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Surgical Implantation of Acoustic Transmitters in *Neolissochilus soroides* and *Channa lucius* and Post-Surgical Wound Observation to Study Fish Telemetry

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ABSTRACT

Telemetry is an emerging method to study fish ecology and a reliable tool that provides useful information for managing and conserving aquatic fauna and river habitats. However, the reliability of telemetry depends on several technical factors. Implantation of the acoustic transmitter is one of the major aspects that ensure the survivability of the animal subject when released into the wild. Studies on the technicalities involving telemetry methods are limited; therefore, this study investigated the surgical insertion of an acoustic transmitter into the peritoneal cavity of *Channa lucius* and *Neolissochilus soroides* or locally known as Bujuk and Tengas. A severity index was used to rate the appearance of surgical wounds observed on the day of release into the river. Fish mortality and complications such as bleeding were not observed in both species post-surgery. The progress and prognosis of wound healing of *C. lucius* were better compared to *N. soroides*, with generally lesser inflammation and more sutures shed. Despite visually severe inflammation on *N. soroides* on day-12 and day-19 post-surgery, there was a good indication that

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skin integuments were healing. This data suggests that *C. lucius* has faster wound healing abilities compared to *N. soroides*. All fishes implanted with the acoustic telemetry method survived up to 244 days upon release into the Tembat River, Hulu Terengganu, Malaysia, and retained the tag throughout the tracking period.

Keywords: Acoustic telemetry, Bujuk, fish surgery, fish telemetry, Hulu Terengganu, Tengas, wound healing

INTRODUCTION

The telemetry study provides precise information on fish movement behaviour and migrations by providing reliable real-time data compared to the conventional method of estimation using the mark-recapture technique (Dudgeon et al., 2015). Aquatic telemetry involves placing a transmitter (an electronic device) on or in the aquatic animal, which autonomously transmits data to data receiver stations (Whoriskey et al., 2019). However, the integrity of information depends on the quality and precision of data obtained (Brownscombe et al., 2019). Conducting a fish telemetry study involves considerations of various crucial parameters that could influence data interpretability. For example, fish survivability post-surgery influences the tracking period and swimming ability in its natural habitat. Thus, surgical skills, post-surgery care, and fish husbandry are important technicalities that indirectly influence the study's outcome (Ammann, 2020; Klinard et al., 2017).

Implantation of telemetry transmitters includes gastric and ovipositor insertion, as well as the external attachment. The most common technique is surgical implantation into the peritoneal cavity of the fish (Crossin et al., 2017) and is reputable for longer retention of the transmitter (Robinson et al., 2021). Nevertheless, peritoneal surgical insertions are comparatively invasive, requiring complex surgical procedures with prolonged duration, and are to be done by a surgeon with sedation (Lopes et al., 2016). Therefore, implantation of a transmitter into the peritoneal cavity of a fish can pose lethal and sub-lethal effects if conducted without experience or appropriate training (Cooke et al., 2013).

The principal premise of tagging procedures in telemetry studies is that tag implantation and device burden do not negatively influence the tagged animals' health, condition, behaviour, or survivability (Vollset et al., 2020). Thus, the increased practice of surgical fish tagging requires a complete understanding of tagging effects (Lopes et al., 2016; Wilson et al., 2017). Currently, most studies in the area are conducted in temperate countries such as Canada, the United States, and several European countries due to their technological advancement in fishery research using telemetry (Klinard et al., 2018; Thiem et al., 2011). However, similar studies are lacking in the tropical region, creating a lack of information on the application of the surgical approach and its recovery process in the local fish species and climate (Mitamura et al., 2006). Furthermore, available literature reviews tag implantation procedures that are species-specific which may vary in shape and size of the implants.

To date, a single study within the local region reports the use of two types of transmitters internally tagged in Mekong Giant Catfish or *Pangasius* sp. (Mitamura et al., 2006). The study was conducted on fishes of the Mekong River, which reported retention of the transmitter and absence of fungal infections post-procedure. Another informative study was conducted by Wagner et al. (2000), which looked at the effect of suture type on the wound healing of rainbows trout (*Oncorhynchus mykiss*). However, differences in climate and species do not favour direct referencing of the data from this study.

Malaysia is a nation with ambient temperature and high humidity and is home to various fish species. These factors may influence the surgical procedure and healing of the surgical wounds on the fish. Therefore, transparent recording of methods will enable future researchers to undertake a complete and informed procedure for a given telemetry study.

In addition to the surgical implantation technique, the predatory (*Channa lucius*) and benthopelagic (*Neolissochilus soroides*) species of fish were selected to study migration behaviour and habitat preferences, respectively (Harrington et al., 2022; Ullah et al., 2022). The *Channa* species have been reported to contain essential amino acids such as glycine, arachidonic acids, and polyunsaturated fats, which help promote wound healing (Kwan & Ismail, 2021). As a result, *C. lucius* may have a faster wound healing rate compared to *N. soroides*.

Proper healing of the surgical wounds on tagged fishes is crucial for their health and tag retention (Jepsen et al., 2002), and this will determine not only the success and interpretability of the study but also prevent financial loss. This study's single V9 VEMCO acoustic tag (Canada) costs approximately MYR 1,146.

This study aims to record the surgical insertion procedure of transmitters in two Malaysian freshwater fish species: *Neolissochilus soroides* and *Channa lucius*. This study also aims to qualitatively assess the healing of surgical wounds inflicted on *C. lucius* and *N. soroides* during the procedure. Wound healing was measured using photographic observation before the fish were released back into the river for telemetry study. Knowledge obtained from this study could aid future telemetry research, especially in the management of post-surgery procedures and research methods.

MATERIALS AND METHODS

Fish subjects used in this telemetry study were native species of the Tembat River, Hulu Terengganu, Malaysia. Using native species reduces the unfamiliarity with the habitat in which the fishes will be released. Five (5) *N. soroides* fishes were caught using a baited fish trap, and the same number of *C. lucius* were caught using a baited hook and line. The fish subjects were then separately kept in a fibreglass tank with the environment mimicking their preferred habitat. The subjects were acclimatised for at least five days to ensure good health.

Stress response, fitness, and health during acclimatisation were observed based on normal and upright swimming behaviour as well as normal opercular movement (Chopin et al., 1996).

Anaesthesia

In preparation for surgery, a 0.25 ml/L clove oil bath was first prepared in a 20 L transparent aquarium. The induction of anaesthesia was observed until stage 5 based on the sedation indication by Keene et al. (1998). A stage 5 of full sedation is indicated when the fish is in a recumbent position in the clove oil bath. The anaesthetised fish was carefully handled for measurement of length and weight prior to surgery.

Fishes were then positioned on a tray fitted with a V-shaped acrylic sheet upholstery to hold the fish upright dorsoventrally (Figure 1). The gills were constantly irrigated to maintain sedation throughout the procedure using a siphon and clove oil bath. The system consisted of a submersible pump placed in the anaesthesia bath that recycles the effluent onto the gills through a small tube (siphon) (Figure 1). The anaesthesia procedure is a modified method recommended by Matin et al. (2009) as well as Neiffer and Stamper (2009).

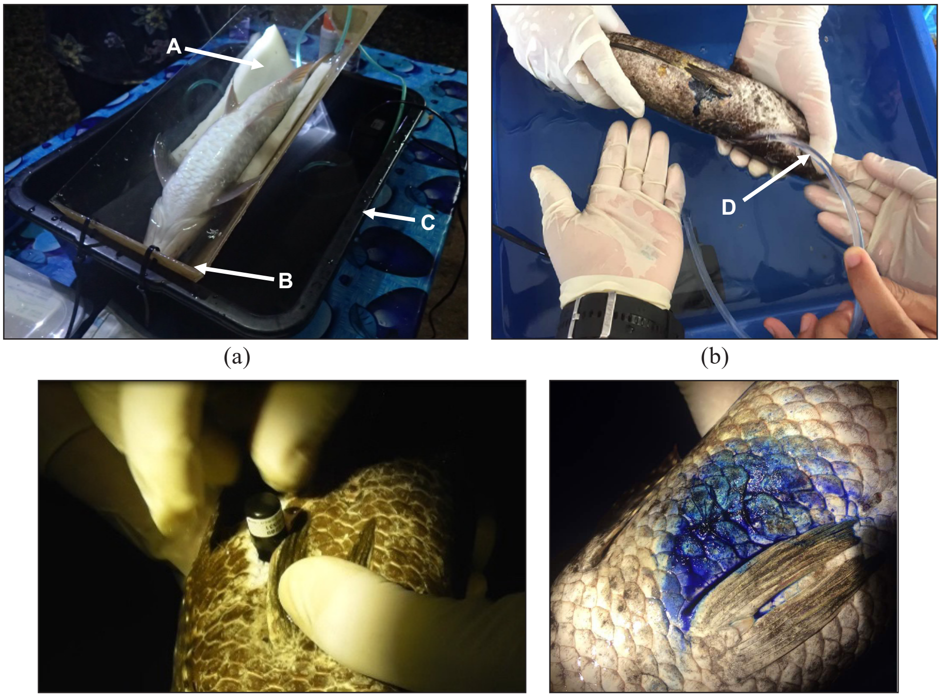


Figure 1. (a) The standard setting of placing a fish in a recumbent position on a sponge-based upholstery (A) in a triangular prism, (B) tray filled with sedative solution, (C) tilted prism to submerge fish's head under water, while the body remained above water for the surgery to be performed septically; (b) Clove oil anaesthetic solution was used to directly irrigate the gills using a siphon hose (D) powered by an aquarium-grade motor pump; (c) The tag was snugly inserted into the peritoneal cavity; (d) The wound was topically treated with an antiseptic solution to prevent infection and promote wound healing

Surgical Implantation of Acoustic Tag

Implantation of both fish species was conducted using different surgical sites based on the unique anatomy of *N. soroides* and *C. lucius*, as reported by Rożyński et al. (2017) and Schoonyan et al. (2017).

The procedure was started with the removal of scales using forceps. Approximately15-20 pieces of scales were removed for *C. lucius* as this species has finer-sized scales. In addition, scales were removed as individual scales have a high resistance to piercing, and the overlapping position of the scales would collectively distribute the puncture over a bigger area (Vernerey et al., 2014).

On *C. lucius*, the incision was made between the posterior of the pectoral fin and the anterior of the anus, where the peritoneal cavity is located (Figure 2). As for *N. soroides*, the incision was made parallel to the midline of the ventral side of the fish between the pectoral and anal fins (Figure 3). A fine incision measuring 3.5 ± 0.12 cm was first made using a surgical blade, followed by turning on the tag using a VEMCO 180kHz Tag Activator (VTA-180k-V9, Canada) and insertion of the acoustic tag into the peritoneal cavity. The V9 VEMCO (Canada) transmitter dimension was 9 × 29 mm and weighed approximately

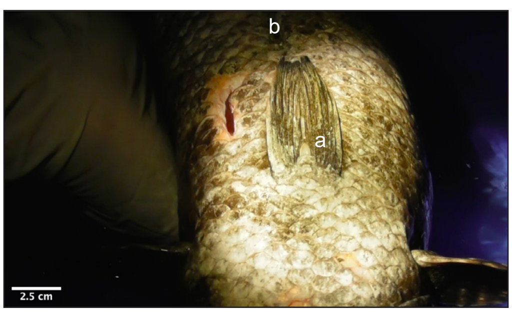


Figure 2. The incision made on *Channa lucius* was located between the pelvic fin (a) and anus (b)

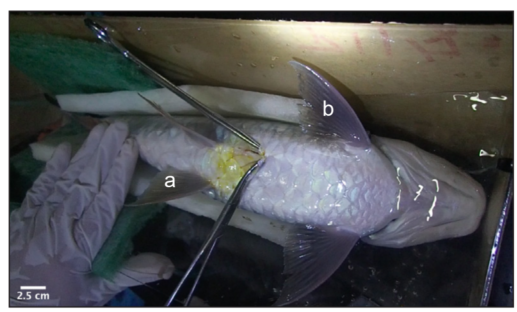


Figure 3. The incision made on *Neolissochilus soroides* was located between the pelvic fin (a) and pectoral fin (b)

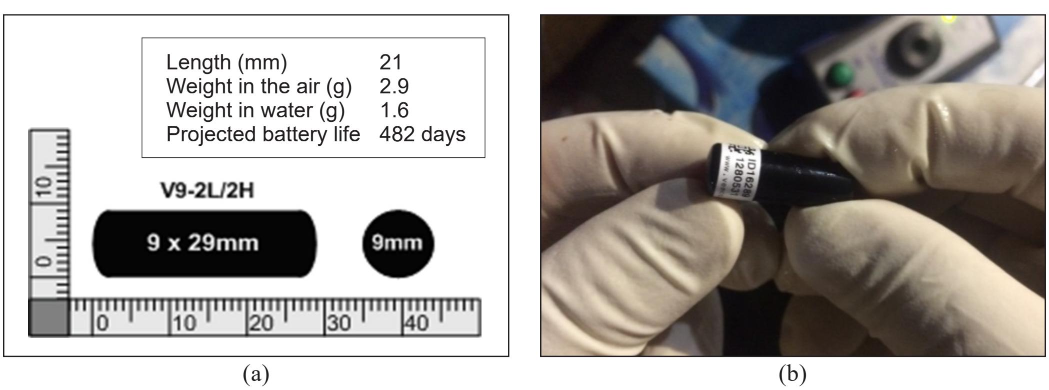


Figure 4. (a) Schematic dimension of each V9 VEMCO acoustic transmitter used in this study; (b) the V9-180khz acoustic transmitter with a tag activator in the background

2.9 g (Figure 4). The incision was closed with three simple interrupted sutures and finished with a surgeon knot without gaping. A topical antibiotic: Biobandage (Hikari, USA), was applied to the wound to prevent secondary infection and promote healing.

Recovery

Immediately after suturing, the fish was transferred to the recovery aquarium for stabilisation and observed until stage 5 of recovery (Keene et al., 1998). At stage 5 of anaesthetic recovery, the fish displayed total behavioural recovery with normal swimming movement. The highly aerated recovery bath was maintained at 24–25 °C to minimise stress and ensure a quick recovery. Injury or fatality was also prevented by securely covering the tanks to avoid fish from escaping. Fishes were kept in these tanks for a minimum of seven days, during which their wound healing was monitored before release into the Tembat River.

Observation of Wound

The wound on each fish was observed after surgery and once on the day of release (between 13-82 days). Repeated examination of the wounds was not conducted to avoid inducing stress. Instead, the wounds were inspected for the number of sutures retained and assigned with an index representing severity value (Adams et al., 1993; Paukert et al., 2001) (Table 1). Adams et al. (1993) suggested the following indexing based on selected parameters:

Maximum wound gape, whereby no gape was scored as 0, <1.0 mm gape as 10, and a gape of >1.0mm as 20.

Wound redness, whereby no redness was scored as 0, redness only near incision and sutures as 10, redness that had expanded beyond sutures as 20, and redness at the entire area around sutures as 30.

Other physiological or physical wound changes were recorded, such as the production of excess mucous on the skin. Consistency of indexing was maintained by assigning the same observer to indicate scores for all fish subjects.

RESULTS

All *C. lucius* and *N. soroides* observed in this study survived the procedure without signs of inflammation and bleeding after surgery. Within 24 hours post-surgery, fish also did not show lethargy or erratic swimming. However, various levels of wound healing

Table 1
Severity index based on the appearance of the surgical wounds of N. soroides *and* C. lucius

Observed parameter	Severity Index
Wound gape	No gape = 0
	Gape < 1.0 mm = 10
	Gape > 1.0 mm = 20
Inflammation (redness)	No redness = 0
	Only near incision and suture = 20
	The entire area around incision = 30

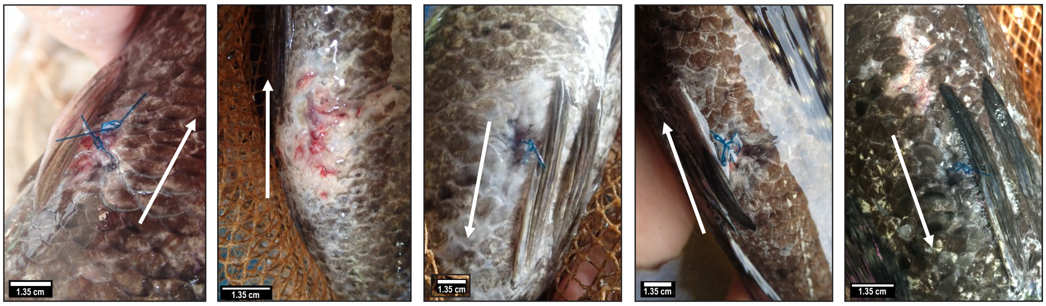
progress were observed from day 13 to day 82, ranging from moderate inflammation, shedding of sutures, and excess mucous production.

The progress of post-surgery wound healing on *C. lucius* between the 1st, 13th, 36th, and 82nd days after surgery were photographically recorded. On the first day post-surgery, the wound typically showed no complications with a clear and clean-cut through the muscle without bleeding. Figures 5 (a), (b), and (c) show the wound progress observed before release on day 13 for subjects ID 16289 and ID 16330, while ID 16312 had shedding of at least two sutures. Although the severity of inflammation was different between subjects, muscle adhesion progressed well without gaping.

However, subject ID 16312's incision wound was moderately inflamed with all sutures shed. Gaping of the wound was also observed at the posterior end of the incision (Figure 5 (b)). Figure 5 (d) shows wound healing progress observed for subject ID16297 on day 36, whereby one suture was shed and slight inflammation was visible. Figure 5 (e) shows the wound healing progress on day 82 for subject ID 16285. Two sutures were shed without severe inflammation and gaping, but mild inflammation was noted in the area where scales were extracted.

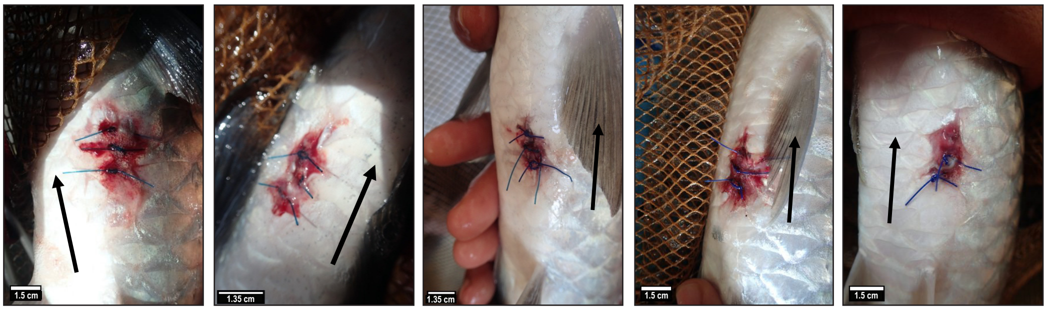
The progress of wound healing in *N. soroides* was observed on the 12th, 13th, and 19th days post-surgery (Figure 6). No complications or bleeding were observed during the surgery, but mild inflammation was seen where the scales were removed. Three sutures were applied to close the surgical wound gap in *N. Soroides*.

Wound incisions on subjects with ID 16342, ID 16174, and ID 16170 were observed on day 12 and 13 post-surgery, respectively (Figure 6 (a), (b), and (c)). These subjects displayed moderate inflammation of the surgical wound while all initial sutures remained intact. The moderate inflammation was accompanied by excess mucous around the suture and where scales were extracted. The surgical wounds of ID 16090 and ID 16089 were observed on day 19 post-surgery. For ID 16090, one suture was shed, and inflammation was observed only at the



(a) (b) (c) (d) (e)

Figure 5. The appearance of the surgical wounds of selected *Channa lucius* was recorded on the day of release. The arrow in the photos indicates the direction of the head: (a) Day 13 – ID 16289; (b) Day 13 – ID 16312; (c) Day 13 – ID 16330; (d) Day 36 – ID 16297; and (e) Day 82 – ID 16285



(a) (b) (c) (d) (e)

Figure 6. The appearance of the surgical wounds of selected *Neolissochilus soroides* was recorded on the day of release. The arrow in the photos indicates the direction of the head: (a) Day 12 – ID 16342; (b) Day 12 – ID 16174; (c) Day 13 - ID 16170; (d) Day 19 – ID 16089; and (e) Day 19 – ID 16090

incision area. Moreover, ID 16089 had all its original sutures intact with one loose suture, but inflammation was observed at the incision site and underneath the scale layers. Excess mucous was also observed on the wound, pectoral fin, and body.

Based on the Severity Index by Adams et al. (1993), the number of sutures retained was higher in *N. soroides* compared to *C. lucius*, while inflammation was more severe in *N. soroides* compared to *C. lucius* (Table 2). As Adams (1993) suggested, fish with higher severity index show lesser progress in wound healing. It was visually comparable, whereby *N. soroides* had inflammation (redness of the wound) extended beyond the incision, while *C. lucius* had very little to no inflammation.

DISCUSSION

Both fish species used in this study have scales as their integument anatomy, particularly of the cycloid type (Farinordin et al., 2017). However, both species differ

Table 2
The severity index assigned to each fish based on observation after 12 to 83 days of surgery for N. soroides *and* C. lucius

Species	Fish ID	Day observed	Total length (cm)	Total weight (g)	No. of suture retained	Wound gape	Inflammation
Channa lucius	ID 16289	13	43	843	2	0	20
	ID 16297	36	41	640	2	0	20
	ID 16330	13	53	1,662	1	0	0
	ID 16285	82	43	745	1	0	0
	ID 16312	13	43	713	0	0	20
Neolissochilus soroides	ID 16170	13	37.5	550	3	0	20
	ID 16090	19	35	659	2	0	20
	ID 16089	19	40	580	3	0	30
	ID 16342	12	41.5	665	3	0	30
	ID 16174	12	43	731	3	0	30

in the size of their scale, which influenced the number of scales extracted for the surgical procedure. For example, *C. lucius* scales are small (0.2-0.3 cm) and densely overlapping, requiring the removal of more scales. Meanwhile, *N. soroides* only required two to three larger soft scales (1-2.5 cm) to be extracted to perform the surgical implantation.

Although scale removal only creates a surface wound, a deeper cut of the muscle tissue required to complete the procedure causes bleeding (Sveen et al., 2020). However, during the incision, minimal bleeding was observed, which is due to blood vessel constriction stimulated by the proinflammatory cytokines. This mechanism happens within a few minutes of wound formation and helps prevent excessive blood loss (Schmidt et al., 2016). Since it was time-consuming to make an incision on *C. lucius* as the scales were small, structurally tough, and dense, the subject must be fully sedated to lessen the stress of the procedure. Comparatively, incisions on *N. soroides* were made much quicker as the fish scales were larger, softer, and pliable, which made extracting the scales easier. It was also observed that *C. lucius* took longer to be fully sedated and recover compared to *N. soroides*. It is due to the ability of *C. lucius* to breathe air while *N. soroides* lack in that capability.

Furthermore, prolonged exposure to stress could delay the wound healing process (Sveen, 2018); thus, the fish were not repeatedly handled for wound monitoring between post-surgery and release. Normally, a surgical wound or a deep wound in teleost will activate a series of healing processes, which includes: re-epithelialisation, inflammation, granulation tissue formation, and tissue remodelling (Sveen et al., 2019). During the process, leukocytes and macrophages are recruited to clear the wound from damaged tissue and drive the repair processes (Richardson et al., 2013). Inflammation is a response to re-epithelialisation and is supposedly an indicator of wound healing (Sveen et al., 2020). Inflammation in adult fish usually starts on day 1 post-surgery to day 65, with marked redness on day 5 to 10 (Schoonyan et al., 2017). Based on the severity index used in this study, most *C. lucius* showed slight inflammatory reaction or redness around the incision area. All *N. soroides* fish showed prominent inflammatory reactions even though the observation was made after 12 days of surgical procedure.

Subjects ID 16090 (*N. soroides*) and ID 16312 (*C. lucius*) exhibited the formation of granulation or repair tissue. Granulation tissue usually grows from the wound's borders and replaces the damaged tissue with time. Granulation tissues contain connective tissue, fibroblasts, myofibroblasts, immune cells, and small blood vessels (Roubal & Bullock, 1988). In fish ID 16090, granulation tissues grew at infringement of the suture. For subject ID 16312, all sutures were shed, and granulation tissue was seen to grow in an unorganised manner. The growth of granulation tissue seen in this study was consistent with data from the literature, particularly Fontenot and Neiffer (2004), Schmidt et al. (2016), and Sveen et al. (2019), whereby granulation occurred from the 6th day to the 75th day post wounding.

Moreover, the complete scale regeneration took place within one month after wounds were inflicted (Richardson et al., 2013). In this study, fish ID 16330 had almost fully regenerated skin, but scales were not regenerated in 13 days. Darker pigmentation was additionally observed on *C. lucius*, ID 16330, which may have been caused by the rapid release of melanin granules, causing pigmentation of the skin around the wound (Rai et al., 2012).

Additionally, all fish had excessive mucous formation on the skin and the wound, which is typical in a wounded fish (Sveen et al., 2020). The mucous gel produced by wounded fish provides physical and antimicrobial protection to the wound surface. The secretion may also enhance wound healing through haemolytic activity and promote vasoconstriction of smooth muscle cells (Akunne et al., 2016).

Another notable observation was the different rate of suture shedding between both species. Shedding of suture occurred on all *C. lucius* and one out of five *N. soroides*. Suture shedding is normal and occurs in other species within 20 days post-surgery (Cooke et al., 2003; Schoonyan et al., 2017) and is also an indicator of healing. In this study, *C. lucius* retained a lesser number of sutures compared to *N. soroides*, while *N. soroides* had significantly more inflammation compared to *C. lucius*. This study also shows, *C. lucius* have a faster wound healing compared to *N. soroides* based on the sutures shed and the inflammation. According to Sveen et al. (2020), the healing of deep wounds is species-dependent, meaning that some species have a much faster healing rate than others. In this case, the faster healing by *C. lucius* may be attributed to the findings by Sahid et al. (2018), in which the study found an active compound produced by channids species that can help accelerate wound healing.

Proper healing of surgical wounds can take up to 100 days, depending on several factors, such as species and local climate (Sveen et al., 2020). Accommodating prolonged timeline was impractical for a telemetry study, while releasing fishes without sufficient recovery may pose a risk of losing the transmitter. Furthermore, losing the transmitter would generate biased data on movement and mortality; a key parameter used in telemetry studies. Therefore, based on the observation in this study, the reasonable but effective duration of time to confine is between 4 to 13 days after surgery for *C. lucius* and 15 to 17 days after surgery for *N. soroides.*

According to Schoonyan et al. (2017), incision closure, inflammation, and the presence of sutures are common indicators of healing rate; thus, it is very important to observe these attributes to ensure fish health post-surgery. In this observation, all subjects showed indications of recovery through shedding sutures, tissue inflammation, and excellent movement agility. Thus, the fish were considered fit and released back into the river for movement and habitat study

(Paukert et al., 2001). The continuity of this study was published in Sharir et al. (2021), whereby all of the fishes were reported to survive and had tag retention for up to 244 days.

CONCLUSION

The assessment of wound severity is an indicator of fish health, and both species in this study responded differently to the surgical procedure. *Channa lucius* showed signs of recovery between 13 to 82 days post-surgery with lesser inflammation and a lesser number of intact sutures compared to *N. soroides*. Although most *N. soroides* demonstrated severe inflammation, survivability was not affected. Nevertheless, both species exhibited a high level of fitness with high agility in swimming behaviour before being released. Based on the current data, surgical tagging of fish is recommended for acoustic telemetry study with retention of the fish in a recovery tank for at least 13 days before being released. This procedure would help ensure appropriate wound healing and survivability in the wild. This duration is also recommended to maximise battery power utilisation of the tags.

The study indicated that the peritoneal insertion of the VEMCO V9 acoustic tag is suitable for *C. lucius* and *N. soroides.* In addition, it may be applicable for other local fishes with similar size and body forms, such as the snakehead species (*Channa micropeltes* and *Channa striata*) and fusiform species (such as *Tor* sp. and *Hampala macrolepidota*). Furthermore, the surgical procedure is proven safe and efficient with retention of the tags, health maintenance, and no reported mortality.

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