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Short term effects of relative tag size and surgical implantation on feeding behaviour, survival rate, plasma lactate and growth rate in juvenile to adult rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Surgically implanted tag attachments provide biotelemetric and biologging data that can be used to investigate the behaviour and physiology of fish in their natural environments. However, to ensure the validity of the obtained data, it is essential to understand the effects of tagging on the behaviour and physiology of the fish. Consequently, a number of studies have examined the effects of surgical implantation of a tag. These studies indicate that the tag should weigh less than 2% of the fish body mass (the so-called "2% rule"). However, there is little information on whether the 2% rule is appropriate for fish in the same species but of different sizes or developmental stages (e.g. juvenile or adult fish). This study investigated the question of whether the ratio of the tag to fish body mass (termed tag ratio here) in the rainbow trout (*Oncorhynchus mykiss*) affected feeding behaviour, survival rate, plasma lactate levels and growth rates. Tag ratios over 3% were found to significantly impair the feeding behaviour at both day 1 and day 8 post-surgery. Survival rate was negatively correlated with tag ratio; there was no effect of fish body mass on survival rate. Our analyses showed that a 5.6% tag ratio was expected to have a 90% survival rate at day 8. Plasma lactate levels and growth rates on day 8 were not affected by the tag ratio or surgical implantation. We conclude from our analyses that the 2% rule may be conservative and is likely acceptable for juvenile stages as well as adult rainbow trout.

1. Introduction

Recent developments and improvements in biotelemetry and biologging technologies have produced a greater understanding of the behaviour and physiology of fish in both their natural environments and under controlled conditions (Cooke et al., 2011, 2013). Many previous studies have employed these technologies in fish with a broad range of body sizes: in salmonids, this approach has been used to track migration of juvenile fish and smolts (e.g. Aarestrup et al., 2002; Welch et al., 2009) and to measure environmental and biotic parameters during the upstream migration of adult fish (e.g. Makiguchi et al., 2011; Miyoshi et al., 2014; Tanaka et al., 2001). Successful completion of this type of experiment is dependent on the tag that is used to provide telemetry: the size of the tag is determined by the required duration of battery life and sensor type of the transmitter; the tag size is also an important factor because of its potential influence on fish behaviour and physiology. Tag size is generally expressed as the ratio of tag weight in air to fish body mass (Cooke et al., 2011). Most fisheries research using biotelemetry and biologging follow a guideline that the tag should be less than 2% of the fish body mass in air ("the 2% rule"), which was proposed by Winter (1983). The 2% rule is widely accepted and used on an empirical basis in many studies. However, Cooke et al. (2011) noted that the 2% rule is somewhat arbitrary as it just one of the means by which to assess tag size since there are no standard scientific criteria that can be applied to different fish species and to fish different body sizes. Indeed, there are some reports that the effects of tagging might vary among fish of different species and sizes (Adams et al., 1998b; Jepsen et al., 2001). As the behaviour and physiology of fish can be potentially impacted by the attachment, it is necessary to understand the negative effects to reduce these to a minimal level in order to produce reliable data in biotelemetry and biologging studies.

Three methods are available for attaching transmitters or dataloggers to fish: external attachment, gastric insertion, and surgical implantation into the body cavity (Bridger and Booth 2003; Deng et al., 2012). In addition, an injection method instead of regular surgery has also been designed (Deng et al., 2015; Liss et al., 2016).

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Surgical implantation is the most commonly used method and has been employed in various fish species including salmonids (Cooke et al., 2011). In salmonids, surgical implantation of an attachment and the influence of the ratio of tag size to fish body mass (hereafter termed "tag ratio") have been investigated for swimming performance (Smircich and Kelly, 2014), feeding behaviour (Adams et al., 1998b), physiology (Makiguchi and Ueda, 2009), and survival (Brown et al., 2010). For example, Brown et al. (1999) reported that a tag ratio up to 12% did not affect swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) with a body mass in the range of 5–10 g. Although there are many reports on the effects of surgical implantation in salmonids, most of these focus on relatively small or juvenile fish and information on the effects of tag ratio were not obtained. Two exceptions are the studies of Martin et al. (1995) and Ivasauskas et al. (2012), but even here the maximum fish body mass was 844 g. In addition, there has been little attempt to systematically investigate the effects of different tag ratios across the body size range of a single species. To remedy this lack of information, we initiated this study to answer the question of whether the 2% rule is applicable in cultured rainbow trout across a developmental range from juvenile to adult fish. To this end, we obtained data on the effect of (1) different tag ratios (0.7–7.07%), (2) fish body mass variation (80–2600 g), and (3) surgical implantation of an attachment on feeding behaviour, survival rate, plasma lactate levels, and growth rate.

2. Materials and methods

2.1. Animals

Cultured rainbow trout that were kept under a natural photoperiod in the Fuji Trout Hatchery Shizuoka Prefectural Fisheries Experimental Station, Shizuoka Prefecture, Japan were used for this study. They were fed daily with commercial trout food pellets; the fish were starved for 48 h before surgery. The water temperature in the Fuji Trout Hatchery is maintained at 9 °C throughout the year. We conducted the experiment from June to October 2012.

2.2. Tagging protocol

To examine the effect of relative tag size, fish body mass and surgical implantation on feeding, survival rate, plasma lactate levels and growth rate, we selected three groups of experimental fish: group 1 (small fish) included 100 fish with a mean body mass of 172 ± 45 g (SD); group 2 (middle size fish) included 90 fish with a mean body mass of 743 ± 193 g; group 3 (large fish) included 40 fish with a mean body mass of 1850 ± 351 g. Each of these three groups was divided into three subgroups: surgically implanted group, sham-control (surgical procedure without tag insertion) group, and control group (subjected only to anaesthesia). In the surgically implanted group, three types of dummy tag were used with small and middle size fish, and two types of dummy tag were used for the large group. The eight types of dummy transmitter were composed of epoxy resin and a steel ball, and were covered with parafilm (Bemis Flexible Packaging, Neenah, Wisconsin) with a flexible antenna made of stainless steel wire (0.27 mm in diameter and 300 mm in length; Makiguchi and Ueda, 2009; Yasuda et al., 2015). In small and middle fish, the three dummy tags were used respectively and in large fish, the two dummy tags were used. The characteristics of the dummy tags are shown in Table 1.

The dummy tags were surgically implanted into fish using a similar method to that described by Makiguchi and Ueda (2009). Fish were anaesthetized using 0·5 ml l^{-1} 2-phenoxyethanol (ethylene glycol monophenylether; Wako Pure Chemical Industries, Ltd, Osaka) in spring water from the Fuji Trout Hatchery. The mass (g) and fork length (nearest mm) of the fish were measured under anaesthesia including the control and sham control group. Fish were placed ventral side up on the surgical pad and an incision of c. 10–20 mm was made from the middle ventral line anterior to the pelvic girdle, and the dummy tags were inserted into the peritoneal cavity. The incision was closed with two independent stitches, which were tightened enough to bring opposing tissue surfaces close together along the length of the incision. The antenna of the tag was pushed through the fish body wall away from the incision using an injection needle (1.4 mm in diameter and 40 mm in length). Polyethylene streamer tags (PST2S; Hallprint Pty, South Australia) were also inserted in the dorsal muscle of each fish to identify the fish from the tag colour. The surgical procedures took 62–205 s (mean time of 102.1 ± 28.8 s) for small fish, 75–187 s (128.2 ± 31.1 s) for middle size fish and 80–303 s (192.0 ± 69.6 s) for large fish. We conducted the surgery but did not insert the dummy for sham tagged fish (20 small fish, 10 middle size fish and 10 large fish). Mass and fork length were measured in anaesthetized control fish (20 small fish, 20 middle size fish and 10 large fish). The sham control procedures took 99–203 s (127.4 ± 21.8 s) for small fish, 35–78 s (52.2 ± 11.8 s) for middle size fish and 80–168 s (103.7 ± 25.7 s) for large fish. To standardize the effect of surgery per se, a single experienced surgeon conducted all surgery. Although only one surgeon carried out the operations, there was a relatively large variation in the time taken for the procedure. To reduce the possible effects of this variation on the analysis, we included study period (June, July, August, and October 2012) as a random factor in the model (see "2.6. *Data analysis*" for details).

Before the experiment began, the fish were allowed to recover from the surgery for 24 h in a mesh cage (H 2.0 × W 2.0 × D 1.0 m) in an outside water tank. After 24 h, the mesh cage was transferred to the experimental tank (H 8.8 × W 7.5 × D 1.0 m) and kept under natural daylight/day length conditions. Each mesh cage contained 20 small fish, or 10 middle size fish, or 5 large fish.

2.3. Feeding behaviour

To examine the effect of the relative size of the tag, fish body mass and surgical implantation on feeding behaviour, we monitored the fish using visual observation by three observers through a polarizing lens on day 1 and day 8 post-surgery. Feeding behaviour was monitored from a distance of 5 m in order not to disturb the fish. One commercial pellet (5.5 mm in diameter, 0.05 g for small and middle size fish and 7.0 mm in diameter, 0.16 g for large fish) was thrown at a time into the experimental tank and the individual that ingested the pellet was identified by the colour of the polyethylene streamer tag and recorded. The experiment was performed for 20 min on days 1 and 8 between 10 and 12 am. We calculated the feeding weight ratio from fish body mass on days 1 and 8 post-surgery. During the interval between feeding observation on day 1 and the start of feeding observation on day 8, we gave the fish commercial pellets once per day and did not record feeding behaviour during this period.

2.4. Survival

The effects of tag size, body mass and surgery on the survival rate were analysed by checking the experimental tanks each morning and removing any dead fish immediately between the end of feeding observation on day 1 and before the start of feeding observation on day 8. Experimental period in the present study is relatively short compared to the previous studies on salmonids species (e.g. Robertson et al., 2003).

However, short term effects of tagging is important to