

## **Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique**

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Approximately 200 km from the mouth of the Fraser River, British Columbia, Canada, adult sockeye salmon *Oncorhynchus nerka*, were gastrically implanted with radio transmitters without anaesthetic. Subsets of the transmitter implanted fish were also biopsied which included drawing blood from the caudal peduncle (3 ml), removal of gill tissue (0.03 g) and quantification of energetic status using a microwave fat meter. Several experiments were used to test the hypothesis that the biopsy had a negligible effect on the subsequent survival and migratory behaviour of transmitter implanted fish. In the first experiment, no difference was found in the survival (both 100%) or tag retention (both 100%) between the two treatment groups (transmitter implanted with and without biopsy) when fish were held in pens for 24 h in the marine environment. Similarly, in other experiments where fish were released to the ocean to resume their migratory journey, no statistical differences were found in the travel times of fish in the two treatment groups, or in the proportion of fish that passed in-river telemetry checkpoints. These results indicated that the handling and biopsy methods produced similar levels of mortality and tag retention as the telemetry treatment alone and that any changes in behaviour between the two treatment groups did not adversely affect migration time. Based upon the evidence provided from the biotelemetry of >300 adult sockeye salmon, it was felt that this general type of approach could be applicable to other fish species.

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## INTRODUCTION

Positional telemetry has revolutionized the understanding of animal behaviour and distribution (Priede & Swift, 1992), particularly in aquatic environments where conventional visual observation techniques are limited (Lucas & Baras, 2000). Positional telemetry, however, provides no information on the physiological or energetic causes, consequences, costs or constraints associated with patterns of behaviour or distribution. A variety of sensors have been developed that enable the continuous telemetering or logging of physiological variables (*e.g.* heart rate, ventilation and electromyographic activity) called 'physiological telemetry' (Butler, 1989; Cooke *et al.*, 2004a). Although physiological telemetry is a powerful tool that has yielded greater understanding of the mechanistic aspects of animal ecology, it has still not been widely adopted by researchers due to cost and technological limitations (Cooke *et al.*, 2004a). An alternative to incorporating physiological sensors into telemetry devices is to couple blood and tissue biopsies with positional telemetry. Positional transmitters are relatively inexpensive compared to physiological telemetry devices providing opportunity to achieve reasonably large sample sizes. Although this approach does not provide real-time data on physiological or energetic status, it does provide an indication of the status of the animal upon release. By coupling assessments of animal position, movement, behaviour and survival, with non-invasive physiological and energetic sampling, it becomes possible to test hypotheses associated with animal condition, behaviour and fate.

A recent programme of research has focused on understanding the migration biology of adult salmon *Onchorhynchus* spp.; (Hinch *et al.*, 2002; Cooke *et al.*, 2004b). Positional telemetry has yielded novel insights into adult salmonid spawning migration patterns including the timing of river entry, travel speeds and mortality (Hinch & Bratty, 2000; English *et al.*, 2003, 2004). These patterns, however, generally do not provide any direct understanding as to the mechanistic basis for variation in behaviour and survival. Thus, the present aim was to obtain non-lethal physiological and energetic assessments of transmitter implanted sockeye salmon *Oncorhynchus nerka* (Walbaum) and then use these physiological data to interpret individual patterns of behaviour and mortality.

Although the concept of releasing transmitter implanted animals that have been biopsied for physiological variables is simple and the potential insight invaluable, only a few published examples where this approach had been employed were located. For those few instances where this technique was used [*e.g.* Weddell seals *Leptonychotes weddellii*: Burns & Castellini, 1996; polar bears *Ursus martimus*: Messier *et al.*, 1992; bluefin tuna *Thunnus thynnus* (L.): Skomal & Chase, 1997; brown trout *Salmo trutta* L.: Aarestrup *et al.*, 2000], only two studies quantitatively assessed the effects of biopsy on the transmitter implanted individuals. Baker & Johanos (2002) evaluated the effects of blood sampling and

other research handling on transmitter implanted endangered Hawaiian monk seals *Monachus schauinslandi* and noted no deleterious effects on resighting, behaviour or condition. Martinelli-Liedtke *et al.* (1999) compared the survival, growth, and physiology of juvenile chinook salmon *Onchorhynchus tshawytscha* (Walbaum) that were implanted with gastric transmitters or implanted and additionally gill biopsied and noted no impairments. Few of these papers provided a detailed suite of methods as to how fishes were handled or sampled, and only Martinelli-Liedtke *et al.* (1999) evaluated the consequences of biopsy on the subsequent behaviour or survival of transmitter implanted fishes.

In the present study larger adult (and thus more powerful) fish than those examined by Martinelli-Liedtke *et al.* (1999) were used. Blood samples were obtained in addition to a gill biopsy. Also, information on energetic status was required although invasive muscle plugs (Wooster *et al.*, 1993) were not taken and instead a non-invasive fat probe was employed that uses microwave signals to assess energetic status (Hendry & Beall, 2004; Crossin & Hinch, 2005). There are many studies that collect blood and gill biopsies from fishes but these typically involve anaesthetizing the fish prior to sampling and are not conducted on transmitter implanted fishes (McCormick, 1993; Iwama *et al.*, 1995). For some telemetry studies, it can be undesirable to anaesthetize fishes due to increases in handling time, recovery and post-operative care, as well as physiological disturbances arising from the anaesthesia. In the case of the migratory salmonids studied, there was the distinct possibility that transmitter implanted fishes could be caught by commercial and recreational fisheries and then processed for human consumption. Anaesthetics currently approved for use in fishes cannot be used in fishes that could be consumed by humans within a short time after application. Alternatively, other anaesthetics can cause severe physiological disturbance that could affect the survival of salmonids (*e.g.* CO<sub>2</sub> or cold-shock). Therefore, it was necessary to develop protocols for biopsy of un-anaesthetized sockeye salmon at sea without compromising the telemetry component of the study (*e.g.* migration speed, success, timing and survival) and while minimizing stress and discomfort for the fish.

In this study, the technique that was developed to efficiently biopsied sockeye salmon are outlined. Using three independent assessments the hypothesis that there are negligible differences in the behaviour, mortality or transmitter expulsion of fish implanted with gastric radio transmitters *v.* those that were implanted with transmitters and also biopsied was tested. Although the specific objective in this study was related to migration biology of sockeye salmon, the approach that was developed should also be applicable to other research.

## MATERIALS AND METHODS

Three separate experiments were conducted to assess the effect of physiological sampling on both short-term and long-term survival of adult sockeye salmon. In each experiment there were two treatments: a control transmitter implanted group and an experimental transmitter implanted and biopsy group. The control treatment involved collecting a scale sample, piercing the adipose fin for a DNA sample with a hole punch, and orally inserting a radio transmitter into the stomach. The biopsy treatment included all of the characteristics of the control fish, as well as the sampling of blood and gill tissues, and measurements with a microwave fat probe meter.

## ETHICAL CONSIDERATIONS

As discussed briefly in the introduction, it was not possible to use anaesthesia for this study for a number of reasons. Ideally, fish would have been anaesthetized as this would have potentially mitigated any pain or discomfort felt by the fish. Furthermore, anaesthesia would have made fish handling, including biopsy and tagging, easier and safer for the researchers. In this specific instance and after consultation with fish health experts, it was determined that the study would be compromised by use of anaesthetics. Standard anaesthetics such as MS-222 require a lengthy holding period due to the possibility of consumption by humans (*i.e.* harvest *via* fishing) or other animals. As migratory fish were being used, extended holding post-surgery was not possible. Another popular anaesthetic, clove oil, has been suggested to interfere with natal homing, which would be problematic for the study of migratory salmonids (Woody *et al.*, 2002). Use of alternative anaesthetics such as CO<sub>2</sub> or cold-shock would have led to additional physiological disturbances and furthermore, worker safety regulations precluded use of large holding facilities on board the vessel (even though one of the largest vessels available was being used) so initial recovery of anaesthetized fish would have been difficult. There was also concern that anaesthetized fish could suffer post-release predation from the abundant marine mammals. Although the technique developed here in consultation with institutional animal care committees was deemed to potentially cause some level of suffering to the fish, the strategies employed were intended to minimize disturbances (*e.g.* keeping fish in water continuously). Furthermore, the data were viewed as important and unattainable with other techniques. Thus, anaesthetic use should be considered in future studies, for fish welfare, unless there are compelling logistic and scientific constraints.

## EXPERIMENT 1: PRE-SEASON TRANSMITTER RETENTION AND MORTALITY ASSESSMENT

The purpose of this experiment was to compare the transmitter retention and mortality among transmitter implanted fish and those which were additionally biopsied. On 26 July 2003, sockeye salmon were captured by purse seine on the *Royal Mariner I* (commercial test fishing purse seine vessel) from the Juan de Fuca Strait (Fig. 1), *c.* 165 km from the mouth of the Fraser River. The timing of the sampling was such that Fraser River sockeye salmon were the target species. To prepare for the sampling day, five sockeye salmon had biopsies and were held in a small tote (a container used by commercial fishing vessels to ship dead fishes on ice) (tote number 1; 89 × 48 × 56 cm, capacity of 239 l) aboard the vessel prior to moving the fish to a holding pen as described below (the biopsy of these five fish referred to as the 'pre-test'). After becoming comfortable with the set up and working conditions, the formal pre-season holding assessment was started. Equal numbers of fish were tagged using the two treatments and then held in a combination of two small totes (tote number 1 and 2; tote 2 was the same as 1) and one larger tote (96 × 120 × 63 cm, capacity of 726 l) with continuous flow-through of sea water for <3 h. Surface water temperatures were *c.* 10° C. Fish were then transported on board the vessel to Sooke harbour (Fig. 1) where the 30 fish were placed into two holding pens (3.5 m × 1.5 m × 1 m, capacity of 5250 l) at equal densities. Fish were held in the pens for a period of 24 h and visually assessed for any mortality and transmitter expulsion. At the end of the 24 h period, fish were individually netted from the pens and examined for transmitter presence. Transmitters were then removed and the fish were released to the ocean.

## EXPERIMENT 2: PRE-SEASON RELEASE ASSESSMENT

The purpose of this experiment was to compare the travel times and survival among control (including transmitter implantation) and biopsied (including transmitter implantation) fish. On 27 July 2003, additional sockeye salmon were captured by purse seine on the *Royal Mariner I* from Juan de Fuca Strait (Fig. 1). Once again, equal

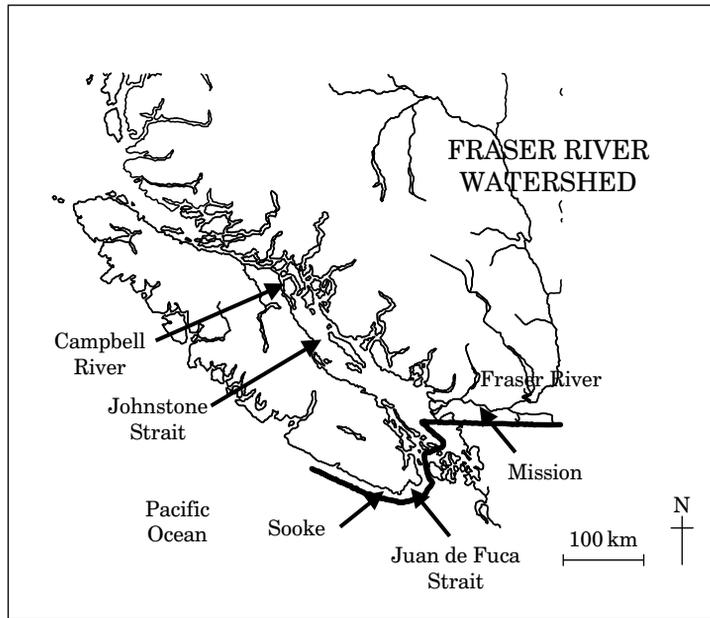


FIG. 1. Map of study site in south-western British Columbia, Canada, and north-western Washington, U.S.A. Experiment 1 and 2 took place in Juan de Fuca Strait while experiment 3 took place in Johnstone Strait. Experiment 1 holding pens were located in Sooke Harbour. The telemetry array was deployed in the Fraser River at Mission, British Columbia.

numbers of fish were tagged using the two treatments, and then held for a 30–40 min recovery period in a large tote (96 × 120 × 63 cm, capacity of 726 l) with continuous flow-through of sea water. Surface water temperatures were between 10 and 13° C. Anecdotal observations were made on the condition of all 52 tagged fish before their release. DNA analyses were not conducted on these fish, and it was assumed that the majority of the fish were bound for the Fraser River since this was the case for DNA analysis of sockeye salmon caught by test fishing vessels operating in the same location on the same day. Even if this assumption was incorrect, it was assumed that because of the randomness of the sampling the stock composition of each group would be similar.

Two radio telemetry stations were set up on the main stem of the Fraser River at Mission, British Columbia (BC) (Fig. 1). Mission is located 85 km upstream from the mouth of the Fraser River, some 250 km from the Juan de Fuca release site. The Mission stations were deployed on each side of the river and each station consisted of two antennas and a data logging radio receiver (SRX\_400, Lotek Engineering Inc., Newmarket, ON, Canada; English *et al.*, 2003, 2004). Detection of individual transmitters was used to assess the relative survival or transmitter expulsion rates of fish in the two treatment groups. The travel times (elapsed time in hours between release and first detection) were also compared. Based on the subsequent detection of 297 transmitters during the 2003 study, the detection efficiency of this receiver station was estimated at 88% (English *et al.*, 2004). In addition, a tag return and reward programme was implemented in the U.S. and Canada to obtain tags from fish captured in commercial, recreational and First Nation fisheries.

### EXPERIMENT 3: IN-SEASON RELEASE ASSESSMENT

The purpose of this experiment was to compare the travel time and survival among control and biopsied fish and it was different from experiment 2 in that it was part of a

formal sockeye salmon behaviour and migration success study (English *et al.*, 2004). Sockeye salmon were captured in Johnstone Strait (Fig. 1) using the test fishery seine vessel, *Sunfisher*, c. 215 km from the mouth of the Fraser River. Fish were collected and released during two distinct periods separated by several days (between 19–22 August 2003, release 1; 26–28 August 2003, release 2). For purposes of these analyses, each of the two sampling periods was considered separately. An attempt was made to release equal numbers of fish in each treatment, as in the pre-season tests. Additional details were similar to those outlined for experiment 2. Surface water temperatures were between 10 and 13° C. The tote used for this experiment was larger (96 × 120 × 126 cm, capacity of 1452 l) than those used earlier. DNA analyses were conducted and there were no differences in the 'run timing group' (stock) composition for each of the treatments during each of the two tagging sessions (English *et al.*, 2004). The in-river receiver arrays at Mission, BC, (Fig. 1) were 300 km from the Johnstone Strait release site and were the same as those deployed for experiment 2. Analyses were the same as those in experiment 2.

## GENERALIZED BIO-SAMPLING AND TAGGING TECHNIQUES

In developing the protocol for biopsying transmitter implanted fish, the expertise of the team members was relied upon in both independently tagging and sampling many species of fish. This background experience was used to develop and test different sampling strategies. All procedures used in this study were developed with approvals and guidance from the Canadian Council on Animal Care administered by the University of British Columbia, Simon Fraser University, and Fisheries and Oceans Canada. The first sampling efforts took place on recently dead sockeye salmon captured on a test fishery gillnet vessel in the lower Fraser River in July of 2003. When comfortable with the biopsy of dead fish ( $n = 4$ ), live sockeye salmon also captured by gillnet in the lower Fraser River ( $n = 5$ ), as well as coho salmon *Oncorhynchus kisutch* (Walbaum) ( $n = 2$ ) and rainbow trout *Oncorhynchus mykiss* (Walbaum) ( $n = 2$ ) at the Simon Fraser University wet laboratory were practiced on. In total, 13 fish were used for practice. The entire sampling team was experienced with gastric transmitter implantation and fish handling. The same individual implanted all of the transmitters within a single experiment. Two equally trained individuals shared the biopsying activities during each experiment, rotating occasionally between holding and biopsy to allow for rewarming of chilled hands and recovery of dexterity.

Following capture, fish were individually netted out of the purse at the side of the boat and placed into large flow-through totes on the boat deck. After all fish required were transferred to the totes (based on the sample size needs and constraints on holding density), individual fish were netted from the totes and placed ventral side up in a 'v'-shaped trough. The trough was lined with foam, contained an integrated measuring tape, and was supplied with flowing sea water that entered the trough and was directed towards the mouth of the fish [Fig. 2(a)]. For experiments 1 and 2, flow-through water was not utilized and instead water was replaced at c. 30 s intervals using buckets. The vigour of fish post-procedure increased after switching to flow through conditions in experiment 3. The trough was angled slightly so that the water was deep enough to cover the entire head of the fish while leaving the caudal peduncle only partially submerged [Fig. 2(a)]. The tagging team consisted of four individuals.

The first step involved restraining the fish in the trough. During preliminary evaluations it was determined that using two sets of wet hands was the best method to restrain the fish without excessive removal of slime or scales. Use of cotton or rubber gloves or a chamois did not improve the ability to handle the fish. One individual always held the head of the fish, gently covering the eyes and keeping the head down. A second individual held the caudal peduncle region and placed their other hand on the mid-section of the fish. The two individuals restraining the fish were positioned on either side of the trough. When the fish was restrained, the third individual stood at the caudal end of the trough and gripped the caudal peduncle region with one hand. At this point, the other individual restraining the caudal region moved their hand slightly anterior to provide room for the



FIG. 2. Photographs illustrating the different components of the biopsy procedures. (a) The fish are restrained in a trough with the anterior portion of the fish (at bottom of photograph) entirely submerged in water. Note the water inflow tube in the lower left hand corner of the trough. The blood was sampled at this time. (b) The fish is restrained while using linesman's pliers to remove a small gill biopsy. The pliers held the sample better than did simple scissors. Note the position of the hands on the fish to open the opercular cavity. (c) The fat probe is held against the side of the fish, which is raised just above the surface of the water. The head of the fish is still submerged.

hand of the blood sampler, but while still assisting with restraining the tail [Fig. 2(a)]. Using their other hand, the vacutainer syringe (38 mm, 21 gauge) was aligned with the caudal haemal arch and when the fish was still, it was plunged into the caudal vessel. Detailed descriptions and diagrams of caudal sampling blood from fish can be found in Houston (1990). The vacutainer (3 ml) was then activated, usually resulting in the immediate collection of blood. On some occasions, the fish would move, bending the needle or terminating the vacuum, or blood did not immediately begin to enter the vacutainer. If subtle adjustments to the position of the syringe did not remedy the problem, the blood sampler then used a new, pre-rigged vacutainer and syringe. If the blood was not drawn within 1 min, the fish was excluded, no transmitter was implanted, and the fish was released. If successful, the blood sampler left the caudal region to place the vacutainer in an ice-water slurry and to dispose of the needle. The individual restraining the tail applied light pressure to the puncture site to facilitate clotting.

Next, the two individuals restraining the fish collected a gill biopsy. The individual restraining the head region used one hand to hold a pair of small sharpened linesman's pliers and when the fish was still, the left operculum was lifted with a single finger and a small (0.03 g) gill biopsy was removed [Fig. 2(b)]. The biopsy was always focused on the first gill arch and included <4 mm of tissue from six to eight filaments. The technique employed resulted in little bleeding and has been used on a number of other fish species

(McCormick, 1993; Schrock *et al.*, 1994). The blood sampler returned with a small vial and took the pliers with the sample. Then they used a pair of forceps to transfer the biopsy into a labelled vial and then placed it in dry ice chips. A fourth individual, responsible for recording information and handling scale and DNA samples, recorded the fork length ( $L_F$ ) of the fish and took a scale sample from the caudal region. A tissue sample was removed from the adipose fin using a hole punch and placed in ethanol (for DNA analysis). The blood sampler then returned with a micro-wave fat probe (Distell Fish Fatmeter model 692, Distell Inc, West Lothian, Scotland, U.K.; Hendry & Beall, 2004; Crossin & Hinch, 2005). This hand-held device houses a microwave oscillator that emits a low powered wave (frequency,  $2 \text{ GHz} \pm 2000 \text{ MHz}$ ; power,  $2 \text{ mW}$ ) that interacts with water in the somatic tissues. Drawing from the strong, inverse relationship between the water and lipid content in fish tissues, microwave sensors convert water concentration to estimates of lipid concentration. The fat probe requires that the fish be held slightly out of water, straight and generally relaxed [Fig. 2(c)]. The probe was placed on the left side of the fish in two locations to obtain measurements of its energetic status.

The final step of the sampling process involved inserting the radio transmitters (MCFT-3A, Lotek Inc., Newmarket, ON, Canada) in the stomach using a plastic tag applicator (Eiler, 1990; Ramstad & Woody, 2003). All transmitters weighed  $16.1 \text{ g}$  in air and  $6.2 \text{ g}$  in water and measured  $16 \text{ mm}$  in diameter and  $51 \text{ mm}$  in length. The antenna trailed out the mouth of the fish and  $30 \text{ mm}$  of tubing from a Floy anchor tag was affixed to the end of the antenna. The individual restraining the head region inserted the transmitter. For all experiments, handlers alternated between control and treatment with each fish. The tagging and sampling procedures were terminated if the entire procedure took  $>150 \text{ s}$ , or if excessive bleeding was noted from the gill biopsy site. Fish that fell onto the boat deck either before or during tagging were not included in the study.

All tagging and sampling was conducted aboard chartered purse seine vessels, thus the sampling conditions were variable. Whenever possible, the crew located calm waters in bays or faced the nose of the boat into the waves and wind to provide some protection from the weather. An important aspect of sampling was to design a layout for bio-sampling gear that allowed gear to be kept dry, sterilized, organized and accessible. A plastic bin with slip-proof material on the bottom was used to hold a number of different water-tight containers with the gear.

## STATISTICAL ANALYSIS

Contingency table analyses were used to assess differences in categorical variables related to survival (response variable) and treatment (factor). Fish captured by commercial, recreational or First Nations fisheries prior to reaching Mission ( $<10$  fish overall) were excluded from these analyses of survival. To test the null hypothesis of no difference in travel times (continuous variable) for control and biopsied fish, two sample *t*-tests were used. Only fish that survived to Mission were used to calculate travel times. Two sample *t*-tests were also used to test for differences in body size, as well as the time required for processing the fish (in seconds from when fish was placed on the table until returned to the tote), the time between processing and release (in minutes), and the total holding time (in minutes from capture to release). These assessments focused on all fish tagged since these data were collected prior to fish captured by the fisheries, dying or otherwise disappearing. All analyses were conducted using JMP 4.0 (SAS Institute, Raleigh, NC, U.S.A.) and were assessed for significance at  $\alpha = 0.05$ .

## RESULTS

### EXPERIMENT 1

Fish from the first set were used to practice the physiological sampling procedures and five of these fish were retained in the on-board revival tote

1 for extended observation (pre-test group). Of these five fish, one died in the revival tote (tote 1). The four remaining fish (all biopsied) were transferred to pens where one died within 1 h. The three remaining fish were alive and apparently healthy 24 h later and had retained their transmitters.

The fish for the formal tag retention and mortality study were tagged starting with the second seine set of the day. A total of 33 sockeye salmon were tagged from sets two to four (six, 13 and 14 respectively). Of the 33 fish tagged, 17 were gastrically tagged without biopsy (controls) and 16 were tagged and biopsied (biopsy treatment) (Table I). The size of the fish was similar for each group (*t*-test, d.f. = 31,  $P = 0.586$ ; Table I). Of the 17 control fish tagged, two died in a small revival tote (tote number 2), leaving 15 for holding in the pen. All 15 of these fish were alive and apparently healthy 24 h later. Of the sixteen biopsied fish, four died in a revival tote number 2. The 12 remaining fish were transferred to pens and they were all alive and 'healthy' 24 h later.

The level of fish mortality in this part of the study was deemed unacceptable and the primary reason for the fish mortalities appear to be related to water flow in the small-totes and the extended on-board holding time. On-board totes number 1 and number 2 were half totes connected in series (*i.e.* the water from tote number 1 flowed into tote number 2). Also, due to rolling of the vessel, the hose supplying water to the totes became partially constricted at times, also affecting the flow into that. In addition, the water entered and drained at the surface so water in the bottom of the tote, where the fish typically stayed, was not properly mixed with the surface water. The problem with the water circulation was revealed after a 2 h holding period when fish were observed on their sides in the bottom of tote number 2. An additional water line was immediately used to add fresh sea water to tote number 2 and 3 and the fish that had lost equilibrium were transferred to the other totes (the transferred fish never recovered). These water circulation problems were addressed for experiment 2 by increasing the flow to tote number 1, removing the hose constriction, adding down pipes in totes 1 and 2 to 'push' the fresh water to the bottom of each tote, and limiting the number of fish held in tote number 1 to a maximum of six. It should be noted that none of the 14 fish held in the large (tote number 3) died during the holding experiment and the two fish that died in tote number 1 were both from the pre-test group where the biopsy technique was being refined.

## EXPERIMENT 2

On 27 July, a total of 52 sockeye salmon (26 control and 26 biopsied) were tagged and released in seemingly excellent condition following a mean recovery period of *c.* 50 min (Table I) and total holding period of *c.* 80 min (Table I). There were no differences in the recovery period (*t*-test, d.f. = 50,  $P = 0.571$ ) or total holding time (*t*-test, d.f. = 50,  $P = 0.916$ ) among treatments (Table I). Both control and biopsied fish were of similar size (*t*-test, d.f. = 50,  $P = 0.564$ ; Table I). For this experiment, the biopsied fish took nearly twice as long to process, than the control fish (*t*-test, d.f. = 50,  $P < 0.001$ ; Table I). Despite the differences in processing time, no differences in the survival of fish between the release site and the Mission telemetry receiver were observed [ $X^2$ , d.f. = 1,  $P = 0.760$ ; Fig. 3(a)]. Seven of 26 control fish (26.9%)

TABLE I. Characteristics and sample sizes of sockeye salmon in each treatment and experiment. The time required for different procedures and holding periods is also given. The procedure time is the time required to biopsy or simply tag the fish. During experiment 1, procedure times and total holding times were not recorded (NA) and recovery time was estimated

Variables	Treatment	Experiment 1	Experiment 2	Experiment 3 (release 1)	Experiment 3 (release 2)
Number of fish processed	Control Biopsy	17 16	26 26	76 76	54 55
Mean $\pm$ s.e. $L_F$ (cm)	Control Biopsy	60.8 $\pm$ 1.0 61.6 $\pm$ 1.1	61.0 $\pm$ 0.7 61.6 $\pm$ 0.7	60.7 $\pm$ 0.3 61.3 $\pm$ 0.4	60.9 $\pm$ 0.3 61.1 $\pm$ 0.3
Mean $\pm$ s.e. procedure time (s)	Control Biopsy	NA NA	55 $\pm$ 4 101 $\pm$ 4	71 $\pm$ 3 94 $\pm$ 4	77 $\pm$ 4 122 $\pm$ 4
Mean $\pm$ s.e. recovery time (min)	Control Biopsy	30-40 30-40	52 $\pm$ 4 49 $\pm$ 4	54 $\pm$ 2 54 $\pm$ 2	54 $\pm$ 5 54 $\pm$ 5
Mean $\pm$ s.e. total holding time (min)	Control Biopsy	NA NA	79 $\pm$ 2 79 $\pm$ 2	96 $\pm$ 2 97 $\pm$ 2	113 $\pm$ 4 113 $\pm$ 4

and eight of 26 biopsied fish (30.8%) were recorded at Mission. In addition, the travel times for fish from the release site were similar [*t*-test, d.f. = 13,  $P = 0.805$ ; Fig. 3(b)]. Controls reached Mission in  $249.6 \pm 42.8$  h (mean  $\pm$  s.e.) and biopsied fish reached Mission in  $265.0 \pm 43.5$  h.

### EXPERIMENT 3

During the first release period (release 1), 152 sockeye salmon (76 control and 76 biopsied) were tagged and released. Fish were provided a recovery period of *c.* 54 min (Table I) and were held for a total period of *c.* 97 min (Table I). There were no differences in the recovery period (*t*-test, d.f. = 150,  $P = 0.912$ ) or total holding time (*t*-test, d.f. = 150,  $P = 0.745$ ) among treatments (Table I). Both control and biopsied fish were of similar size (*t*-test, d.f. = 150,  $P = 0.214$ ; Table I). For this experiment, the biopsied fish took *c.* 20% longer to process than the control fish (*t*-test, d.f. = 150,  $P < 0.001$ ; Table I). Despite the differences in processing time, no differences in the survival of fish between the release site and the Mission telemetry receiver were observed [ $X^2$ , d.f. = 1,  $P = 0.769$ ; Fig. 3(a)]. Survival was uniformly high, near 70%. In addition, for those fish that survived to Mission, travel times for fish from the release site were similar between control and treatment groups [*t*-test, d.f. = 101,  $P = 0.091$ ; Fig. 3(b)]. Surviving fish reached Mission in 10 to 11 days on average.

Similar trends were observed in the second release period (release 2). A total of 109 sockeye salmon (54 control and 55 biopsied) were tagged and released (Table I). Fish were provided a recovery period of *c.* 54 min (Table I) and were held for a total period of *c.* 113 min (Table I). There were no differences in the recovery period (*t*-test, d.f. = 107,  $P = 0.998$ ) or total holding (*t*-test, d.f. = 107,  $P = 0.930$ ) among treatments (Table I). Both control and biopsied fish were the same size (*t*-test, d.f. = 107,  $P = 0.631$ ; Table I). For this experiment, the biopsied fish took on average *c.* 45 s longer to process than did control fish (*t*-test, d.f. = 107,  $P < 0.001$ ; Table I). Despite the differences in processing time, no significant differences in the survival of fish between the release site and the Mission telemetry receiver were observed [ $X^2$ , d.f. = 1,  $P = 0.345$ ; Fig. 3(a)]. Nonetheless, survival tended to be higher for control fish (70%) than biopsied fish (62%). Travel times for fish from the release site that survived to Mission were similar [*t*-test, d.f. = 70,  $P = 0.354$ ; Fig. 3(b)]. Surviving fish in both groups reached Mission in 9 to 10 days on average.

### DISCUSSION

A prevailing hypothesis at the onset of this study was that biopsy might induce substantial stress on radio-tagged fish, making them more susceptible to mortality (either direct or indirect from predation), alterations in behaviour, or tag expulsion after release. Collectively, the independent evidence obtained using migratory adult sockeye salmon indicated that it was possible to collect physiological and energetic data from individuals implanted with transmitters without causing significant deleterious effects. Consequently, further analyses of marine

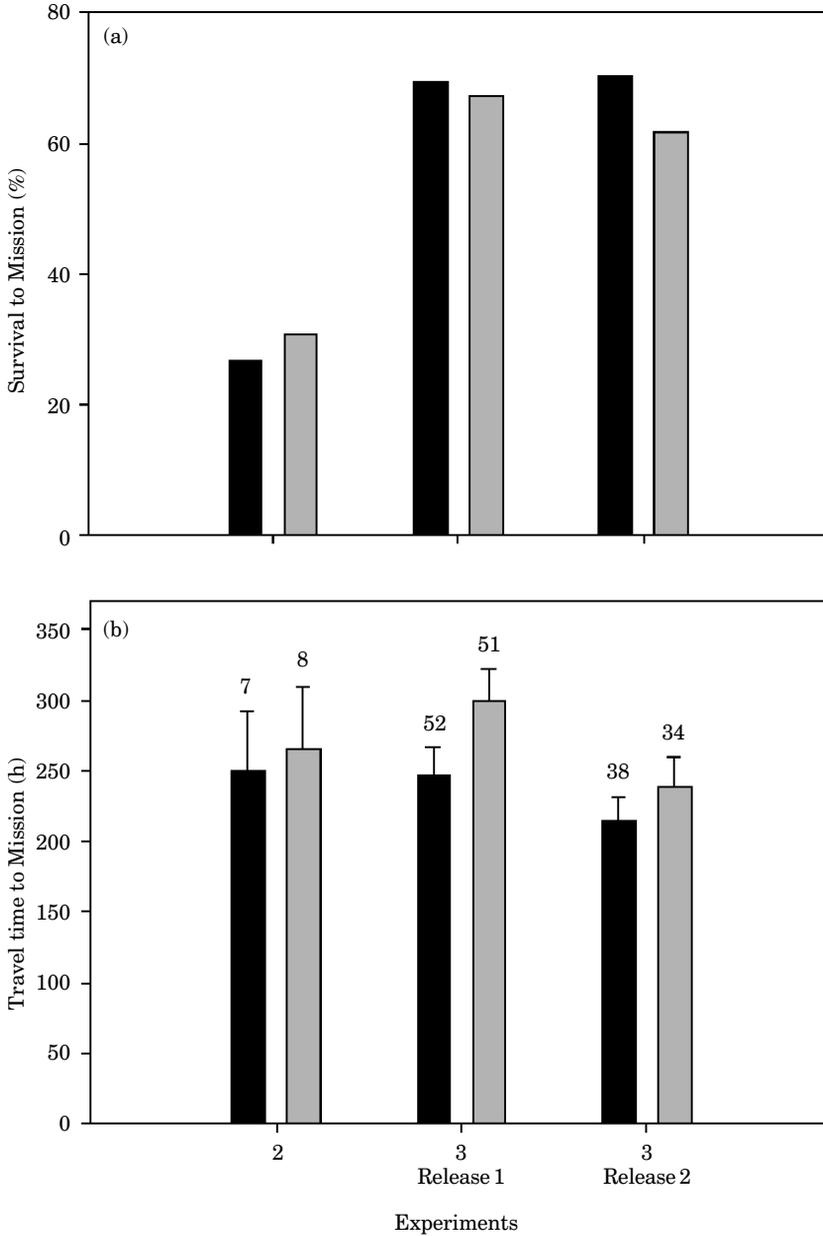


FIG. 3. (a) The survival to Mission and (b) mean  $\pm$  s.e. time of arrival at Mission for control (■) or biopsy (▒) sockeye salmon. Sample sizes are presented above each bar, representing the number of fish in each group that survived to Mission and for which it was thus possible to calculate travel times.

and in-river survival patterns of sockeye salmon using this data-set can be conducted using all radio-tagged fish, without making further distinction based on their biopsy history. In addition, this approach could be applied to fishes or

other animals. What is evident from the processing of several hundred adult sockeye salmon is that the attention to and upgrading of methodological details improved the ability to handle fish successfully and reduce fish loss (28% survival for experiment 2 *v.* 65% survival for experiment 3), although it cannot be certain that animal predation and fish capture by fishers did not differ for the two sampling sites and times.

The assessments revealed that the 24 h transmitter retention and survival of sockeye salmon was uniformly high for both biopsied and non-biopsied individuals. In fact, all fish from the holding study in experiment 1 survived the 24 h retention period and no fish regurgitated transmitters. Consistent with the present findings, gastric transmitter retention in migratory adult sockeye salmon in other studies has been high. In a controlled experiment, sockeye salmon in Alaska had an overall gastric retention rate of 98% over a period of 15 to 35 days (Ramstad & Woody, 2003). In a field experiment in British Columbia, Groot *et al.* (1975) noted 100% retention of upriver migrating sockeye salmon when tracked for 2 days.

In the present study, there was no mortality during the 24 h holding period for either of the treatment groups in the holding study component of experiment 1. Some mortality was observed during the initial pre-test period when fish were held in small totes, but this was attributed to poor water quality (probably hypoxia) related to insufficient water exchange in the totes. Although the holding period for experiment 1 was only 24 h, mortality levels for gastric-tagged fishes, including sockeye salmon, have also been quite low. Ramstad & Woody (2003) noted that mortality was *c.* 2% for gastric tagged and *c.* 3% for controls sockeye salmon. In fact, gastric implantation for adult migratory salmonids is the preferred approach because it is regarded as having negligible impacts on migration behaviour or ability (Eiler, 1990). In the only study to assess the effects of transmitter implantation and biopsy on fishes, Martinelli-Liedtke *et al.* (1999) reported that there was no difference in tag retention or mortality between fishes that were gastrically implanted and those that were additionally biopsied. In their study, biopsy was restricted to the gills (using the protocol described by McCormick, 1993). Although the biopsy in the present study also included blood sampling and non-invasive fat probing, a significant deleterious effect on mortality or tag retention among the two treatment groups was not observed.

Martinelli-Liedtke *et al.* (1999) state that their laboratory study must be cautiously applied to field studies due to increased presence of pathogens, environmental variation, recovery potential and predation. To overcome the limitation of laboratory or controlled experiments, field assessments were also conducted in the present study. Although it was not possible to explicitly assess mortality of released fish in the field, relative mortality could be inferred based upon knowledge of harvest rates and DNA stock structure, and proportions of fish in the different groups that reached a predetermined check-point. The results from both pre-season and in-season comparisons between sockeye salmon tagged, and those tagged and biopsied, indicated that there were no significant differences in mortality. Also, in both a preliminary and in-season comparison of travel times (time required to travel from oceanic release point to the most downstream in-river checkpoint), no differences between fish that were just

tagged and those that were tagged and biopsied were noted. Minor differences in travel times among fish tagged in the two different release groups of experiment 3 were to be expected and were more likely to be due to the timing of releases than the stocks tagged. Migration rates can vary by several days between early and later migrants of a single stock.

Although there was no statistical difference in either mortality or travel time of field released fish, there was a consistent trend towards a *c.* 5% difference in mortality and travel times, being higher for those fish that were additionally biopsied. It is not unreasonable to expect a slight negative consequence arising from the additional sampling (consisting of additional handling and the physical process of removing blood and gill tissue) relative to non-sampled fishes. The level of difference between treatment groups might vary depending upon the fish size, species, gender, water temperature, tagging conditions and 'bio-sampler' experience and should be considered when implementing this type of biopsy programme. Also, based on evidence from the pre-trial in experiment 1, it appears that biopsied fish may be more sensitive to poor water quality (*i.e.* hypoxia) than control fish immediately post processing. This is a reasonable expectation if it is assumed that stress is cumulative. Longer handling may translate into greater oxygen debt and higher metabolic rates, increasing oxygen demands and thus increasing their sensitivity to low oxygen conditions (Farrell *et al.*, 2000). Thus, water quality during and after the procedure seems to be an important component of ensuring healthy fishes and successful biopsy.

The single biggest factor slowing the biopsy process was 'thrashing' fish that made it difficult to collect blood, obtain gill tissue and log fat probe readings but holding fish in dorsal recumbency tended to overcome this problem. In some instances, anaesthetic use may ameliorate it. Anaesthetics, however, may themselves alter migration and alter the physiological status of research variables of interest, as well as increasing handling time and potentially introducing 'contaminated' individuals into the food chain. Such decisions require consideration of biological, ethical and logistical constraints. The most variable component of the tagging process was the blood collection. Although most fish bled immediately, some fish took >30 s to obtain blood and in others blood was never successfully collected. Since the latter was an infrequent event (about one fish in 15; S.J. Cooke, pers. obs.) and fish abundance high, fish from which blood could not be rapidly collected (*i.e.* <60 s from time of first needle insertion or 120 s until it was time to insert the transmitter) could be released.

The concept of linking individual behaviour and fate with energetics and physiology is one that has eluded researchers for some time (Altmann & Altmann, 2003) despite that inter-individual variation has been recognized as an important concept (Bennett, 1987). The techniques outlined in this paper can be used to generate results that may provide mechanistic understanding of observed behaviour and mortality. Instead of just determining what an animal does, the additional biological information can be used to determine how they do it. An added strength of this approach is that it allows insight into factors that are linked to mortality, which is especially relevant to migration research. Conventional lethal physiological sampling from a population that suffers mortality during its migration, means that the sampling progressively becomes

limited to those fishes that have succeeded in completing a specific part of the migration, providing little insight into what happened to those that were unsuccessful and died.

At present, there are very few examples of researchers using biopsy of transmitter implanted fishes. Long-line by-catch mortality of pelagic fishes and sea turtles in the Pacific Ocean using archival pop-up satellite transmitters has been undertaken by Musyl *et al.* (2002). Blood samples collected on animals have been used to assess biochemical correlates of mortality to better understand by-catch stress and mortality. In another study, researchers sampled blood from marine, pelagic fishes following recreational angling and then released them with ultrasonic telemetry transmitters (Skomal & Chase, 1997, 2002). These data revealed the biochemical alterations associated with mortality and aberrant behaviour. Although there are reasonably few examples linking telemetry with other biopsies, and even fewer studies actually assessing whether that type of sampling has negative consequences, this approach will probably become more common where ecology and conservation problems demand mechanistic insight. Techniques in field physiology (Goldstein & Pinshow, 2002; Costa & Sinervo, 2004) are evolving rapidly so that animals can be studied in more natural environments. The present findings reveal that using careful preparation and practice to develop technique it is possible to sample multiple physiological and energetic variables from transmitter implanted fishes without deleteriously affecting animal behaviour.

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