Effects of external and surgically implanted dummy radio transmitters on mortality, swimming performance and physiological status of juvenile masu salmon *Oncorhynchus masou* (Brevoort)

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Short title

Transmitter attachment effects on masu salmon

Abstract

The effects of external and surgically implanted radio transmitters on juvenile masu salmon were examined. External attachment of transmitters significantly decreased the survival of fish and caused a decreased U_{crit} compared with surgical implantation. Although plasma cortisol, plasma glucose, and hematocrit values did not differ significantly among groups, it appears that the most suitable transmitter attachment method for juvenile masu salmon may be surgical implantation.

Keywords : *Oncorhynchus masou*, nano-tag dummy radio transmitter, surgical implantation, survival, critical swimming speed

Biotelemetry has been widely used for studies on migration patterns, behaviour and physiology of fish in their natural environment (Cooke *et al.*, 2004). To accomplish successful telemetry studies, understanding of potential negative effects of transmitter attachment must be understood to ensure that the survival, physiology and behaviour of fish are not affected. In general, transmitter attachment techniques basically include three main categories; external, stomach and surgical implantation (Mellas and Haynes, 1985; Bridger and Booth, 2003). From past studies, implanted transmitters have been found to not significantly affect the survival, growth or swimming performance of juvenile salmonid species (Moore *et al.*, 1990; Adams *et al.*, 1998; Jepsen *et al.*, 2001), suggesting that surgical implantation is best for long term studies. However, these studies also imply that the effects of transmitter attachment method may vary among species and fish size. Jepsen *et al.* (2001) showed that plasma cortisol, glucose and lactate levels of chinook salmon *Oncorhynchus tshawytscha* (Walbaum) smolts surgically implanted with transmitters returned to normal levels a few days post surgery, and concluded that the presence of implanted transmitters were not chronically stressful. However, there are no studies examining the effect of radio transmitters on survival, behaviour and physiology of juvenile masu salmon *Oncorhynchus masou* (Brevoort).

Recently a miniature coded radio transmitter, nano-tag (NTC-3-1) has been

developed at the Lotek Engineering Inc., allowing biotelemetry studies on the behaviour of smaller fish. This transmitter is suitable size (0.8 g in air, 14.5 mm in length, 6.3 mm in width, 4.5 mm in height) for the attachment to smaller fish such as salmon parr and smolts and is expected to aid in studying the behaviour of juvenile masu salmon in their natural environments. The objective of this study was to investigate the effects of external and implanted attachment methods on the survival, swimming performance and physiology of juvenile masu salmon and to determine the most suitable attachment method of radio transmitters for this species.

The experimental animals were 2-year-old masu salmon (11.6-19.6 cm) reared in an outdoor culture tank (1.6 m diameter, 0.5 m water depth) at the Toya Lake Station, Hokkaido University under a natural photoperiod. The culture pond is continuously supplied with spring water (8.7-11.9 °C) with a flow of 27 l min^{-1} and volume set to 1.0 m^3. In order to reduce cost, we used dummy nano-tag radio transmitters (same size and weight as normal tag) with a flexible antenna made of stainless steel wire (0.27 mm in diameter, 30 cm in length) for all tagged fish.

Procedures for the implantation of radio transmitters into fish were similar to those of Moore *et al.* (1990). Briefly, fish were anesthetized using FA100 (eugenol; Tanabe Seiyaku Co. Ltd, Osaka, Japan) at a concentration of 0.5 ml l^{-1} in spring water from

Toya Lake Station. Fork length and weight were measured and fish were placed ventral side up on a surgical table. In order to identify fish, passive integrated transponder (PIT) tags (Destron Fearing Corporation, USA) were inserted in the dorsal muscle of each fish. The control group was marked with PIT tags only. All surgical equipment and the dummy transmitters were cleaned by soaking in 70 % ethanol. The transmitter was inserted through an incision of approximately 10 mm which was made on the middle ventral line anterior to the pelvic girdle. The incision was closed with three stitches, which were tightened enough to closely bring opposing tissue surfaces together along the length of the incision. The antenna of the transmitter was pushed through the fish body wall away from the incision using an injection needle (1.4 mm in diameter, 40 mm in length). A small amount of an aminoglycoside antibiotic (Akiyama Seisakujyo Co. Ltd, Tokyo, Japan) was applied around the closed incision to avoid inflammation. Procedures for surgical implantation took 3.6-5.8 min (average 4.5 min). Externally tagged fish were anesthetized and placed upright on the surgical table. Two hypodermic needles (3mm diameter) were pushed through the dorsal musculature positioned anterior to the dorsal fin to secure the transmitter. A plastic wire was fixed to the transmitter using nylon ties and epoxy resin with small silicon pads, which were attached to minimize abrasion (Herke and Moring, 1999). The wire was passed through

the needles, the needles were removed, and the wire was tied on the opposite side. Procedures for the externally tagged group took 1.0-1.8 min (average 1.3 min).

Survival experiments were conducted from 16 October to 24 December in 2006 (68 days). Fish were randomly selected and divided into four experimental groups; (1) control anesthetized but not tagged, (2) surgically implanted using dummy nano-tag, (3) sham-tagged surgery but no tag and (4) externally tagged using dummy nano-tag. Following the attachment procedures, the fish were transported to the outdoor culture tank described previously, where all groups were held together. The fish were fed commercial trout pellets (Oriental Yeast Co. Ltd, Tokyo, Japan) once daily. The tank was checked daily during feeding and any dead fish were removed, dissected, and any damage to tissue inflammation and tag position. After 68 days, all fish were anesthetized, and measured and weighed.

In order to examine critical swimming speed (U_{crit}) and the physiological effects of transmitter attachment, swim trials were conducted and fish were held for two days with no feeding before they were tagged. After two days recovery from surgery, three fish from each group were introduced into the swim chamber, and acclimated for at least an hour before the experiment under a water velocity 0.5 BL s^{-1}, according to the average length of the fish. Some tests were conducted to include different groups of fish.

Following the hour acclimation, water velocity was increased 0.5 BL s^{-1} every 30 minutes until all of the fish fatigued. Fatigue was defined as when the fish could no longer maintain its position and collapsed against the back screen. During the U_{crit} trial, the fatigued fish were removed so they would not impede the other fish in the swimming section, and the time and the water velocity were recorded. Relative U_{crit}, in body length per second, was calculated using the formula described by (Brett, 1964):

$$U_{crit} = U_p + (T_p\ T_i^{-1}) \times U_i$$

where U_p is the velocity at which the fish last swam for the full period, U_i is the velocity increment (0.5 BL s^{-1}), T_p is the time in minutes that the fish was able to swim against the water velocity which produced fatigue, and T_i is the time between velocity increments (30 min). The U_{crit} trials were conducted at the National Salmon Resources Center, Chitose, Hokkaido. The U_{crit} of all groups were measured using a swim chamber with a volume of 180 l and a swimming section of 111.5×49.5×35 cm (SOC-10, Japan Aqua Tec Co. Ltd, Nagasaki, Japan). The Chitose River water (7.7-7.9°C) entered the swim chamber before each trial. Water flow in the swimming section was generated by a voltage-controlled motor and propeller, where the voltage was calibrated against water velocity. Water velocities at selected motor frequencies were verified using an impeller connected to a pre-calibrated frequency counter. During the trials, the antenna of

dummy transmitters attached to fish did not tangle with one another. The maximum-cross-sectional area of the fish was less than 5 % of the cross-section of the swim chamber, so the solid blocking effect caused by the fish was ignored (Bell and Terhune, 1970; Thorstad *et al.*, 2000).

In order to study the effect of transmitter attachment on physiological states during swimming, fish were allowed to recover for two days. Three fish were then selected from the experimental groups, introduced into the swim chamber, and acclimated for an hour before the experiment under a water velocity of 0.5 BL/s. After the acclimation, water velocity was increased to 2.0 BL/s for 30 minutes. All fish could continue to swim against the encountered flow for 30 minutes. Following the swim trial, fish were rapidly anesthetized and blood samples were collected from the caudal vasculature using 0.55×25 mm heparinized syringes. Plasma glucose and hematocrit were measured on whole blood immediately after sampling. Plasma glucose was measured using blood-sticks (Ascensia, Bayer Corporation, USA) of 2-10 μL blood samples. Hematocrit was read after centrifugation of blood in capillary tubes at 3500 rpm for 5 min. The remaining blood was centrifuged at 3000 rpm for 15 minutes and plasma was stored at -30 °C until plasma cortisol analysis was conducted. The plasma cortisol level was measured in duplicates by time-resolved fluoroimmunoassy (TR-FIA) described by

Yamada *et al.* (1997). Cortisol measurement was acquired in a single assay (N=24) and the intra-assay coefficients of variation were 7.2 %. All statistical analyses were performed using R statistical software (http://www.r-project.org). One-way analysis of variance (ANOVA) was used to compare means of U_{crit}, plasma glucose, cortisol and hematocrit value. Post-hoc multiple comparisons were made with Bonferroni's method. For the survival experiment, survival analysis was used to evaluate the effect of the transmitter attachment. Survival time was calculated as the number of days from the start of the experiment until the death of the fish. Survival distributions were described using the Kaplan-Meier method. The Cox proportional hazard model was used for evaluation of differences between treatment and control groups.

Over the experimental period (68 days), mortality was observed in all groups. However, mortality rates were less than 30 % except in the externally tagged group. Mortality in the externally tagged group was observed from 7 days post surgery and a total mortality of 83% (20 of 24 fish) occurred 68 days post surgery. The Kaplan–Meier survivor curves were plotted for each treatment and control groups and showed a substantially lower probability of survival on externally tagged fish (Fig. 1). Mortality rates were not significantly different between the control and implanted groups (p=0.40), or between the control and sham-tagged groups (p=0.89). However, the mortality rate of

the externally tagged group was significantly higher than the control group over the 68 days (p=0.002). Further, externally tagged fish displayed wounds and inflammation in the vicinity of the attachment wire. Healing of surgical incisions and no infections were observed in any fish from the implanted and sham-tagged groups. Although expulsion of the implanted dummy transmitter through the body wall was observed for three individuals during the experiment, the body wall around the incision had healed well, indicating that the death of fish may not be due to transmitter expulsion. Table I shows the length and weight of each treatment and control group at the time of surgery and 68 days post surgery. Some of the identification tags in all groups were lost during the experimental period, and thus growth rates were compared among treatment and control groups excluding the growth data of these fish. A Kruskal-Wallis test showed that there were no significant differences in growth rate among the implanted, sham-tagged, externally tagged and control groups (p >0.05, df=3). However, tendency of higher U_{crit} for implanted and sham-tagged was observed.

The U_{crit} for the externally tagged fish was significantly lower than the implanted and sham-tagged groups (p=0.022 and p=0.006 respectively), but no significant differences were found among the control, implanted and sham-tagged groups (p>0.05, Table II).

There were no significant differences in either plasma glucose, hematocrit or plasma

cortisol among the treatment and control groups of fish 30 minutes after swimming performance trials (p>0.05, Table III). However, there was a tendency for plasma cortisol levels to be higher in the implanted and sham-tagged groups, which might result from a breach of the peritoneum.

Mortality of fish tagged externally, significantly increased compared with that of the control group, suggesting that the external attachment of transmitters is not a suitable method for long-term biotelemetry studies on juvenile masu salmon. Further, growth rates were not significantly different between the implanted and control groups. Lacroix *et al.* (2004) reported that although surgical implantation of tags into juvenile Atlantic salmon *Salmo salar* L. affected the growth of fish approximately 180 days after tagging, growth rates of surgical implantation was not different from that of control fish 316 days post surgery. Our results suggest that surgical implantation of the transmitters may be preferred for studies tracking juvenile masu salmon over longer time frames of at least 2 months. All dead fish from the externally tagged group displayed wounds and inflammation around the dummy transmitter. Previous studies also have indicated that external transmitters cause severe muscle damage, dorsal scale loss around the transmitter (Mellas and Haynes, 1985) and irregular swimming and rubbing of the external transmitter along tank sides and bottom causing eventual transmitter loss

(Bridger and Booth, 2003). Further the authors also suggest that external transmitters may act as a lure, which attract the attention of other cultured fish at an aquaculture stocking density. This attraction might be one of the reasons for high mortality in externally tagged fish.

The U_{crit} of the externally tagged fish significantly decreased compared with that of the implanted and sham-tagged groups. This result suggests external transmitters increase the effort required for fish to maintain a given swimming speed, an effect that may be caused by increased water drag effects. External tagging has been known to cause significantly lower swimming speed in Atlantic salmon smolts (McCleave and Stred, 1975) and decrease U_{crit} and increase oxygen consumption in Atlantic cod (*Gadus morhua* L.) (Steinhausen *et al.*, 2006). The U_{crit} was not significantly different among the implanted, sham-tagged and control groups two days after surgery. This indicates that surgical implanting does not affect the swimming performance of juvenile masu salmon. Therefore, transmitters used in this study, representing 2.0-3.9 % of the fish's body weight, may be suitable for use on juvenile masu salmon (FL; 14.9 ± 1.6 cm). Adams *et al.* (1998) noted that surgically implanted radio transmitters representing 2.2-5.6 % of the fish's body weight did not affect the U_{crit} of juvenile chinook salmon >12 cm. The U_{crit} values between masu salmon (3.38 ± 1.21 BL/s) and chinook salmon

(3.82 ± 0.79 BL/s) were similar, suggesting that the surgical implantation is most applicable for other juvenile salmonid species in biotelemetry studies. Surgically implanting transmitters has been found to have no significant effects on the swimming ability of Atlantic salmon within 7 to 14 days after tagging (Moore *et al.*, 1990) and in adult rainbow trout *Oncorhynchus mykiss* (Walbaum) 7 days after tagging (Mellas and Haynes, 1985). However, Adams *et al.* (1998) reported that the swimming performance of juvenile chinook salmon was affected by surgically implanted transmitters a day after surgery but not after 21 days. Further, Jepsen *et al.* (2001) showed that physiological states (which are related to stress) of chinook salmon smolts returned to control levels a few days post surgery.

The 30 minutes of endurance swimming did not significantly affect plasma cortisol, glucose and hematocrit reading of fish two days after surgery. Similarly, Thorstad *et al.* (2000) showed that externally and surgically implanted transmitters did not affect the plasma glucose and hematocrit value of adult Atlantic salmon after an endurance swim trial. Therefore, although transmitter attachment may indeed be stressful for juvenile masu salmon, physiological effects of surgical transmitter implantation appear to be relatively low. These results imply that U_{crit} increased and physiological stress indicators decreased as the period of recovery increased, and two days may be all that is required

for surgical recovery. However, there was a tendency for higer U_{crit} values and higher plasma cortisol concentration for implant and sham-tagged groups. Jepsen *et al.* (2001) found surgical tagging increased plasma cortisol levels 24 h after tagging in chinook salmon smolts. Milligan *et al.*(2000) found that elevation of plasma cortisol levels in response to stress prolonged the recovery period from exhaustive exercise in rainbow trout and the stimulus for cortisol release resulted from not the exercise but post-exercise inactivity. Wang *et al.* (1994) indicated that post-exercise inactivity leads to long recovery times. Thus, we speculated that cortisol levels were higher in response to surgery and the fish increase their swimming speed (U_{crit}) to prevent the long recovery period.

In this study, we found that surgical implantation of the dummy radio transmitters did not affect the survival, U_{crit} and physiological response of juvenile masu salmon. In conclusion, the surgical implantation method is suitable for juvenile masu salmon and the nano-tag radio transmitter could be applicable for use in biotelemetry studies on this species.

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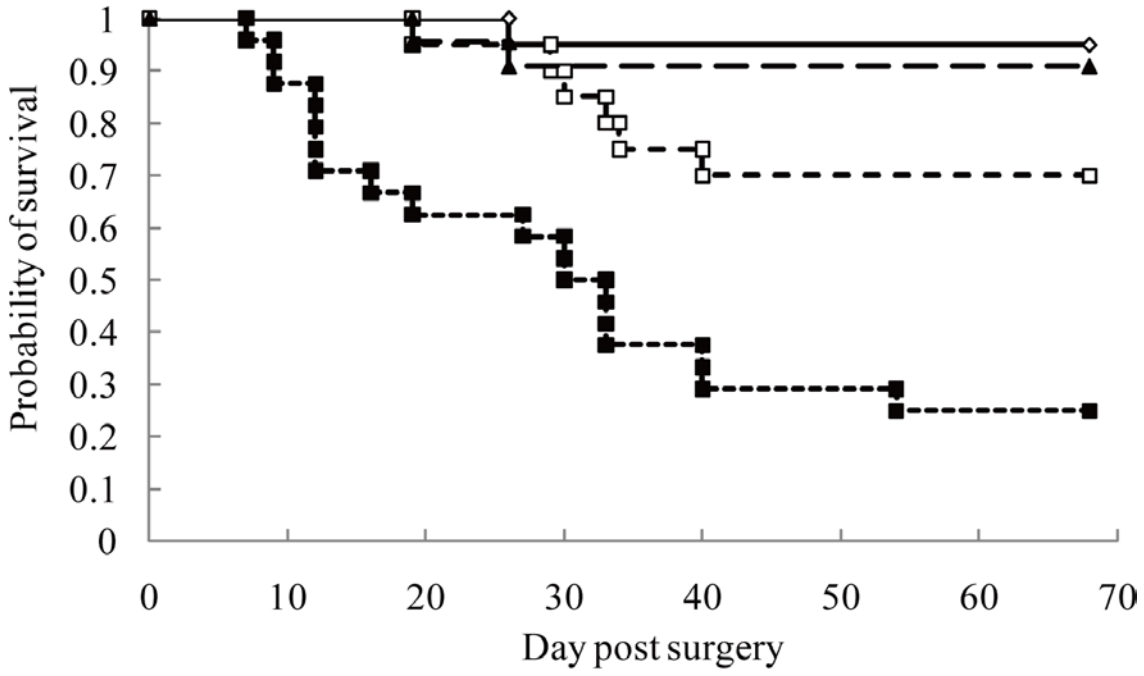

Figure legends

Fig 1. Kaplan–Meier survivor curves for juvenile masu salmon of treatment and control groups. Symbol shapes denote each group: control (◇), externally tagged (◆), implanted (□), and sham-tagged (▲) group.

Table 1. Mean ± standard error (S.E.) of weight and fork length of juvenile masu salmon immediately and 68 days post surgery

Group		days			
		0	*N*	68	*N*
		Mean±S.E.		Mean±S.E.	
Control	fork length (cm)	13.8 ± 0.19	20	14.3 ± 0.12	19 (17)
	weight (g)	27.2 ± 1.05		30.8 ± 1.02	
Implanted	fork length (cm)	14.2 ± 0.27	20	15.4 ± 0.55	14 (12)
	weight (g)	30.7 ± 1.81		35.9 ± 3.00	
Sham-tagged	fork length (cm)	13.8 ± 0.34	22	14.9 ± 0.26	20 (17)
	weight (g)	28.4 ± 2.54		32.3 ± 1.41	
Externally tagged	fork length (cm)	14.3 ± 0.27	24	14.4 ± 0.95	4
	weight (g)	31.6 ± 1.90		36.4 ± 7.25	

Table 2. Mean ± standard error of mean (S.E.) of critical swimming speed (U_{crit}, BL/s) weight and fork length of juvenile masu salmon. Means followed by different letters are significantly different ($P < 0.05$).

Group		Mean±S.E.	*N*	U_{crit} (BL/s)
Control	fork length (cm)	14.1 ± 0.48	6	2.75 ± 0.24 ab
	weight (g)	25.7 ± 3.22		
Implanted	fork length (cm)	14.9 ± 0.60	7	3.38 ± 0.46 a
	weight (g)	30.7 ± 2.99		
Sham-tagged	fork length (cm)	14.8 ± 0.49	6	3.71 ± 0.46 a
	weight (g)	30.3 ± 5.10		
Externally tagged	fork length (cm)	15.0 ± 0.60	7	1.78 ± 0.22 b
	weight (g)	37.6 ± 3.55		

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Table 3. Mean ± standard error of mean (S.E.) of physiological parameters (plasma cortisol, glucose and hematocrit) weight and fork length of juvenile masu salmon

Group		Mean±S.E.	Plasma cortisol (ng/ml)		Plasma glucose (mg/dl)		Hematocrit (%)	
				N		*N*		*N*
Control	fork length (cm)	14.7 ± 0.24	179.5±13.59	6	116.3±17.23	6	41.3±1.84	6
	weight (g)	28.9 ± 0.94						
Implanted	fork length (cm)	15.4 ± 0.58	305.7±119.74	5	149.7±25.47	5	39.0±1.55	5
	weight (g)	36.2 ± 5.46						
Sham-tagged	fork length (cm)	15.1 ± 0.46	269.1±76.06	8	184.3±25.19	8	37.6±2.94	8
	weight (g)	32.9 ± 2.86						
Externally tagged	fork length (cm)	15.8 ± 0.80	189.1±70.79	5	136.0±12.17	6	42.4±1.06	6
	weight (g)	39.6 ±5.99						

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