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Abstract

We used both on-shore holding and field releases of wild fish to evaluate the effect of using intracoelomic surgical implantation of small radio transmitters (4 g, 9.5 mm diameter, 26 mm length) on adult mountain whitefish. In the holding study, all fish survived the 48 hr period following surgery. Significant mortalities were noted on days four and five of the experiment; however, tagged fish were no more likely to die than control fish. Incisions showed signs of healing and had no macroscopic inflammation. The times until exhaustion during forced swimming trials were similar between tagged and control fish. However, after fish were held for five days, exploratory activity levels were depressed in both groups, emphasizing the potential consequences of retaining this species for even short periods of time. When we implanted twelve mountain whitefish with tags (0.7–3.0% of body weight) and released them in the Columbia River, we had reasonable success at tracking movements of these fish over three seasons and there was no evidence of tagging mortality after release. We suggest that using the protocols described here, it is possible to implant adult mountain whitefish with electronic tags but advise that fish should not be held for more than several hours post-implantation to minimize stresses of handling and captivity. This finding is consistent with the growing body of literature suggesting that pre- and post-surgical care is an important component of successful transmitter implantation.

Introduction

Mountain whitefish (*Prosopium williamsoni*) are a widely distributed salmonid in western North American rivers, ranging from the Colorado River to northwestern Canada (Behnke 2002). In comparison to most western salmonids, little is known about the biology of mountain whitefish (McPhail and Troffe 2001). Even basic information on seasonal movements, population dynamics and habitat use of mountain whitefish is limited (but see Pettit and Wallace 1975, Thompson and Davies 1976, Alexander et al. 2006, Meyer et al. 2009, Lance and Baxter 2011) which can make it difficult to manage the species (McPhail and Troffe 2001). Although biotelemetry studies have the potential to address many of the knowledge gaps for this species, mountain whitefish are reputed to be sensitive to sampling and handling which creates some challenges for surgical implantation of transmitters. This may partly explain why there have been few studies using telemetry on this species.

Intracoelomic implantation of telemetry tags via laparotomy (i.e., incision through abdominal wall to access the coelom) is generally regarded as the most appropriate approach for long-term biotelemetry and biologging applications in fish (Jepsen et al. 2002, Bridger and Booth 2003). Although more invasive than external tagging, surgical implantation does not affect drag forces while swimming and enables animals to be tracked over long time periods. The assumption among all telemetry studies is that subjects do not incur behavioural or physiological impairments as a result of surgical procedures (i.e., that the behaviour of the tagged individuals is representative of untagged conspecifics; Bridger and Booth 2003, Thiem et al. 2011). The growing interest in fish

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welfare (see Mulcahy 2003) has promoted even greater scrutiny of surgical procedures. No tagging effect studies exist for mountain whitefish and the use of surrogate species to develop tagging protocols is discouraged (Ebner et al. 2009). As such, there is a need to conduct tagging validation studies for this species. There are a wide range of endpoints that can be measured to evaluate potential tagging effects although the best approach is to use multiple endpoints that evaluate lethal and sublethal effects (e.g., healing, swimming activity, and performance; Cooke et al. 2011a). There is also merit in combining controlled holding studies with field validations to ensure that data are relevant to field scenarios (Cooke et al. 2011a).

Our objectives were to: (1) evaluate short-term (five day) mortality and incision healing of radio tagged whitefish relative to control subjects; (2) evaluate the relative change in exploratory activity before and after surgical implantation and holding; (3) evaluate the effect of transmitters on prolonged swimming performance as measured from chase tests on tagged versus control fish; (4) determine the feasibility of locating mountain whitefish released into the wild for the purpose of documenting medium to long term seasonal movement patterns. We also provide detailed methods to enable researchers to emulate these procedures for future tagging studies of mountain whitefish.

Study Area

This study was conducted on the Upper Columbia River, downstream of the river's source – the Rocky Mountain Trench in British Columbia, Canada. Specifically, the study site was between the Revelstoke Dam (51°02'56" N, 118°11'37" W) and the downstream Arrow Lakes Reservoir. Water flow in this section of the river is controlled by hydropeaking operations.

Methods

Fish Collection Procedures

All fish were collected by boat electroshocker and transferred to a holding tank (diameter = 243 cm, depth = 90 cm and volume = 2839 L) while awaiting either surgery (field release study) or activity tests (holding study). Small coded radio transmitters were used (Model MST-930; 9.5 x 26 mm; 4 g; Lotek Wireless, Newmarket, Ontario) for fish released into the wild (0.7 to 3.0% body mass). For the holding experiment, dummy transmitters were created to mimic the dimensions and weight of the real tags used in the field study. The only difference was that the tag coating for the MST-930 tags was a polyethylene sleeve while the dummy tags were sealed with a synthetic plastic coating (Plastidip International, Blaine, MN, U.S.A.) certified by the manufacturer to be physiologically inert when cured.

Mountain whitefish were anaesthetized in 60 ppm clove oil (emulsified in EtOH at a ratio of 1 part clove oil to 10 parts EtOH; Anderson et al. 1997) bath until reflexes were absent and opercular movements were slow and irregular (i.e., stage 5; Summerfelt and Smith 1990). Fish were then transferred to a v-shaped plastic surgery trough in a supine position. Water was continuously pumped across their gills with a maintenance bath of 30 ppm clove oil. Using a scalpel (number 3 blade, rounded cutting point), a small ~12 mm incision was made in the ventral body surface, posterior to the pelvic girdle, slightly off midline. A 16 gauge needle was then inserted externally in an anterior direction exiting through the incision. A blunt, bladeless scalpel handle was used to protect the viscera and to guide the needle prior to its withdrawal after the antenna was passed through it. The transmitter was then placed gently into the body cavity and positioned at the incision site. Three simple interrupted sutures (Ethicon PDS II absorbable monofilament, 3/0) were used to close the incision. The same intermediately rated surgeon (~120 previous surgeries; Cooke et al. 2003) performed all surgeries. The fish were then placed back into the holding tank (details of holding conditions below) until the fish recovered and exhibited "normal" swimming behavior, at which time they were released back into the river (for field releases) or left in the holding tank (for holding experiment).

Holding Experiment

On 13 October 2010, twelve mountain whitefish (fork length = 226–375 mm; weight = 130–588 g) were collected (water temperature at time of capture was 9–10 °C) and held for 12 hr before their first activity test (details of activity tests below). Following this test, surgeries were performed on six fish. Both tagged and control fish were then held for five days before being tested again. On 20 October 2010, a replicate study was conducted using a new collection of six tagged and six control fish (fork length = 253–315 mm; weight = 169-346 g), which were held and tested using the same methods as the first set. Two replicate studies were conducted to reduce tank density.

Fish were held in the circular tank on the shoreline of the Columbia River with continuous flow-through water pumped from the river at 7570 L hr^{-1}. Tank water was replaced every ~30 min with the intention that it would mimic river water. The water was aerated using a water pump to recirculate tank water into a diffused spray over the water surface. Water temperature in the holding tank ranged from 7.0–9.6 °C.

Mountain whitefish eat both benthic macroinvertebrates and zooplankton in this system (Perrin and Chapman 2010) and therefore had access to zooplankton suspended in fresh intake water throughout the experiment. The tank was covered with a partly-translucent material to dissuade mammalian and avian predators while still allowing partial light.

Upon completion of the experiment (or at the time of death) mountain whitefish were killed and their incision site was evaluated for macroscopic inflammation using a six point scale, which incorporated an evaluation of incision closure and inflammation (see Wagner and Stevens 2000). The general condition of all fish (mortalities and survivors) was assessed visually for the presence of fungus, as well as internal examinations of macroscopic lacerations and punctures to the viscera. Detailed pathological examinations were not conducted.

Activity Tests

One at a time, approximately 12 hr after capture, whitefish were dip netted from the common holding tank and placed into an annular swim flume—a circular polyethylene tank (122 cm in diameter) with black lines dividing the tank into eight equal segments (Nixon and Gruber 1988; Fobert et al. 2009). A video camera (Sony HDD 2000) was secured to a tripod above the swim flume. The number of black lines traversed in a circular pattern was enumerated for 15 min (Champagne et al. 2010). This test allowed fish to swim spontaneously and continuously while exploring their novel environment and served as a proxy for routine activity. Each fish was returned back to the holding tank and the same test was performed again five days later. Immediately after the second exploratory activity test, a separate test was also performed in which the length of time each mountain whitefish could endure being chased by hand in the annular swim flume was recorded (Portz 2007, Fobert et al. 2009). The time of exhaustion was defined as the time at which three deliberate, consecutive tail grabs could be performed on the subject without an active reflex (Kieffer 2000, Portz 2007). Portz (2007) determined that there was a strong positive correlation between swim speeds generated during forced swim trials of chinook salmon in swim flumes (i.e., critical swimming speeds) and those generated using the annular flume chasing protocol used in this study. All activity tests were performed at the same time of the day (between 1400-1700) inside a covered canopy to control for light intensity.

We compared mortality, exploratory activity and time until exhaustion among trials to determine if the results from both trials could be grouped for further analysis. Categorical mortality data (i.e., died or did not die) were evaluated using a logistic regression to determine if the presence of a tag or fish size had an effect on mortality. Due to small sample sizes, activity data were compared using Mann-Whitney U test for time until exhaustion and the Wilcoxon signed-rank test for comparing exploratory activity on the same individuals before and after tagging.

Field Tagging and Tracking

On 9 and 13 October 2009, twelve mountain whitefish (fork length = 251–307 mm; weight = 162–361 g) were collected from up to 7 km downstream of Revelstoke Dam, but were all released at the surgery site, about 2 km downstream from the dam. Water temperature at time of capture was 11–12 °C. These fish were implanted with small MST-930 coded transmitters following the same surgical procedures described above and tracked in the field for three seasons (Fall of 2009, Winter and Spring of 2010). Once released, these fish were free to swim downstream of the river into Arrow Lakes and if this occurred, were subsequently not trackable (radio signals are attenuated by deep water).

For the field study, fish were located each day and night for 14, 11, and 20 days during Fall, Winter, and Spring tracking sessions, respectively. Tracking was conducted from shore using successive gain techniques with a 3-element Yagi antenna and a Lotek SRX600 receiver. Once a fish was located, the operator stood on shore immediately adjacent to a location and recorded a GPS point (Garmin 60CS). Each session lasted approximately 3 hr starting at midnight and noon each day (i.e., twice daily). A map of the study area was divided into 100 m sections and numbered progressively downstream starting at Revelstoke Dam. Waypoints from each tracking session were mapped and the location corresponding to river segment was recorded (e.g., 3700 m from the dam).

Results

Holding Experiment

No mortalities occurred during, immediately after, or within 48 hrs after tagging (Figure 1). Mortalities (11 of 24 total subjects) were noted on days 4 and 5 post-surgery. No significant difference in mortality occurred among trials (Fisher Exact Test, $P = 1.0$). When data from both trials were combined, there was no significant difference in mortality rate between tagged and untagged fish ($\chi^2(1) = 0.17$, $P = 0.68$; Table 1), even when controlling for the size of the fish ($\chi^2(2) = 3.96$, $P = 0.14$; Table 1).

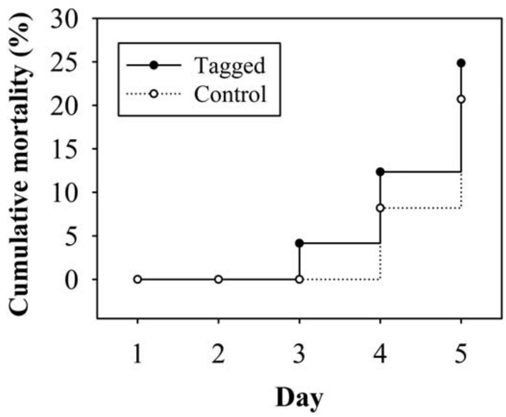

Figure 1. Cumulative mortalities of mountain whitefish during five-day holding experiment following intracoelomic radio transmitter implantation (n = 24). Solid line represents tagged individuals and hatched line represents control fish. No significant difference in mortalities was observed between tagged and control fish.

There was no evidence of macroscopic inflammation at the site of incision or the site of suture entry for any tagged fish, so it was not possible or necessary to use the quantitative incision scoring scale developed by Wagner and Stevens (2000). Incisions were typically closed, but if pulled, the incision could easily be split back open. No fungus was observed on either survivors or mortalities and no lacerations or punctures could be found on any organs.

TABLE 1. Parameter estimates for logistic regression model: Effects of tagging and fish length on mountain whitefish mortality. Step 1 represents the effect of tagging alone and Step 2 represents the effect of tagging when controlling for fish size.

			95% CI for Odds Ratio			
		B(SE)	Lower	Odds ratio	Upper	Sig.
Step 1	Constant	0				
	Treatment	-.336(.822)	0.143	0.714	3.579	P > 0.05
Step 2	Constant	8.705 (5.257)				
	Treatment	-.260 (.889)	0.135	0.771	4.401	P > 0.05
	Size	.032 (0.019)	0.932	0.968	1.006	P > 0.05

Note: $R^2 = 0.152$ (Cox & Snell), 0.203 (Nagelkerke). Model $c^2(1) = 3.956$, $P > 0.05$

To compare exploratory activity before and after tagging, we grouped surviving fish that were tagged (n = 6) and control (n = 7) fish as there was no significant difference in median activity rates between these two groups (U = 13.00, z = 13.00, P = 0.25). Activity was significantly depressed after the five day holding period (Median = 3.9 lines/min) compared to before holding (Median = 6.5 lines/min), T = 11.00, r = -0.55, P = 0.03. Prolonged swimming capacity of tagged individuals (Mdn = 114 min), as determined by the chase test, did not differ significantly from the control group (Mdn = 90 min), 5 days after tagging occurred, U = 21.00, z = 21.00, P = 1.00.

Field Study

In the first few days following release, the majority of the implanted fish (11 of 12) remained in close proximity to their release point and did not move more than 200 m. Some mountain whitefish made small up- and downstream movements (up to 5.9 km downstream and 1.2 km upstream) during the following 14 days of monitoring. One fish was never located after release and another was only located for a few days before disappearing. No tags were found to be static in position, which suggested that tag expulsion or mortality did not occur within the study area. Approximately 120 days after release, six mountain whitefish were located each day and night during the winter session; whitefish made comparatively fewer movements at this time and water temperature was between 2–3 °C. The following Spring of 2010 session, six whitefish were located every day and every night for 20 days (water temperature 5–10°C). Two of these fish had not been located in the study area during the previous Fall or Winter suggesting that they were residing in the downstream reservoir during previous tracking sessions. Two other fish that had been found during both previous seasons were missing eight months later and not found during the final tracking session.

Discussion

We adopted techniques commonly used for the implantation of radio tags in smolts and other fish (as reviewed in Wagner et al. 2011). There were no notable issues with the implantation or the surgical procedure and we did not observe any immediate mortality during surgery or in the 48 hours post-surgery. However, we did observe high levels of mortality in the holding experiment four and five days post surgery. Control fish died at similar rates than implanted ones, suggesting that the mortality observed was a result of a combination of stressors associated with capture, handling and captivity. Despite minimizing handling and providing fish with cover and river water while in captivity, food and environmental conditions experienced by fish in the tanks were certainly different than in the river. All mountain whitefish tested exhibited a diminished performance in the activity test after five days of holding, and this supports the assertion that these fish are sensitive to handling stress, and that pre- and post-surgical holding should be minimized. Stressful holding conditions have been shown to affect locomotor activity (e.g., Jones et al. 1985; Lemly and Smith 1985) and this may have caused the depressed activity observed in mountain whitefish after holding. Indeed, our results are consistent with the growing body of literature that recognizes that pre- and post-surgical care and conditions can be more important than the surgical procedures themselves and must be considered as a part of the integrated care of fish during tagging (Cooke et al. 2011a, Oldenburg et al. 2011).

We also examined several sublethal endpoints of surgical recovery related to wound healing. Although our experiment was relatively short in duration, our experimental subjects experienced no macroscopic inflammation in the five days of observation, which is encouraging considering infection does occur in some tagged fish (e.g., Wagner and Stevens 2000) and that inflammation can become apparent immediately (Wagner et al. 2011). The temperature of the holding water was quite low during the experiment (7.0–9.5 °C), which has been shown by other authors to reduce risk of infection (Jepsen et al. 2002). We also observed no evidence of pressure necrosis or tag expulsion and the relatively smaller fish did not experience significantly more mortality than larger fish, suggesting that tag size relative to body size

(0.7-3% of body weight for our experimental subjects) was not a significant issue in this study.

No mortalities were observed in the mountain whitefish released with transmitters into the wild. Although we were not able to directly observe these fish, they did make small but regular movements up and downstream of their previous locations which suggest that they did not succumb to death following their release.

While holding mountain whitefish in captivity was a challenge, our results suggest that the intracoelomic implantation of telemetry tags via laparotomy is possible in this species. Although the ratio of tag size/body size did not influence mortality, we recommend that the smallest possible transmitters be used for mountain whitefish (or any other species for that matter). Finally, tagged fish should only be held for the minimum amount of time before and after surgery, certainly no longer than 48 hr, using well-oxygenated water and close to the ambient temperature of their capture location.

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