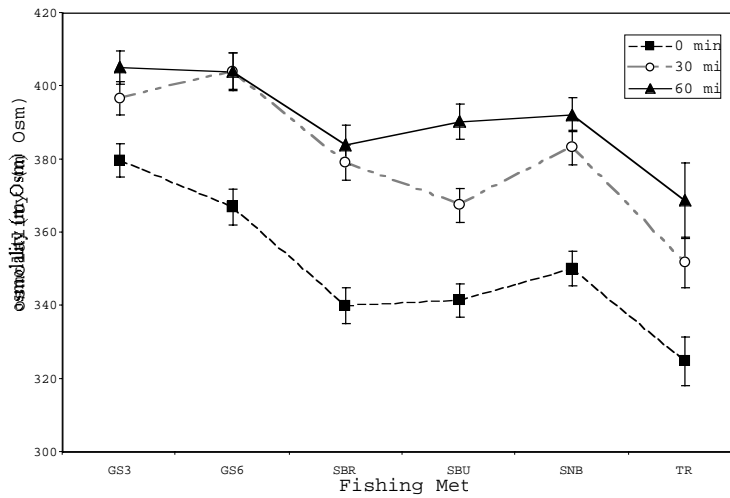


Figure 6. Plasma osmolality as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

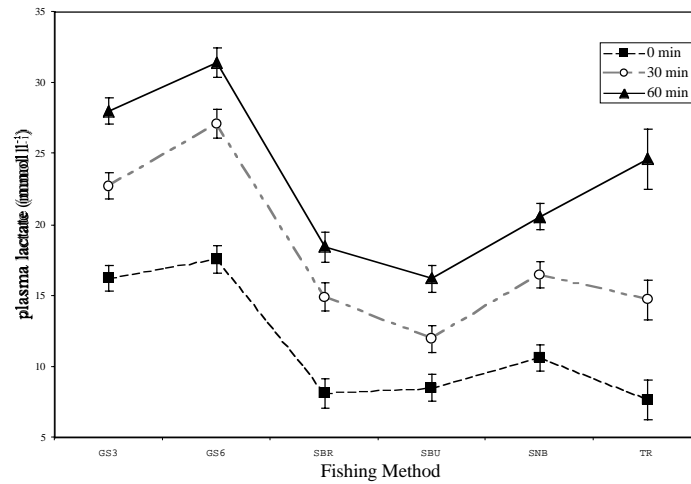


Figure 7. Plasma lactate as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

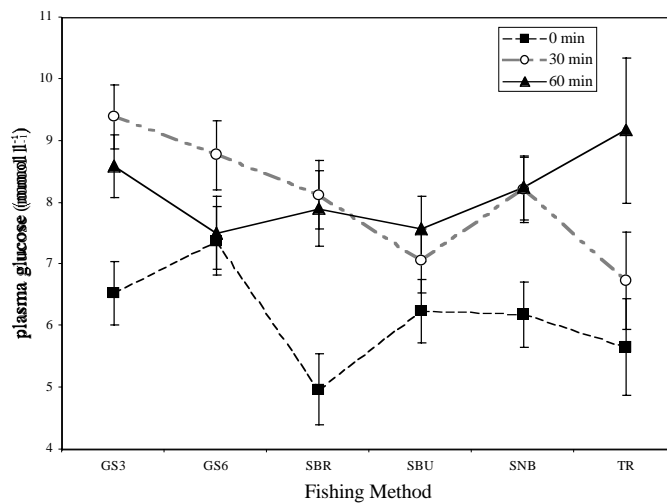


Figure 8. Plasma glucose as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

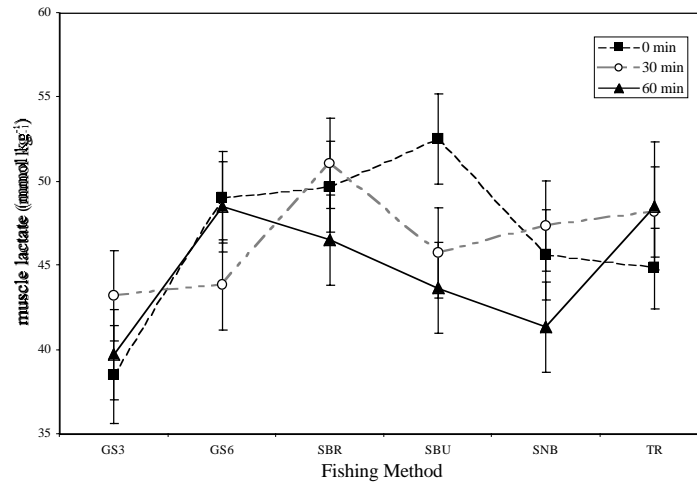


Figure 9. Muscle lactate as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

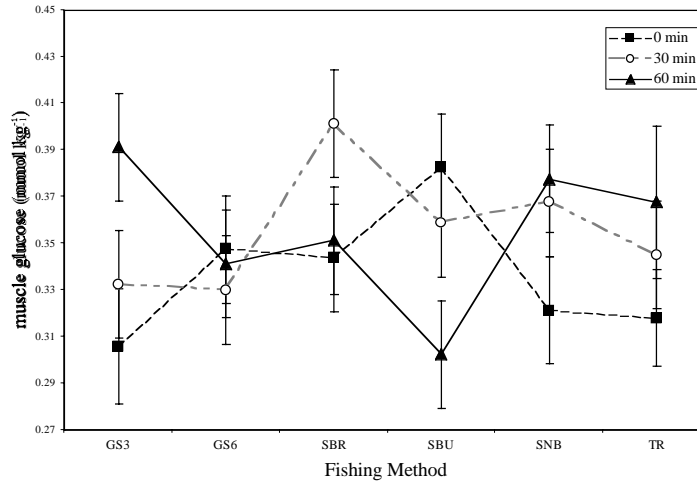


Figure 10. Muscle glucose as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

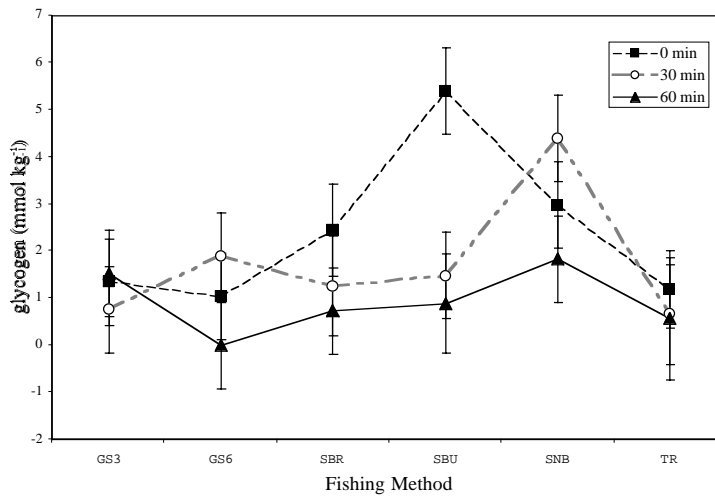


Figure 11. Muscle glycogen as a function of fishing method and recovery time. Symbols for fishing method and recovery time as described in Figure 2.

Table 1. Differences for coho response variables between gear types.

Response Variable	Gillnet ^a	Seine ^a	Level of significance ^b	Gillnet ^a	Troll ^a	Level of significance ^b	Seine ^a	Troll ^a	Level of significance ^b
plasma chloride (mEq ^l ⁻¹)	146.9	141.2	**	146.9	144.4	NS	141.2	144.4	NS
plasma sodium (mEq ^l ⁻¹)	179.6	168.4	***	179.6	164.5	***	168.4	164.5	NS
plasma potassium (mEq ^l ⁻¹)	4.1	3.7	NS	4.1	4.3	NS	3.7	4.3	NS
osmolality (mOsm)	373.2	343.8	***	373.9	324.9	***	343.8	324.9	NS
hematocrit (%)	51.85	48.5	NS	51.9	44.6	NS	48.5	44.6	NS
plasma lactate (mmolkg ⁻¹)	16.9	9.1	***	16.9	7.7	***	9.1	7.7	NS
plasma glucose (mmolkg ⁻¹)	6.9	5.9	NS	6.9	5.7	NS	5.9	5.7	NS
muscle lactate (mmolkg ⁻¹)	44.2	49.3	NS	44.2	44.9	NS	49.3	44.9	NS
muscle glucose (mmolkg ⁻¹)	0.3	0.4	NS	0.3	0.3	NS	0.4	0.3	NS
muscle glycogen (mmolkg ⁻¹)	1.2	3.7	NS	1.2	1.2	NS	3.7	1.2	NS

^a Least Squares Means for holding time equal to zero.

^b Two-way Analysis of Variance with Interactions using the data from all holding times ($\alpha = 0.05$ with additional protection for multiple comparisons); NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2. Differences for coho response variables between holding times.

Response Variable	0 ^a	30 ^a	Level of significance ^b	0 ^a	60 ^a	Level of significance ^b	30 ^a	60 ^a	Level of significance ^b
plasma chloride (mEq ^l ⁻¹)	143.6	151.9	***	143.6	152.3	***	151.9	152.3	NS

plasma sodium (mEq ^{l-1})	171.4	185.4	***	171.4	190.9	***	185.4	190.9	***
plasma potassium (mEq ^{l-1})	4.0	3.5	*	4.0	3.7	NS	3.5	3.7	NS
osmolality (mOsm)	350.5	380.4	***	350.5	390.7	***	380.4	390.7	**
hematocrit (%)	48.9	50.1	NS	48.9	49.8	NS	50.1	49.8	NS
plasma lactate (mmol ^{l-1})	11.5	18.0	***	11.5	23.2	***	18.00	23.2	***
plasma glucose (mmol ^{l-1})	6.2	8.1	***	6.2	8.2	***	8.1	8.2	NS
muscle lactate (mmolkg ⁻¹)	46.7	46.6	NS	46.7	44.7	NS	46.6	44.7	NS
muscle glucose (mmolkg ⁻¹)	0.3	0.4	NS	0.3	0.4	NS	0.4	0.4	NS
glycogen (mmolkg ⁻¹)	2.4	1.7	NS	2.4	0.8	*	1.7	0.8	NS

^a Least Squares Means.

^b Two-way Analysis of Variance with Interactions ($\alpha = 0.05$ with additional protection for multiple comparisons); NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3. Differences for coho response variables between visually assigned fish condition.

Response Variable	1 ^a	2 ^a	Level of significance ^b	1 ^a	3 ^a	Level of significance ^b	2 ^a	3 ^a	Level of significance ^b
plasma chloride (mEq ^{l-1})	149.2	153.4	NS	149.2	150.4	NS	153.4	150.4	NS
plasma sodium (mEq ^{l-1})	181.5	175.6	NS	181.51	187.9	**	175.6	187.9	NS
plasma potassium (mEq ^{l-1})	3.5	4.6	NS	3.5	4.0	NS	4.6	4.0	NS

osmolality (mOsm)	371.9	358.7	NS	371.9	389.9	***	358.7	389.9	*
hematocrit (%)	48.2	43.2	NS	48.2	48.2	NS	43.2	48.2	NS
plasma lactate (mmol ⁻¹)	15.6	11.0	NS	15.6	22.9	***	11.0	22.9	***
plasma glucose (mmol ⁻¹)	7.4	6.2	NS	7.4	7.8	NS	6.2	7.8	NS
muscle lactate (mmolkg ⁻¹)	46.0	51.2	NS	46.0	45.1	NS	51.2	45.1	NS
muscle glucose (mmolkg ⁻¹)	0.4	0.4	NS	0.4	0.3	NS	0.4	0.3	NS

^a Least Squares Means.

^b Single-factor Analysis of Variance (Model 1) ($\alpha = 0.05$ with additional protection for multiple comparisons); NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. Least squares means phosphocreatine levels (mmolkg⁻¹) for gillnet, seine and troll at 0, 30 and 60 minute holding times. Figures in brackets represent standard errors.

Gear type	Holding time (minutes)		
	0	30	60
Gillnet (GS3)	3.31 (1.26) n = 8	0.48 (1.26) n = 8	5.29 (1.26) n = 8
Seine (BR)	1.55 (1.26) n = 8	1.51 (1.26) N = 8	0.47 (1.26) N = 8
Troll	4.32 (.99) n = 10	0.06 (1.11) n = 8	

Discussion

Hematocrit

Only 3 of 303 fish had Hct values below the normal Hct range for salmonids (Gallaughner and Farrell, 1998). This result indicates that the capture and handling procedures that were used in this experiment did not cause bleeding sufficient to cause anemia at least during the duration of this experiment. But it should be noted that only fish of conditions 1 –3 were sampled. Anemia is known to reduce the critical swimming speed of salmonids (Gallaughner *et al.*, 1995). The overall mean Hct value (~ 50%) is higher than values previously reported for salmon sampled by acute puncture methods under a number of different conditions (Gallaughner

and Farrell, 1998). However, since we did not sample fish prior to capture we cannot assess to what degree the elevated Hct was caused by stress-related responses such as adrenergic cell swelling, splenic transfusion and fluid shifts (Gallaughner and Farrell, 1998). Elevated plasma catecholamine levels (adrenaline, noradrenaline) following exhaustive swimming (Gallaughner and Farrell, 1998; see also Figure 1, Milligan, 1996) are thought to be involved in the red cell swelling, spleen transfusion and fluid shifts associated with exhaustive swimming (Gallaughner *et al.*, 1992).

Hematocrit values may also be influenced by sexual maturity. For example, Thorarensen and Davie (unpublished observations, see Gallaughner and Farrell, 1998) have reported higher than normal Hct values (sampled via cannulae) in sexually mature rainbow trout (36% and 29% for males and females, respectively). Relatively high Hct values (ranging from 28.8 - 33.8 %), were obtained (via indwelling cannulae) in mature Albern Inlet sockeye salmon (Farrell *et al.*, 1998). In a related study with mature sockeye salmon, Hct values were higher still ($43.2 \% \pm 8.0$ sem) in fish sampled via acute puncture after two repeat exhaustive swimming sessions (Farrell and Tierney, unpublished observations).

Therefore, we suspect that the unusually high Hct levels observed in this study are in part associated with the sexually mature condition of these wild fish, but are also indicative of the extreme physiological stress associated with the capture and sampling methods.

Frequently, exhaustive swimming under laboratory conditions results in significantly elevated Hct levels followed by a decline to pre-exercise levels after a 1- 2 hour recovery period (e.g., swim tunnel, Gallaughner and Farrell, 1998; manual chasing, Wood *et al.* 1983). Evidence of hemoconcentration has also been observed after laboratory induced exercise in wild Atlantic salmon, with a return to pre-exercise conditions after a 4-hour recovery period (Wilkie *et al.*, 1997). In the present study, Hct values measured after the 30- and 60-minute recovery periods in the blue boxes were not different from 0 minute samples taken immediately after capture. This could be explained in several ways, one of which is the possibility that the fish are experiencing ongoing stress in poorly designed recovery boxes. However, we have shown previously that elevated hematocrit levels are not prohibitive for swimming (Gallaughner *et al.*, 1995).

Muscle Metabolites

The initial source of energy for muscular contraction during exhaustive swimming is provided by the hydrolysis of phosphocreatine and ATP. As a result, PCr and ATP levels normally decline precipitously with exhaustive exercise, although the degree of decline is variable (see Figure 1, Milligan, 1996). The decline in PCr stimulates muscle glycogen to be broken down to lactate with the release of ATP (see Figure 1, Milligan, 1996). Muscle lactate therefore increases with exhaustive exercise, and some of this muscle lactate is released to the blood, causing a slower rise in plasma lactate levels, some of which continues well after the exercise bout has been completed.

In the current study we observed extremely low levels of PCr ($< 5 \text{ mmolkg}^{-1}$) for all gear types immediately following capture. These muscle PCr levels are comparable to the low values reported in other studies where muscle was sampled using similar procedures following exhaustive swimming in rainbow trout (Dobson and Hochachka, 1987; Pearson *et al.*, 1990; Ferguson *et al.*, 1993; Kieffer *et al.*, 1994; Milligan, 1996; Burgetz *et al.*, 1998) and Atlantic salmon (Wilkie *et al.*, 1997) and angling in Atlantic salmon (Wilkie *et al.*, 1996). Interestingly, Wilkie *et al.* (1997) demonstrated greater depletion of PCr in Atlantic salmon at 12 °C compared with 18 or 23 °C. The water temperature in the recovery boxes in the present study was between 12 and 13 °C. Furthermore, Ferguson *et al.* (1993) have shown a positive correlation between PCr depletion and larger body size in rainbow trout ranging in length from 8 to 54 cm. The coho salmon in our study ranged from 53.5 to 80.0 cm in length.

Surprisingly, we did not observe significant increases in PCr levels during the 1-hour recovery period in the blue boxes. Laboratory studies with rainbow trout and properly designed holding boxes clearly show partial recovery of muscle PCr and ATP within an hour of exhaustion (Pearson *et al.*, 1990; Milligan, 1996) and

similar results have been observed for wild Atlantic salmon during recovery from angling (Booth *et al.*, 1995; Wilkie *et al.*, 1996) and manual chasing (Wilkie, 1997). Again, it is possible that in the present study the activity and related stress of confinement in the recovery boxes may have prevented the restoration of these muscle energy stores. However, PCr is known to be highly labile and difficult to preserve during both sampling and analysis (Milligan, 1996). Therefore, the possibility exists that the low initial levels of PCr, as well as the absence of any recovery of PCr levels, may be related more to the difficulty in getting an accurate sample in the field without anaesthetizing the fish (Wang *et al.*, 1994) than to the degree of muscular exhaustion (see below). We did, however, find an increase in PCr in the gillnet fishery, suggesting that the blue box had improved the metabolic status of these fish after 60 minutes. It is not clear why the gillnet fishery stands out in this way. We are in the process of performing more PCr measurements to improve the statistical power of these data comparisons and we are examining individual data for differences in handling.

As expected muscle glycogen levels were very low ($2 - 4 \text{ mmolkg}^{-1}$) immediately following capture in all gear types, similar to levels reported in a number of studies following exhaustive exercise in rainbow trout (Stevens and Black, 1966; Pagnotta and Milligan, 1991; Ferguson *et al.*, 1993; Kieffer *et al.*, 1994; Wang *et al.*, 1994; Milligan, 1996) and angling (Booth *et al.*, 1995; Brobbel *et al.*, 1996; Wilkie *et al.*, 1996) and manual chasing (Wilkie *et al.*, 1997) in Atlantic salmon. That there was no evidence of resynthesis of glycogen during the periods of recovery in the blue boxes is not surprising. Evidence of glycogen resynthesis within 1 – 2 hours of recovery following exhaustive exercise in rainbow trout and angling in Atlantic salmon is variable and may be related to a number of other factors. For example, Wilkie *et al.* (1997) reported slower glycogen resynthesis in wild Atlantic salmon at 12°C compared with 18 and 23°C , and Stevens and Black (1966) demonstrated that re-exercise after a bout of exhaustive exercise followed by a recovery caused even greater depletion of muscle glycogen with a slower resynthesis. Although the lack of recovery of muscle glycogen in the coho we sampled could easily be explained either by the short period of holding in a blue box relative to the normal time needed for metabolic recovery, or by the extreme level of fatigue causing a delayed resynthesis, we have another concern. Fish in blue boxes were frequently observed to roil continuously and even perform more violent movements propelled by 1-2 tail flips. These activities suggests that some level of recovery had occurred in the blue box, most likely a recovery of the blood oxygen status. However, the burst activity powered by tail flips is typically fueled anaerobically, and would preclude glycogen and PCr resynthesis. A redesigned blue box that would limit these activities thereby preventing further depletion or repeated depletion of muscle energy reserves would be more useful in properly assessing the potential for metabolic recovery in blue boxes.

Muscle glucose levels were also extremely low ($0.3 - 0.4 \text{ mmolkg}^{-1}$) and did not differ between gear types or increase after a 1-hour recovery. Pearson *et al.* (1990) observed significantly higher muscle glucose levels in rainbow trout immediately after exhaustive exercise ($\sim 4 \text{ mmolkg}^{-1}$) compared with resting levels (1.0 mmolkg^{-1}) and a continuing increase during a 6 hour recovery period. However, Milligan and Pagnotta (1991) observed a significant decrease in muscle glucose levels immediately after exhaustive exercise in the same species followed by a rapid increase during recovery. Wang *et al.* (1994) observed no difference in muscle glucose levels in resting compared with exhaustively exercised fish ($\sim 4 \text{ mmolkg}^{-1}$). The levels of muscle glucose observed in the present study provide no evidence for recovery in the blue boxes although based on laboratory studies (see Figure 1) they might not have been expected to change.

As glycogen is utilized to fuel muscle contraction, lactate accumulates in the muscle. Upon recovery, the muscle lactate levels decline as a result of resynthesis of muscle glycogen. The muscle lactate values reported here ($44 - 49 \text{ mmolkg}^{-1}$) did not differ between gear types and are similar to those observed in Atlantic salmon after angling (Wilkie *et al.*, 1996; Brobbel *et al.* 1996) and rainbow trout after induced exercise (see Figure 1, Milligan, 1996; Burgetz *et al.*, 1998). However, we did not observe evidence of a decline in lactate levels over the 30- and 60-minute recovery periods. Booth *et al.* (1995) and Wilkie *et al.* (1996) observed a decline in muscle lactate after 2 hours of recovery from angling in Atlantic salmon, whereas Brobbel *et al.* (1996) observed a similar decline after recovery in kelts but not bright Atlantic salmon. In rainbow trout, Milligan (1996) reports a significant decline in muscle lactate 1-hour after

exhaustion from induced exercise. Routine resting muscle lactate values were restored after 12 hours of recovery. Therefore, it is possible that the muscle lactate levels did not decline in the present study after 60 minutes of recovery either because there had not been sufficient time for a significant resynthesis of glycogen, or the continued activity of the fish in the recovery boxes prevented resynthesis. Interestingly, muscle lactate levels for the gillnet 30 minute soak time samples tended to be lower than the levels for the 60 minute soak time. Since this difference was not statistically significant, we are currently measuring lactate in additional tissue samples to increase the statistical power.

Stevens and Black (1966) found in rainbow trout that if muscle lactate levels did not increase beyond around 45 mmolkg^{-1} following a 15 second burst swim, muscle lactate could recover to around 24 mmolkg^{-1} after approximately 1 hour. These fish could then complete another 15 second burst swim. Following the second swim, muscle lactate rose to more than 56 mmolkg^{-1} but with little recovery over the next 1 hour such that the fish could not burst swim again. Thus, as with muscle glycogen resynthesis, recovery of elevated muscle lactate after exhaustive exercise, is influenced by both time and exercise intensity.

Plasma Lactate and Glucose

As muscle cell lactate levels increase lactate diffuses out of the muscle and into the blood space, resulting in a steady increase in plasma lactate levels, peaking at 2 hours post-exhaustion. Further recovery involves a gradual decline plasma lactate levels until they reach pre-exercise levels at 8 hours post-exhaustion (Wood *et al.*, 1983; Milligan, 1996).

The values we observed for plasma lactate immediately after capture ($7 - 17 \text{ mmol}^{-1}$) fall within the range for rainbow trout after induced exercise as summarized by Milligan (1996; see Figure 1), and are similar to the levels reported forty years ago for troll-caught coho (Parker *et al.* 1959) and chinook (Parker and Black, 1959) salmon, also sampled via acute puncture. Plasma lactate levels are significantly higher than those observed by Farrell *et al.* (1998) for mature sockeye salmon following two exhaustive swims in a swim tunnel. Some of this difference can be attributed to the less stressful blood sampling method (via a cannulae inserted into a blood vessel). The use of cannulation techniques for blood sampling is not practical for field studies such as this. The levels of blood lactate reported here are higher than those reported for Atlantic salmon after angling (Booth *et al.*, 1995; Brobbel *et al.*, 1996; Wilkie *et al.*, 1996) where the fish were anaesthetized before sampling.

For all gear types the plasma lactate levels increased after 30 minutes and again after 60 minutes of recovery in the blue boxes. A similar pattern has been described for coho (Parker *et al.*, 1959) and chinook (Parker and Black, 1959) salmon after capture in the troll fishery, wild Atlantic salmon (Booth *et al.*, 1995; Brobbel *et al.*, 1996, Wilkie *et al.*, 1996), and natural rainbow trout (Pankhurst and Dedual, 1994) after angling, and rainbow trout after induced exercise (Milligan, 1996). Of interest is the finding by Parker *et al.* (1959) that simulated troll fishing in fresh water resulted in a much smaller increase in blood lactate compared with troll-caught coho in seawater. Most importantly, Ferguson and Tufts (1992) very clearly demonstrated that when fish are exposed to air for only 60 seconds following an exhaustive swim, the increase in plasma lactate during recovery is much greater and occurs at a greater rate compared with fish that do not experience air exposure. This may in part explain why the lowest plasma lactate levels observed in this study were those for the troll fishery at time 0. These fish were released directly off the hook and were stunned at the surface of the water with a minimum of air exposure. Both gillnet and seine caught fish were exposed to air before being released.

A study conducted simultaneously by Kriebberg *et al.* observed that after a 24 hour netpen recovery, plasma lactate levels had declined to values within the high end of range of those reported in the literature. This observation is consistent with the decline in plasma lactate described in Figure 1 in this document. The plasma lactate values measured in netpen-held fish, however, did not reflect the true recovery status of the fish because in all cases the fish were dipnetted out of the net pens before being sampled via caudal

puncture. This capture method is stressful and, as shown by the data, results in a modest elevation of plasma lactate compared to the level expected in resting fish sampled in a less stressful manner.

Parker *et al.* (1959) reported that the delayed mortality of coho held in net pens after troll capture showed no significant decrease in plasma lactate from the elevated post-exhaustion levels.

There were significantly higher levels of plasma lactate for gillnet compared with seine and troll gear. This difference may be accounted for in one of two ways. Blood lactate levels are known to increase with degree of muscle fatigue. For example, blood lactate levels were significantly higher in a natural population of rainbow trout that were angled for 15 compared with 5 minutes (Pankhurst and Dedual, 1994). Therefore, the higher plasma levels could reflect a greater level of exhaustion during capture compared with the other fishing methods. This explanation is not consistent with the similar muscle lactate concentrations, however. A more plausible explanation, is that gillnetted fish would have become exhausted some time before landing, and so lactate diffusion from the muscle was further underway at landing compared with the other gear types of fishing. Although the values for fish captured via troll appeared to be lower than those captured via seine the differences were not significant owing to the relatively small sample for troll caught fish.

Blood Glucose

Blood glucose levels did not change significantly and this finding is consistent with the findings of Booth *et al.* (1995) for angled Atlantic salmon. Pagnotta and Milligan (1991) have demonstrated similar low levels of blood glucose immediately following exhaustive exercise in rainbow trout, and a very rapid return to pre-exercise levels during recovery.

Plasma Osmolality and Ion Concentrations

It is important that the electrochemical potential is maintained across muscle cell membranes for the process of muscle contraction. However, ion imbalances are known to be associated with exhaustive swimming in salmonids and this could contribute to muscle fatigue (e.g. see Wood *et al.*, 1983, Holk and Lykkeboe, 1998) and even post-exhaustion mortality (Parker *et al.*, 1959; Wood *et al.*, 1983). Swimming to exhaustion in both seawater and freshwater have been shown to produce osmotic imbalances in both chinook salmon and rainbow trout. Typically, osmolality levels are significantly elevated following exhaustive swimming in seawater, continue to increase slightly during the first hour of recovery, and then decline to pre-exercise levels at 24 hours. The osmolality values observed in the present study at time 0 (324 – 374 mOsm) are similar to those observed for both seawater rainbow trout and chinook salmon after exhaustive swimming in a swim tunnel. (Gallaugh, 1994; Gallaugh, Thorarensen, Kiessling and Farrell, unpublished observations). A similar range of values has been observed in wild Atlantic salmon after manual chasing and during recovery (Wilkie *et al.*, 1997). The exercise-induced elevations in plasma sodium (165 – 180 mEq⁻¹), chloride (141 – 147 mEq⁻¹) and potassium (3.7 – 4.3 mEq⁻¹) ion concentrations observed in the present study are similar to those observed by Booth *et al.* (1995) in wild Atlantic salmon after angling and by Wood *et al.* (1983) in rainbow trout after exhaustive swimming. Booth *et al.* (1995) noted a decline to pre-exercise levels for all ions measured after a 2-hour recovery and Wood *et al.* (1983) observed significant declines in Na⁺ and Cl⁻ but not K⁺ after a 1-hour recovery. In the present study, there was evidence of restoration of potassium ion levels during recovery.

Despite all these metabolic measurements, the single most important recovery test was not performed in these studies. No information was collected on the ability of fish to swim again immediately after capture and after recovery in blue boxes. Such studies should be performed in the future, and before any final recommendations are made regarding the acceptability of non-retention methods.

Assessing Physiological Stress and Fatigue associated with Non-Retention Commercial Fishing Practices

Only fish of conditions 1, 2 and 3 were used in this experiment. Plasma sodium concentrations, osmolality, and lactate levels were all significantly higher (indicating greater levels of stress) in fish of condition 3 compared with condition 1 (see Table 3). This correlation with physiological markers of stress supports the use of the visual criteria currently used to assess fish condition.

Overall in this study the observations are consistent with a severe level of physiological stress and fatigue following capture regardless of fishing method. The values for some of the markers we used were as extreme as have been measured previously. With this degree of stress, we would expect to see significant problems with swimming capacity if the fish were released immediately after capture. One concern is that the oxygen stores in the blood and tissues are depleted, a problem likely to be acute if there was significant air exposure during capture. Without repletion of these oxygen stores it is unlikely that any appreciable level of swimming activity is possible. Repletion of oxygen stores is likely to take only a matter of minutes however, if the fish have access to good quality (clean and well aerated), cool (<18C) water. Furthermore, if there is a water current to help gill irrigation, then recovery would be faster still. The incidental observation that fish were “brighter and less lethargic” after being held in the blue boxes would be consistent with this type of recovery. A second concern is metabolic recovery, and this would have two components. Foremost, there is the recovery of critical muscle metabolites, such as ATP and PCr, that fuel burst activity. If these metabolites are not restored, then it is unlikely that fish can power their muscles for escape time manouvers with a powerful series of tail flips. Also, there is the longer term metabolic recovery, one that would permit a full range of swimming abilities. In this regard, the earlier work of Stevens and Black (1966) is particularly informative. They were able to show a muscle lactate threshold, beyond which fish could not repeat a 15 second bout of burst swimming activity. In our experiments, the muscle lactate levels clearly approached or exceeded the threshold levels reported by Stevens and Black (1966). We would conclude therefore that the fish we studied would have had only a limited ability for burst swimming and escape manouvers if they had been released immediately after capture. Interestingly, although the results were not statistically different, the 30 minute soak time for the gillnet fishery resulted in muscle lactate levels that were below this threshold, while the values for the 60 minute soak were above this threshold. In addition, and again in the absence of statistically significant changes as a function of recovery time, the muscle lactate levels for the seine fishery were around the muscle lactate threshold reported by Stevens and Black (1966) after a 60 minute recovery. Since this is suggestive of muscle recovery, we are performing more muscle lactate analyses to increase the power of the statistical comparisons.

There is evidence that the method of capture used by the gillnet fleet is generally more stressful than for the seine and troll fleets. Further, some of the variables showed trends indicating less stress associated with capture by troll compared with seine and gillnet gear but conclusions were hampered owing to the low ‘n’ for samples taken from the troll fleet. However, any differences observed between gear types are relatively small compared with the overall level of severe stress observed for all gear types.

The observation that within the seine fleet, the procedure of ramping is more stressful than for brailing or brailing with a modified bunt supports the observation of a higher mortality rate for all species of seine caught salmon, including coho, in fish landed via ramping compared with brailing (J. O. Thomas and Associates, 1997). Also, for the gillnet fishery a 60 minute soak time may prove to be more stressful than a 30 minute soak time, based on additional analysis of muscle lactate.

We cannot be conclusive about the value of blue recovery boxes. Good gill ventilation during capture is unlikely for any of the capture methods, but for differing reasons. However, it is likely that the blue boxes help restore blood oxygen status because the fish can perform adequate gill ventilation with aerated water inside the blue boxes. This possibility should be confirmed experimentally. Some of the markers we used should have shown signs of recovery within 1 hour, if the blue boxes had indeed promoted extensive recovery (e.g., Hct, K^+ , Cl^- , Na^+ , muscle lactate, PCr and glycogen). However, with the exception of K^+ for all fish, and muscle PCr for the 60-minute recovery period with gillnet fishing, this was not the case. At best it appears that the blue boxes did not make matters worse.

The present study has provided important insights into the metabolic status of fish landed in simulated commercial fishery operations. We have performed a rather broad analysis of the data and ignored any differences that may or may not have existed as a result of fish capture on different vessels and with different sets, and as a result of fish processing by different people. The data set simply did not lend itself to such fine resolution analysis. However, we did perform visual inspections of the data, and failed to notice any large, consistent deviations related to these factors. Thus, all fish were highly fatigued and stressed upon capture and sampling differences, if they existed, would have been small. We are confident about the quality of the data from the blood samples. Given the large numbers of fish that needed to be sampled in confined conditions, we were pleased with the data obtained. Similar constraints existed for the muscle sampling. While we are confident about the quality of muscle lactate, muscle glycogen and muscle glucose values, we are less confident about ATP and PCr measurements. Muscle PCr and ATP are extremely labile and it is well known from previous, more thorough laboratory studies that low values can be obtained because of poor sampling techniques. We sampled to the best of our ability under the conditions. However, the possibility exists that we underestimated the true ATP and PCr values because of the fish struggling during the muscle sampling procedure. Such an error would be unfortunate because these two markers give us the best indication of immediate recovery. To preclude such uncertainty in the future, it would be better to use a biopsy needle to gather tissue samples and eliminate the need to recapture the fish for muscle sampling. This sampling method could be part of future studies. As noted already, a poorly designed blue box would be particularly problematic when assessing the potential for recovery using the markers PCr and ATP

We were also concerned about the adequacy of the blue box design in promoting effective recovery. Since laboratory experiments demonstrate that well-designed holding facilities can promote recovery even from levels of fatigue near to those that we observed in the present study, we suggest a better blue box design be used for future studies. Fish should be less able to roil about, and receive a significant water flow to irrigate the gills. Milligan (1996) has shown that slow to moderate aerobic swimming post-exhaustion can promote a more rapid recovery in rainbow trout (see Figure 12), as did Gallaugher and Thorarensen (unpublished observations). Therefore, the level of recovery we expected to see for fish placed in blue boxes may simply be related to the poor design of the blue box itself. We recommend that experiments be performed in a laboratory environment to design a better blue box, and that this blue box be used in future studies on board commercial vessels. It is important to note that successful metabolic recovery could be promoted by holding fish in net pens (see simultaneous study of Kriebert *et al.* Who observed that plasma lactate was restored to near normal levels after 24 hours). We expected these findings because fish held in netpens would have had the benefit of slow swimming or water currents to assist in their gill ventilation. It is equally important to note that post-exhaustion mortality, a phenomenon often observed up to 8 hours after capture, was not significant in the netpen studies.

Owing to unforeseen problems with hemolysis of blood samples, we cannot assess the benefits of the net pens in the process of recovery from the capture-induced osmotic disturbances. Data were unavailable. We expected to see decreases in hematocrit, osmolality, and restoration of the ion imbalances.

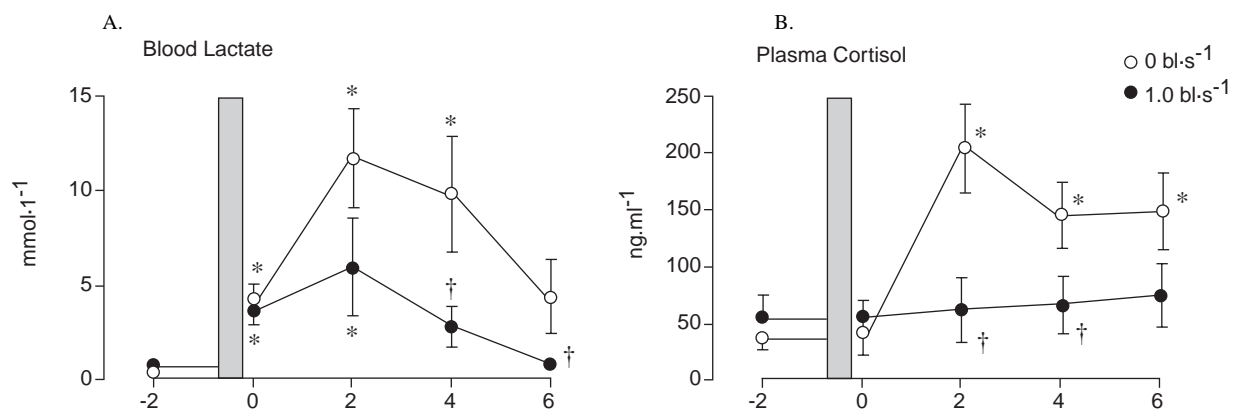


Figure 12. The influence of slow aerobic swimming following exhaustive exercise on blood lactate (A) and plasma cortisol (B) (from Milligan, 1996). $\text{bl}\cdot\text{s}^{-1}$ is body lengths per second.

Conclusion

Facts Discovered from Experimental Work

1. Almost 99% of fish sampled came on board with (a) the high hematocrit (% red blood cells) and (b) the low number of fish with a visual condition rating of either 2 or 4 (those scores that identify cuts or bleeding). Therefore these fish were not anemic and suffered little to no blood loss. This finding demonstrates that commercial vessels can, with appropriate care, technique and gear, land fish in a condition that is conducive to recovery.
2. No fish died in the blue-box recovery tanks and fish looked livelier, less lethargic and brighter after being held in blue boxes. It is likely that this improvement of condition comes about because the blue-box recovery tank can promote repletion of blood and tissue oxygen stores.
3. A number of physiological indicators of stress were correlated with the visual criteria levels, indicating that these criteria are useful in predicting some of the stress effects of capture and handling.
4. The indices of metabolic status from blood (plasma glucose and lactate) and tissue samples (glucose, lactate, glycogen, phosphocreatine, ATP) suggest that all coho were in a state of extreme fatigue when first landed on board the vessel, regardless of gear type. Given this metabolic state, it is our view that the potential of this fish to swim to escape predators if released immediately on capture, would be very poor.
5. The indices of osmoregulatory status from the blood samples (plasma sodium, chloride, potassium and osmolarity) suggest that while there were significant disturbances, these were not as severe as the muscle fatigue. It is our view, that this reflects good standards of fish capture and handling that minimize skin, scale and gill damage.
6. Coho held for 24 hours in a net pen following capture by seining methods showed signs of recovery, although sampling fish from a netpen introduces additional stress. Mortality was <3%. Thus, there is evidence that, given the correct recovery conditions and time, commercially caught coho can recover from extreme muscular fatigue and stress and show little post-capture mortality. Minimizing air exposure during handling may have been important in this result.
7. Holding coho for 30 min in a blue-box recovery tank resulted in significant increases in most plasma variables (sodium, chloride, lactate, glucose and osmolarity), but plasma potassium decreased significantly. After 60 minutes, plasma lactate, sodium and osmolarity increased further. These variables were not expected to recover within one hour given the extreme level of muscular fatigue. The decrease in plasma potassium is indicative of the situation not getting worse.
8. Holding coho for 30 or 60 minutes in a blue-box recovery tank resulted in no significant changes in muscle metabolic status, except for the improvements of muscle phosphocreatine with gillnet samples. It was expected that muscle lactate would decrease and muscle phosphocreatine and glycogen would increase during the early stages of recovery. It is possible that the level of fatigue was so extreme that recovery would have taken much longer than one hour. However, we believe that two other factors contributed to this apparent refractoriness. Foremost, the design of the blue-box recovery tanks could not effectively prevent further struggling, which may have delayed recovery. Secondly, the muscle sampling technique may not have been adequate to detect recovery of phosphocreatine (probably the best early indicator of metabolic recovery).
9. Statistical differences were observed for some variables between different gear types and between fishing methods, but from a physiological standpoint the differences tended to be small since all fish were extremely fatigued. Notably, seine fishing using ramping versus brailing methods and gillnet

fishing using 60 versus 30 minute soak times were more stressful to coho. In addition, plasma sodium, chloride, osmolarity, and lactate levels of coho caught by gillnet were greater at landing than for coho caught by seine and troll gear. This difference is likely explained by differences between gear types in the time between actual exhaustion of the fish when it encountered the fishing gear and landing on the vessel.

Recommendations for Future Work

1. Laboratory experiments should be performed to test a better design for the blue-box recovery tank for use on board commercial vessels. Since it is known that recovery from fatigue can be promoted in other salmonid species in laboratory experiments, a proven design should be used in future experiments involving commercial fishing vessels. These laboratory experiments could also develop a simple but effective field measure of swimming performance.
2. Blue-box recovery tank experiments should be repeated in a commercial fishery. Recovery should be followed for two hours on board the vessel and for 48 hours using netpens. A concerted effort must be made to minimize uncontrolled variability among box dimensions, loading rates, water flow characteristics and fish-handling details or the chance for reliable conclusions will be reduced.
3. The same suite of metabolic and osmoregulatory variables should be measured. However, a biopsy needle should be used for tissue sampling to minimize the handling of fish and air exposure, and to maximize the opportunity to detect metabolic recovery in muscle tissue.
4. The ability of fish to swim at various times post-capture should be measured directly rather than inferred from indices of metabolic status. It is possible that a correlation can be established that identifies metabolic thresholds for effective swimming.
5. The present observer rating system, while useful, was incapable of detecting the extreme physiological exhaustion found in all coho. The observer scale should be expanded to include a more accurate assessment of behaviour/liveliness that could be correlated to objective physiological measures of performance capacity. Perhaps this could be done in conjunction with lab tests of alternate recovery tank designs.

Recommendations for the Fishing Industry

1. The types of fishing methods and fish handling used in the Alberni Inlet experiments resulted in 24-h survival of coho salmon. This clearly demonstrates that the industry has the capabilities to handle fish in such a way that recovery could be promoted. Reducing or eliminating air exposure of fish probably played a key role in this improvement. We recommend specifics such as the following be considered for non-retention fishing: minimal time out of water for fish, small brailer-loads, small sets, dumping seine sets if need be due to gear foulup or too many fish, and sustained good flow in recovery tanks.
2. We recommend continued preference be given to the use of brailing over ramping methods for seine gear given the demonstrated benefit with respect to stress of non-retention fish.
3. We recommend continued preference be given to the use of a gillnet soak time of 30 minute over 60 minute soak times given the demonstrated benefit with respect to stress of non-retention fish.
4. We recommend the continued use of recovery tanks for non-retention coho until superior alternatives have been developed and field-tested.
5. We recommend a redesign of the standard recovery box to include partitions that produce compartments for holding the fish and a unidirectional flow of cool, clean water of high oxygen saturation, preferably such that the fish swim slowly into the water current.

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