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Electronic Tag Retention and Survival of Giant Salamanders after Surgical Implantation

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Abstract: Expansion of the habitat occupied by the hybrid giant salamander (Japanese×Chinese giant salamander, *Andrias* spp.) in the Kamo River of Kyoto Prefecture, Japan, has been a serious problem for the conservation of the Japanese giant salamander, *A. japonicus*. Thus, the movement and behavior of hybrid giant salamanders must be determined to quantify their ecological impact. Biotelemetry facilitates the long-term tracking of this species in rivers if transmitters are surgically implanted successfully. However, to use this approach successfully, it is important to understand the effects of surgical implantation and the healing process after surgery on animals. Nine hybrid salamanders were surgically implanted with dummy transmitters, while three individuals were incised without implanting any transmitters as the control treatment. Three months of observation after surgery showed that all individuals survived and retained transmitters. No hernia was observed in implanted individuals, and surgical incisions completely healed in one to two months. Body weight increased in all individuals, with no significant difference being observed in the weight gain and growth rate of implanted versus control individuals. The present study demonstrated the successful retention of implanted dummy transmitters in giant salamanders for three months without severe effects, suggesting the utility of biotelemetry for monitoring individuals in the wild.

Key words: *Andrias* spp.; Biotelemetry; Growth; Surgery; Transmitter

Introduction

Giant salamanders (*Andrias* spp.) are the world largest amphibian species living in Japan and China, and play an important role as predators in river ecosystems (Okada et al., 2008). The Japanese giant salamander, *A. japonicus*, is distributed in rivers in the west-

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ern parts of Honshu, Shikoku, and Kyushu islands in Japan (Kobara, 1985; Browne et al., 2014). The species is protected as a Special Natural Monument of Japan because of its scientific significance. However, the habitats used by this species are being destroyed, leading to the continued decline in its population size (Tochimoto, 2005a). As a result, the Japanese giant salamander is listed as Near Threatened in the International Union for Conservation of Nature (IUCN) Red List (Kaneko and Matsui, 2004). After the Chinese giant salamander *A. davidianus* was imported to Japan in the 1970s, the two species have hybridized, complicating efforts to conserve the Japanese giant salamander. Hybrid salamanders are frequently observed, mainly in the Kamo River in Kyoto Prefecture, possibly further contributing to the reduction in habitat availability and population size of native Japanese giant salamanders (Matsui et al., 2014; Nishikawa et al., 2016). Although Japanese giant salamanders might migrate upstream during the breeding season (Tochimoto, 2005b; Taguchi, 2009), detailed information on the movement and behavior, including reproductive migration, of native versus hybrid giant salamanders have not been reported. It is difficult to obtain this information because giant salamanders are active at night (nocturnal), moving underwater and using rocks and other structures as refuges (Okada et al., 2008). Yet, it is important to study the movement and behavior of native Japanese giant salamanders and the hybrids in the river to understand how each use the river environment to identify potential areas of conflicting use and to ensure that appropriate habitat is conserved for Japanese giant salamanders.

Bio-logging and biotelemetry using electronic tags (i.e., radio transmitters, ultrasonic transmitters, and data storage tags) provide effective techniques for tracking aquatic animals (Naito, 2004; Hussey et al., 2015), and could be used for giant salamanders. However, these techniques require the external attachment or implantation of electronic tags to track the movement and behavior of released animals. While external attachment is relatively easy and provides less initial stress to animals (Jepsen et al., 2015), surgical implantation is preferable for long-term tracking as it reduces drag forces (thus, minimizing energetic cost to animals), reduces the likelihood of tag loss, and reduces environmental entanglement (Jepsen et al., 2002; Cooke et al., 2011). Surgical implantation of transmitters in the abdominal cavity is widely used in fishes (Jepsen et al., 2002; Cooke et al., 2011) and amphibians (Madison et al., 2010). An electronic tag weighing less than 2% of the body weight of a target animal is generally accepted in studies of fishes. It is also recommended that tag retention and the effects of surgical implantation should be evaluated prior to deployment (Cooke et al., 2011). The effects of electronic tags on survival, growth rate, swimming, and feeding behavior have been reported to vary according to the species and the size of target animals (e.g., Lucas, 1989; Makiguchi and Ueda, 2009; Yasuda et al., 2015; Makiguchi and Kojima, 2017).

It is particularly important to evaluate the effects of surgical implantation on amphibians, as such information remains limited (Madison, 1997; Goldberg et al., 2002), with electronic tags of different weights being used (e.g., ≤5% of the body weight [Goldberg et al., 2002; Bodinof et al., 2012a, b]; ≤10% of the body weight of most individuals [Jehle and Arntzen, 2000]). With respect to giant salamanders, radio transmitters (≤5% of body weight) have been implanted in the abdominal cavities of the Chinese giant salamander and hellbender *Cryptobranchus alleganiensis*, the other genus of Cryptobranchidae. Hernias from the surgical incision or dehiscence of the incision was observed in some individuals (Bodinof et al., 2012a, b; Marcec et al., 2016). Transmitters might fall out through surgical incisions or abraded wounds, body walls, and intestines (Summerfelt and Mosier, 1984; Marty and Summerfelt, 1986; Baras and Westerloppe, 1999; Meyer and Honebrink, 2005) as observed in fishes.

To evaluate the potential success of tracking giant salamanders over long periods through bio-logging and biotelemetry, we examined: (1) the retention of electronic tags and their effect on survival, growth, and feeding, and (2) the healing process after surgical implantations. Hybrid giant salamanders were monitored for three months after the surgical implantation of dummy transmitters in their abdominal cavities. Healing was evaluated through the visual examination of the suppuration of surgical incisions and infection with aquatic fungi, as well as by examining damage to subcutaneous tissues and internal organs through dissection at the end of the study. Dummy transmitters have been widely used to assess the retention rate of transmitters and healing process following surgery in fishes (Lucas, 1989; Meyer and Honebrink, 2005; Makiguchi and Ueda, 2009; Fabrizio and Pessutti, 2007; Hall et al., 2009; Makiguchi and Kojima, 2017).

Materials and Methods

Study Animals and Surgery Procedure

Twelve hybrid giant salamanders were captured in the Kamo River as study animals (average±standard deviation of total length: 76.9±7.8 cm; body weight: 2708.3±875.8 g before surgery). The present study was conducted under permits issued by the Japan Agency of Cultural Affairs to K. Nishikawa in 2011–2017 for research in Kyoto City (No. 420) and 2015–2018 for research in Kyoto Prefecture (No. 710). Salamanders were confirmed as hybrids by genetic identification using microsatellite markers developed by Yoshikawa et al. (2011, 2012). The dummy transmitters were similar in size shape and weight to V9 pingers (φ9 mm×45 mm, 5–6 g in air; Vemco Inc., Canada) that can be used for ultrasonic telemetry >1.5 years (https://vemco.com/products/v7-to-v16-69khz/). The dummy transmitters were made from vinyl chloride cylinders in which epoxy resin-encapsulated lead weights were included to make them weigh the same as V9 pingers. Dummy transmitters were then covered with beeswax. The weight of a dummy transmitter was 0.1–0.3% of an individual.

Before surgical implantation, each captured hybrid giant salamander was housed for 1–72 days in a plastic tank in the laboratory or in a container in the Kyoto Aquarium, Kyoto Prefecture in the same manner as during the subsequent observation period. After anaesthetizing the study animals with MS-222 (1 g/1 L) for 30–40 minutes, incisions were made approximately 1 cm toward the inguinal part from the center on the right side of the abdomen. Nine animals were surgically implanted with the dummy transmitters disinfected with ethanol (Implantation group; total length: 76.8±8.2 cm; body weight: 2555.0±888.6 g) and sutured, whereas three individuals were incised and sutured without implanting any transmitters (Control group; total length: 77.2±7.9 cm; body weight: 3168.3±797.9 g). Surgical incisions were sutured with 3–5 stitches using ELP silk suture thread and needle (cutting edge, 1/2 circle, 37 mm; Akiyama-seisakusho Co., Ltd., Tokyo). The seam spacing was about 2 mm.

Evaluation

After surgery and post-anesthetic recovery, the study animals were kept for an observation period of 90–102 days at the Kyoto Aquarium. They were housed in separate containers (100.2 cm×62.0 cm×30.0 cm; water depth 15.0 cm; usually under dark conditions), and were fed twice a week on thawed frozen freshwater minnows of the subfamily Leuciscinae (22.0–94.0 g). If food was not consumed immediately, it was left until the next day. The water in the containers was filtered with silica sand and wool and allowed to circulate and flow constantly (38 L/min at a maximum). Water temperature and pH were kept at 17.0–18.9°C and 5.0–8.0, respectively. The survival of individuals, retention of dummy transmitters, hernia, infection of aquatic fungi, and suppuration of surgical incision were visually observed every day by gently lifting and twisting the posterior part of body without taking individuals out of the water. Refusal of food (i.e., existence of left-over food until the next day of feeding) was recorded for each feeding event. In addition, individuals were taken out of the water once a month and their incisions were photographed to evaluate the healing process. At the end of the observation period, all individuals were weighed with no stomach contents, and the weight gain (i.e., the difference in weight before surgery and at the end of the observation period) was compared between the implantation and control groups using a Student's t-test. Daily growth rate was calculated as: Growth rate=weight gain/(body weight before surgery×days after the surgery) ×100.

Differences in growth rate were tested by analysis of covariance (ANCOVA), which included the treatment and body weight before surgery and the interaction of the two as explanatory variables, through linear modeling in R v3.2.4 (R Core Team, 2016). In addition, all individuals were euthanized using 2-phenoxyethanol or Nembutal and dissected to investigate any damage to internal organs and the final position of dummy transmitters, and to sex the animals by directly observing the sexual organs. The euthanasia of hybrid individuals was required for the conservation of Japanese giant salamanders by removing genetic contamination from the river. We preserved the euthanized hybrids as specimens in Graduate School of Human and Environmental Studies, Kyoto University for further studies on their morphology.

Results

Retention and Effects of Implanted Dummy Transmitters on Growth and Feeding

During the observation period, all individuals survived and no salamander expelled the dummy transmitters. All individuals with and without implanted dummy transmitters successfully fed at more than 65% of the feeding events (Table 1). As a result, all individuals showed an increase in body weight during the observation period (Table 1). The average±SD weight gain of the individuals of the implanted and control groups was 617.9±252.0 and 435.7±376.3 g, respectively.

Table 1. Summary of study animals, growth, and feeding events.

Treatment	ID	Experimental period (Observation period at Aquarium)	Total length (cm)	Body weight (g)		Weight gain (g)	No. of feeding/ events (percentage)	Sex
				Before surgery	At euthanasia			
Implantation	ID1	107 (102) days	86.1	3855	4644	789	28/29 (96.6)	Male
	ID2	107 (102) days	72.7	2055	2892	837	29/29 (100.0)	Male
	ID3	107 (102) days	77.0	2635	3618	983	29/29 (100.0)	Female
	ID4	102 (91) days	87.8	3815	4000	185	21/26 (80.8)	Male
	ID5	102 (91) days	81.3	3085	3422	337	26/26 (100.0)	Female
	ID6	102 (91) days	70.9	1665	2224	559	26/26 (100.0)	Female
	ID7	102 (91) days	69.5	1745	2278	533	26/26 (100.0)	Female
	ID8	102 (91) days	82.0	2610	3192	582	25/26 (96.2)	Male
	ID9	102 (91) days	63.5	1530	2286	756	26/26 (100.0)	Female
Control	ID10	109 (90) days	83.0	3200	3500	300	23/26 (88.5)	Female
	ID11	109 (90) days	80.5	3950	4096	146	17/26 (65.4)	Male
	ID12	109 (90) days	68.2	2355	3216	861	25/26 (96.2)	Female

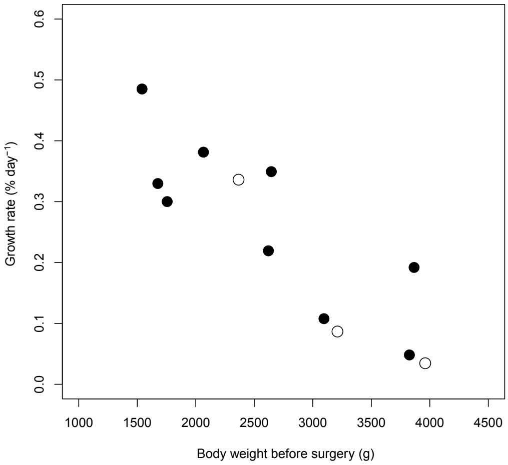

FIG. 1. Relationship between growth rate and body weight before surgery. Implantation and control groups are shown by filled and hollow circles, respectively.

Weight gain was not significantly different between groups (Student's *t*-test, $p>0.05$). Refusal of food tended to occur in larger individuals (Table 1), and the growth rate decreased significantly with increasing body weight ($F=7.00$, df=1, 8, $p<0.05$), without significant effects of treatment (main effect of treatment: $F=0.41$, df=1, 8, $p=0.54$; interaction: $F=0.61$, df=1, 8, $p=0.46$) (Fig. 1).

Healing after Surgery

No hernias, suppuration of surgical incision, and aquatic fungi infection were observed in the implantation and control groups. In some individuals, the suture thread fell away from the surgical incision as healing progressed. Monthly external observation showed that the surgical incisions mostly closed in one to two months, whether the dummy transmitters were implanted or not (Figs. 2 and 3a). Dissection at the end of the three-month observation period confirmed that the surgical incisions in the subcutaneous tissue were closed (Fig. 3b), and no damage or bleeding was observed in the internal organs of the implantation group.

Location of Dummy Transmitter in the Abdominal Cavity

At the end of the observation period, the dummy transmitters were located between the intestinal membranes (ID1 and ID6; Fig. 4a), between the stomach and lung (ID2; Fig. 4b), behind or next to the intestinal tract or stomach (ID3, ID5, ID8, and ID9; Fig. 4c), between the stomach and intestine (ID4; Fig. 4d), or between the intestine and pancreas (ID7; Fig. 4e). Digested materials were observed in the stomach and rectum, but no dummy transmitters were found inside the digestive tracts, recta, or body walls.

DISCUSSION

The survival of all individuals for three months after the surgery confirmed that the procedure (anesthesia and surgery) used in this study did not have any fatal effects on the hybrid giant salamanders. Although Bodinof et al. (2012a, b) and Marcec et al. (2016) sometimes observed hernias or dehiscence of incisions in Ozark hellbenders and Chinese giant salamanders, we did not. Hernias or dehiscence of incisions might have been avoided because of the smaller relative weight of dummy transmitters to animal body weight (0.1–0.3%) in this study compared to previous studies (1–5% maximum; Bodinof et al., 2012a, b; Marcec et al., 2016).

Dummy transmitters were neither expelled from the surgical incisions nor taken into digestive tracts or body walls of the giant salamanders. Although expulsion from the digestive tract or body wall has been reported in fish in experimental ponds or tanks (Summerfelt and Mosier, 1984; Baras and Westerloppe, 1999; Penne et al., 2007), these expulsion pathways were not used in giant salamanders, at least not for the first three months. The results suggest that expulsion from the surgical incisions or abraded wounds is a main expulsion pathway. Thus, it is important to promote the healing of incisions after surgery to reduce the effects of surgical implantation and to confirm the retention of

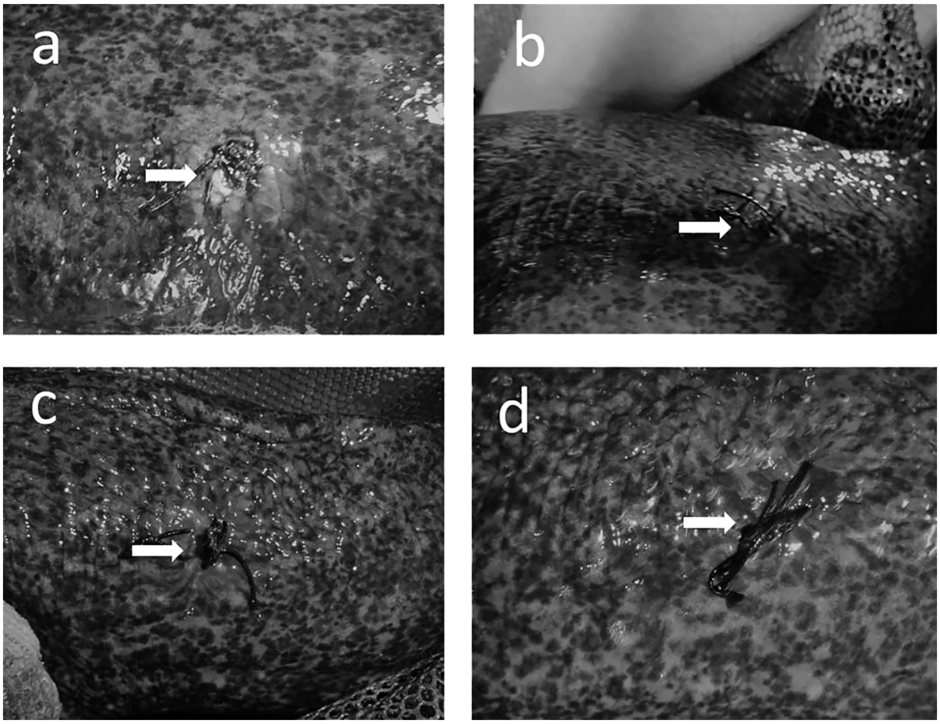

Fig. 2. The healing process of surgical incision of ID4 that is indicated by arrows. (a) 10 days, (b) 22 days, (c) 60 days, and (d) 94 days after surgery.

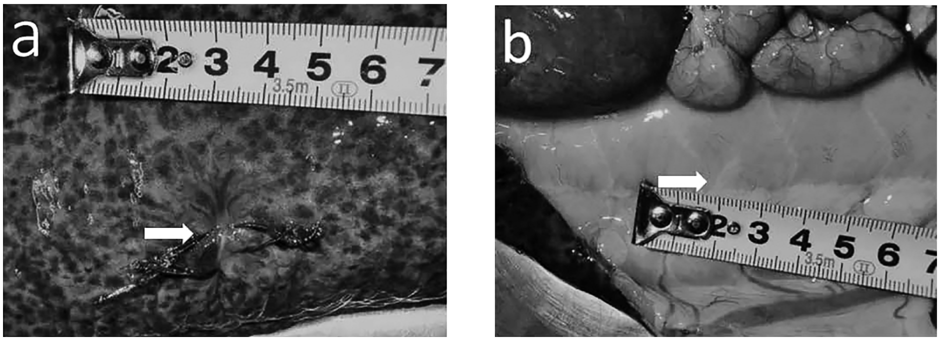

Fig. 3. Surgical incision, indicated by arrows, that was observed from the outside (a) and inside (b) when ID4 was dissected 102 days after surgery. The surgical incision was closed inside, and ID4 had a scar inside indicated by an arrow.

electronic tags.

This study detected no significant effect of the implantation of dummy transmitters on the growth of giant salamanders. A decrease in growth rate in relation to body weight was indicated in both the implantation and control groups, that is a phenomenon widely reported from many animals (e.g., von Bertalanffy, 1957). The refusal of food was recorded for several individuals from both treatment groups; however, an increase in the body weight of all individuals indicated that

FIG. 4. Internal organs at the end of observation period and location of dummy transmitter (φ9 mm×45 mm) of (a) ID1, (b) ID2, (c) ID8, (d) ID4, and (e) ID7. The left side of panels indicates the anterior part of a giant salamander except panel (b) where the upper side indicates the anterior part. Abbreviations are as follows: in: intestine; li: liver; lu: lung; pa: pancreas; st: stomach.

the refusal of food is normal and did not affect growth. Carnivorous ectotherms generally have low food requirements, and that might result in the refusal of food (Barboza and Hume, 2006). In fact, the refusal of food is often observed in captive giant salamanders without any surgical incisions (S. Seki, unpublished).

The present study confirmed the successful retention of implanted dummy transmitters in giant salamanders for three months, with no severe effects on their survival, growth, or feeding behavior. To prevent the expulsion of electronic tags from a surgical incision or an abraded wound and to confirm the retention of electronic tags in giant salamanders, the closure of surgical incisions after surgery is important. Therefore, to use bio-logging and biotelemetry techniques successfully on giant salamanders, it is important to promote healing after surgery. Under the captive conditions of this study, where individuals could be housed in separate spaces, with enough food, and filtered water, the closure of surgical incisions of giant salamanders occurred within one to two months after surgery. Keeping and feeding animals under captive conditions before release, or capturing and releasing them to the sites where there is sufficient space to live and sufficient food, may be important for the successful application of biotelemetry devices. Closure of the inner skin can be a criterion for releasing the individuals. Alternatively, tight suturing of an incision may be important for avoiding dehiscence, particularly when prolonged captivity is impractical before release. It should be noted that prolonged captivity of wild individuals might induce stress that compromises healing (Martin, 2009; Archie, 2013) and possibly limit chances for their seasonal behaviors (e.g., breeding activity). Thus, long-term captivity after surgery is not always the best procedure, and avoiding surgery and captivity during the breeding season should be considered. In addition, because giant salamanders can live for more than 60 years (Browne et al., 2014), the effect of possible long-term retention of transmitters should be further investigated and efforts to miniaturization of transmitters should be continued. The results of this study could be applicable to studies using similar-sized radio transmitters and data storage tags.

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