Vol. 32: 21–29, 2023
https://doi.org/10.3354/ab00759

AQUATIC BIOLOGY
Aquat Biol

Published April 19

Effect of surgical staples and stitches on the critical swimming speed of *Sebastes schlegelii* after implantation of ultrasonic pingers

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ABSTRACT: Implantation of ultrasonic pingers in fish is invasive in nature, as it bisects epidermis and skin tissues, with negative health impacts on the individual. Taking Korean rockfish *Sebastes schlegelii* as a model species, we compared the difference between using surgical sutures and disposable-staple sutures so as to minimize the impact caused by implantation. The results showed that (1) the critical swimming speed of *S. schlegelii* changed significantly 2 h after surgery but did not change significantly 1 d after the operation; (2) both suture methods can effectively close the wound; and (3) it takes less time to suture the wound with disposable skin staples than with a surgical needle. The abdominal incisions of the experimental fish were clean, the fish showed no inflammation or necrosis after suture by the 2 methods, and they remained healthy for 7 d after operation. We conclude that disposable skin staples are well suited for minimally invasive surgical suturing.

KEY WORDS: *Sebastes schlegelii* · Ultrasonic transmitter · Disposable skin staples · Critical swimming speed

1. INTRODUCTION

Ultrasonic biotelemetry is a technique that uses a system composed of a transmitter and a receiver to analyze how environment and habitat affect animal behaviors. The transmitter sends out ultrasonic signals, which are identified and stored by the receiver. The data signals are then processed by a computer to offer information such as position and depth (Liu et al. 2021). Ultrasonic telemetry has been widely used in modern fisheries as well as in fish monitoring studies in aquatic environments such as marine habitats, lakes, reservoirs, estuaries, aquaculture nets, etc. (Clark & Green 1990, Taylor et al. 2013, Binder et al. 2018, Núñez-Rodríguez et al. 2018, Hou et al. 2019). The technology monitors the behavior of fish by placing an ultrasonic pinger either outside or inside the body cavity. Depending on the size and species of fish and study objectives, different fixation methods are used, including stomach insertion via injection, dorsal fin suspension, and *in vivo* implantation (Bridger & Booth 2003, Hussey et al. 2015). Once the ultrasonic pinger is fixed, the ultrasonic signal from the marked fish can be received. The acquired data are then processed to obtain the changes in the underwater behavior of the marked fish, including swimming speed and the horizontal and vertical positions of movement (Juell & Westerberg 1993).

In 1956, the dorsal fin suspension method was first used to monitor the movements of chum salmon *Oncorhynchus keta* and silver salmon *O. kisutch* (Trefethen 1956). Due to the large size of the early ultrasonic pinger, they were mostly suspended on the body of adult fish for tracking. For bonito *Katsuwo-*

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nus pelamis, the ultrasonic pinger was fixed using the ventral implantation method and the bonito resumed feeding 3–4 d after the implantation procedure (Yuen 1970). The dorsal fin suspension method is relatively simple, but the transmitter is easily detached from the fish body, so the *in vivo* implantation method is preferred over the dorsal fin implantation method for long-term behavioral tracking of fish (Moore et al. 1990).

Many kinds of ultrasonic pingers are currently available. Researchers have suggested that heavier ultrasonic pingers may affect the swimming performance, growth, survival, or behavior of fish (Jepsen et al. 2005), so it is necessary to pay attention to the proportion of the weight of the ultrasonic pinger to fish body mass, which must not exceed 2% in air, a standard known as the '2% rule' (Lee et al. 2013). A study on the ability to adjust the volume of the swim bladder pointed out that the value must not exceed 1.75% in water (Summerfelt & Mosier 1984). In practical studies, the mass of the ultrasonic pinger selected for testing should be minimal to reduce its impact on fish (Zale et al. 2005). However, some researchers believe that the weight does not affect the swimming speed or physiology of the fish (Brown et al. 2006). For example, when the ratio was ~6–8%, the critical swimming speed of juvenile rainbow trout *O. mykiss* was not affected (Brown et al. 1999), and a ratio of ~7–8% did not affect the survival of Coho salmon *O. kisutch* (Chittenden et al. 2009).

The implantation of an ultrasonic pinger by minimally invasive surgery is common in studies of fish tracking and behavioral monitoring. Different incision positions and suture techniques in ultrasonic beacon surgical implants are directly related to the survival rate of the fish (Panther et al. 2011). Standardized implantation techniques can improve fish survival and retention of ultrasonic pingers, and the use of sterile instruments for surgical suturing can effectively reduce the infection rate and speed up wound healing (Wagner et al. 2011). Minimally invasive surgery could reduce the impact on fish by reducing the number of sutures and by adjusting the position of the sutures (Miller et al. 2014). In addition, the use of different materials to suture wounds could influence the recovery of fish (Deters et al. 2010). Deters et al. (2010) used different types of sutures on juvenile Chinook salmon *O. tshawytscha*, including non-absorbable (Ethilon) and absorbable (Monocryl) monofilament sutures, and other non-absorbable materials (Nurolon and silk), and the results showed that different suture materials had different effects on suture retention, incision openness, pinger retention, tissue inflammation, and tissue ulceration. In contrast, Jepsen et al. (2008) studied the effect of different suture types on the survival and growth of brown trout *Salmo trutta* after they had an ultrasonic pinger implanted during field surgery. The results showed that the wounds healed better when absorbable filament was used for the sutures, and the rate of ejection of the pinger from the body was lower. Other methods to close surgical wounds include tissue adhesives, but wounds sutured this way are prone to dehisce at a later stage because the adhesive does not bond well enough to the skin (Rożyński et al. 2017). Li et al. (2019) used disposable skin staples to suture the implantation wound of *Carassius auratus*, concluding that the sutures were effective and wounds showed no necrosis within 7 d of the trial. Cooke et al. (2003) studied the effect of minimally invasive surgical implantation on incision healing, growth, and survival in juvenile largemouth bass *Micropterus salmoides* and found that test fish survival was related to proficiency of suture and that higher proficiency could improve the efficiency of suturing and reduce operation time.

Sebastes schlegelii (family Scorpaenidae, order Scorpaeniformes) is mainly distributed in the Northwest Pacific (Xu et al. 2018). In China, it is one of the most important economic fish species and is mainly distributed in the Bohai Sea, the Yellow Sea, and the East Sea regions (Feng et al. 2021). Ultrasonic telemetry helps elucidate the movement characteristics of tracked fish. At present, there are many studies on the effect of ultrasonic pinger implantation and sutures on fish, but there is no report on this kind of research related to *S. schlegelii*. Therefore, the present study investigated the effect of different suture methods on *S. schlegelii* implanted with an ultrasonic pinger, which will provide a reference for the selection of appropriate surgical suture methods for this fish when implanting ultrasonic pingers.

2. MATERIALS AND METHODS

2.1. Study animals

Sebastes schlegelii (n = 100) were purchased from a breeder in Dalian, Liaoning Province, China. Based on visual assessment, the body surface of the fish showed no damage. Before the experiment, the fish were acclimated in a cylindrical fiber-reinforced plastic tank with a radius of 0.50 m, water depth of 0.80 m, water temperature of 20.41 ± 0.87°C, and salinity of 25 ppt for 3 d. The fish were fed with sinking bait once a day at 08:00 h. The bait amount was 20% of the fish body mass. About 6 h after feeding, the bottom of the tank was cleaned of bait residue and feces using a bottom suction device before changing 50% of the tank water.

2.2. Experimental treatment

After the acclimation period, 25 active *S. schlegelii* were haphazardly selected and evenly divided into 5 groups: (1) control (without any surgical treatment), (2) needle- or (3) stapler-sham (no ultrasonic pinger was implanted, and the incision was closed with a suture using a surgical needle or disposable skin staples, respectively), (4) needle- or (5) stapler-implantation (after ultrasonic pinger implantation, the incision was closed with a suture using a surgical needle or disposable skin staples). Overall mean ± SE total length was 20.24 ± 1.56 cm, and overall mean body weight was 166.20 ± 12.77 g (see Table 1 for group means).

The sham and implantation groups underwent minimally invasive surgery on the abdomen. The surgical equipment and ultrasonic pingers were sterilized by soaking in 75% alcohol and then rinsed with distilled water to minimize adverse irritation to the body wall after implantation. The fish were anaesthetized using MS-222 at a concentration of 100 mg l^{-1} (Guan et al. 2011). After anesthesia, a fish is unbalanced, lying on its side underwater, and has no tactile response to external stimuli (touch with a glass rod) (Guan et al. 2011). Fish were placed dorsal side down on a custom-built surgical table with a groove (Fig. 1). The abdomen of the fish was wiped with 75% medical alcohol. We then made a ~2 cm long incision with a scalpel 2 cm anterior to the anus (Fig. 1a). For the 2 implantation groups, the ventral cavity of the fish was opened using sterilized forceps, and, following the method of Moore et al. (1990), an ultrasonic pinger was inserted into the abdominal cavity with an inclination angle of approximately 30° (Figs. 1b & 2). In the 2 sham groups, the incision was closed without implanting a transmitter; other operation steps were the same as above.

In the needle-sham and needle-implantation groups, we used a sterilized semicircular stainless steel suture needle (Fig. 3a) and surgical thread for wound closure, applying the interrupted suture method as described by Li et al. (2001) and the instrument knotting method (Fig. 1c). For the stapler-sham and stapler-implantation groups, we used a disposable skin stapler (DSS-W-35, Shenkang Medical Equipment

Table 1. Mean (±SE) body length, body mass, and critical swimming speed (V_{crit}; before [Pre-OP] and after surgery on the treatment fish), as well as ultrasonic transmitter to body mass ratio of *Sebastes schlegelii* in the 5 experimental groups (see Section 2.2 for descriptions of the groups). ANOVA of repeated measurements between V_{crit} in different groups and at different time points showed that the overall data conform to sphericity (see Section 3.2). The results of main effects are: F_{time} =142.068, p_{time} = 0.000; F_{group} = 11.909, p_{group} = 0.000; $F_{time \times group}$ = 5.715, $p_{time \times group}$ = 0.000. Compared with different time points in the same group, superscript 'a' means that other time points are compared with 0 h, 'b' means that other time points are compared with 2 h, and 'c' means that other time points are compared with 1 d, $p < 0.05$. In the comparison between different groups at the same time point, superscript 'A' means that other groups are compared with control, 'B' means that other groups are compared with needle-sham, 'C' means that other groups are compared with stapler-sham, and 'D' means that other groups are compared with needle-implantation, $p < 0.05$. na: not applicable (no transmitter implanted)

Group	Total body length (cm)	Body weight (g)	Transmitter to body mass ratio	V_{crit} (cm s^{-1}) Pre-OP (0 h)	2 h	1 d	3 d	7 d
Control	20.16 ± 1.62	162 ± 12.49	na	56.52 ± 2.80	53.24 ± 1.17	53.74 ± 1.16	55.53 ± 1.49^{b}	56.22 ± 2.21^{b}
Needle-sham	19.54 ± 1.19	169.2 ± 5.74	na	57.36 ± 1.77	46.27 ± 2.99^{aA}	53.64 ± 1.57^{ab}	55.65 ± 2.51^{b}	56.46 ± 2.63^{bc}
Stapler-sham	20.76 ± 1.85	158.6 ± 10.05	na	53.94 ± 1.98^{AB}	43.36 ± 1.12^{aA}	51.64 ± 1.76^{b}	53.84 ± 0.54^{bc}	53.44 ± 1.00^{bAB}
Needle-implantation	19.68 ± 1.53	173.6 ± 13.71	1.62	59.61 ± 2.83^{C}	46.87 ± 3.40^{aAC}	56.11 ± 1.31^{bABC}	57.51 ± 0.58^{bC}	57.85 ± 1.29^{bC}
Stapler-implantation	21.08 ± 0.74	167.6 ± 13.05	1.68	57.83 ± 0.70^{C}	43.73 ± 1.59^{aAD}	56.02 ± 2.47^{bABC}	57.23 ± 1.55^{bC}	57.43 ± 1.81^{bC}

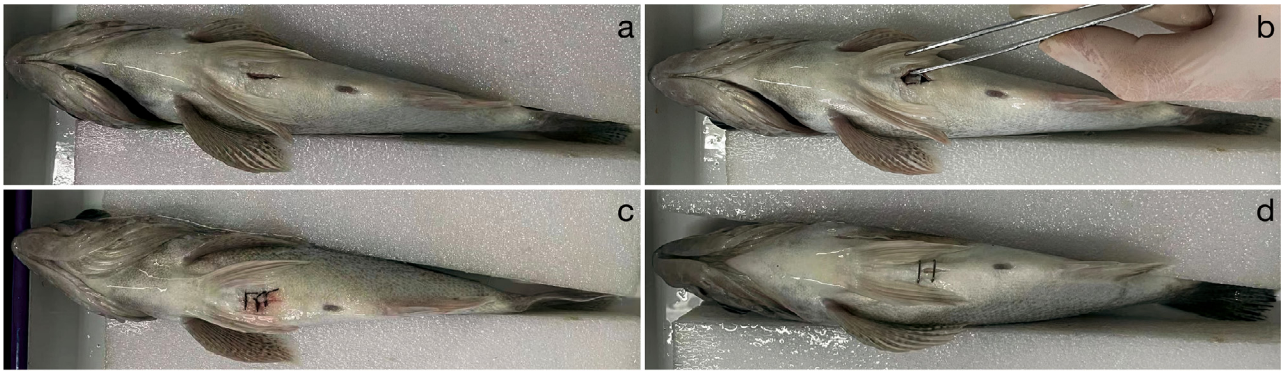

Fig. 1. Minimally invasive surgery performed on *Sebastes schlegelii*. (a) Minimally invasive laparotomy; (b) ultrasonic pinger implanted in the abdomen; (c) incision closed with needle and thread; (d) incision closed with disposable skin staples

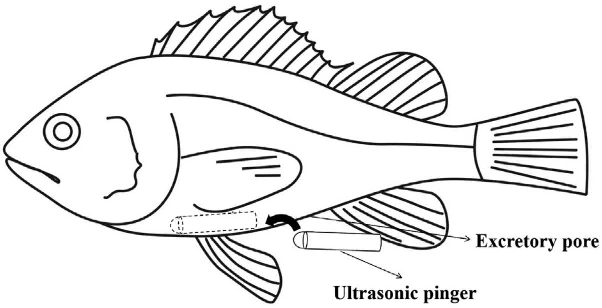

Fig. 2. Schematic diagram of ultrasonic pinger implanted into abdominal cavity of *Sebastes schlegelii*

Co., China) (Fig. 3c) for wound closure (Fig. 1d). Suture lines (Fig. 3b) and metal staples (Fig. 3d) only passed through the epidermis. To keep a wound tightly closed after surgery, 3 suture knots need to be completed with needle and thread, while only 2 metal staples are needed to close a wound. After suturing, the wounds were cleaned and disinfected using cotton balls soaked in alcohol before erythromycin ointment was evenly applied to the incision site with a medical swab. The fish were then placed in an oxygen-enriched water bath for recovery from the anesthesia. The time required for surgical suture

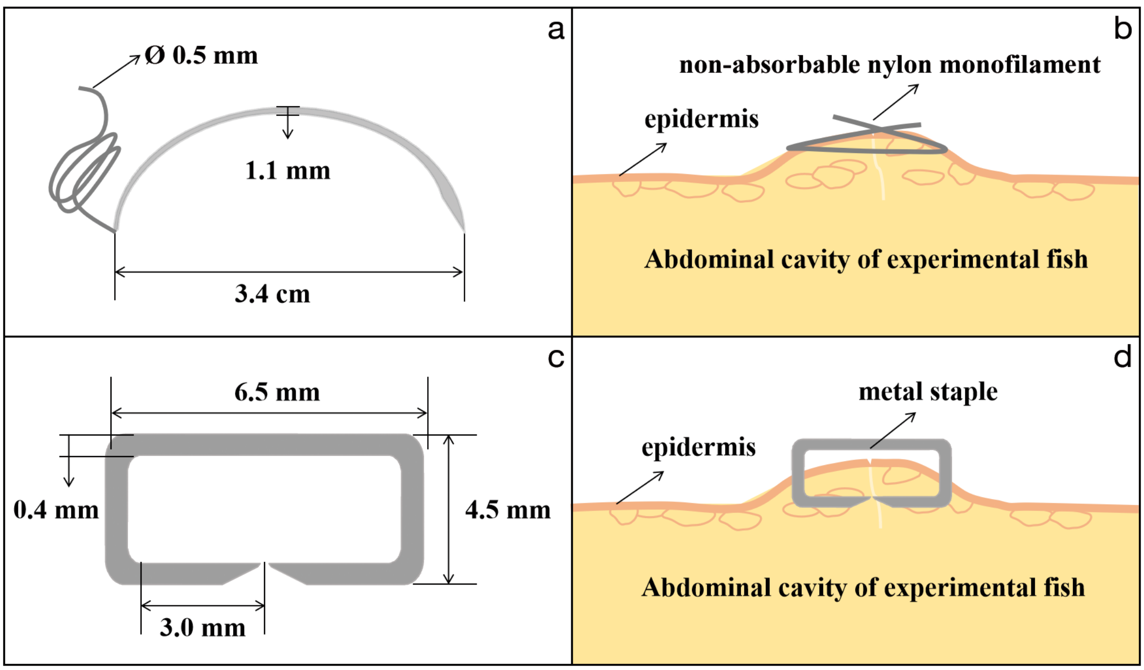

Fig. 3. (a,c) Suture materials and (b,d) suture sites for experimental fish wounds after implantation of ultrasonic pingers. (a) Semi-circular stainless steel suture needle; (b) cross-section through fish epidermis incision closed with a suture needle; (c) stainless steel metal staple; (d) as in (b) but for suture staple. ∅ : diameter

(from the start of work with suture instruments to the end of the wound suture) was recorded for each fish using a stopwatch. After the operation, we checked whether there were sutures or metal staples falling off the fish every day.

The ultrasonic pingers we used were cylindrical, ~35 mm in height, and 10 mm in diameter (FRX-4002 type, FUSION). Their emitting frequency was 60 kHz, and the sound pressure level was 155 dB (re 1 µPa). At an emission interval of 1 s, battery life was 2 d (Fig. 4). The ratio of ultrasonic pinger to fish body weight was <1.7 for both implantation groups (Table 1).

We measured critical swimming speed (V_{crit} in cm s^{-1}) in all groups before the operation time and 2 h, 1 d, 3 d, and 7 d after operating on the sham and implantation fish. Before each swimming speed test, fish were kept without food for 24 h and were then placed one by one into a custom-built Steffensen-type fish swimming-ability measuring tank (X. Yu et al. 2017) (Fig. 5). Fish were allowed to acclimate to the tank for 20 min at a flow rate of 13.0 cm s^{-1}. After this time, the flow rate was increased by 10 cm s^{-1}. If the fish could swim at that speed (i.e. 23 cm s^{-1}) for 20 min, the flow rate was increased by another 10 cm s^{-1}. This was repeated until the fish were too fatigued to continue swimming and stopped, at which point the swimming test was ended. The absolute V_{crit} (L. Yu et al. 2017) was then calculated as:

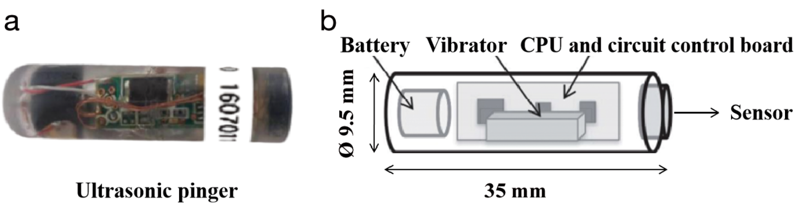

Fig. 4. (a) Actual appearance and (b) schematic of 60 kHz FPX-1030 ultrasonic pinger

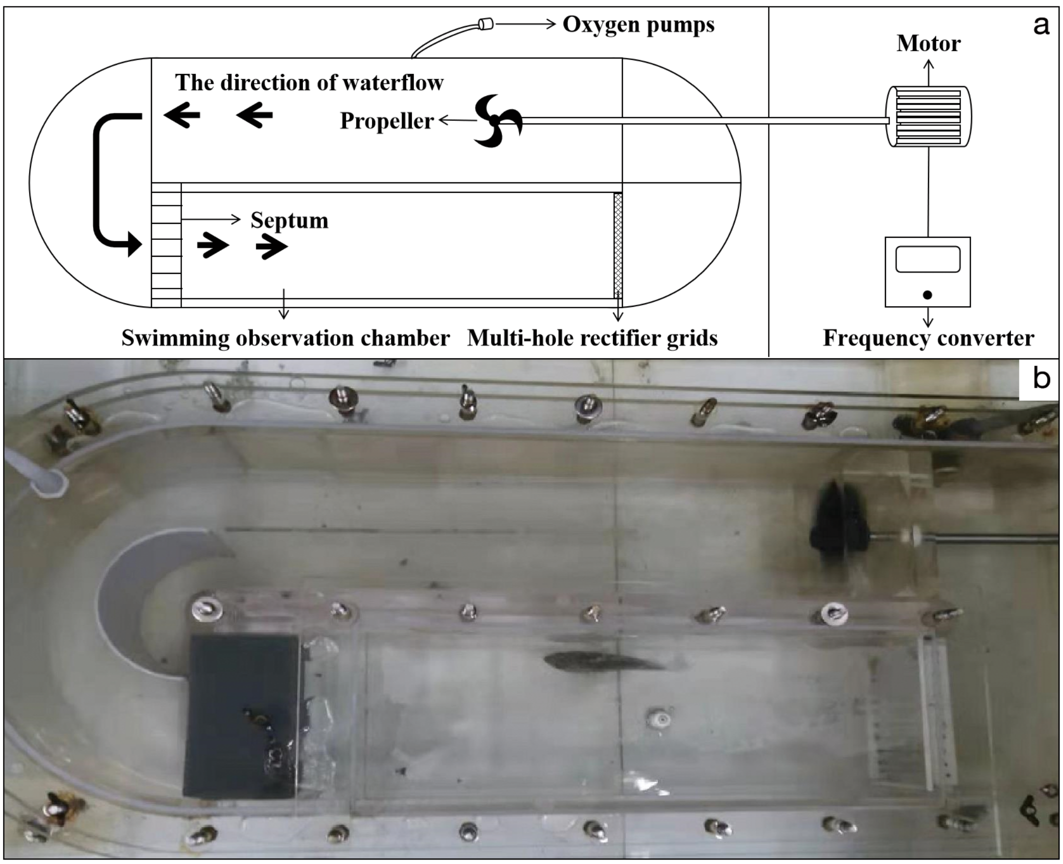

Fig. 5. Steffensen-type fish swimming-ability determination tank. (a) Schematic of the device. Multi-hole rectifier grids are baffles on one side of the swimming observation chamber, with evenly distributed holes above them, through which water can circulate. (b) Top view of the device during the experiment, showing a fish in the swimming observation chamber (L × W × H: 50 × 15 × 15 cm)

$$V_{crit} = V_1 + (t_1 / t_2) \times V_2 \quad (1)$$

where V_1 is the maximum water flow velocity (cm s^{-1}) when the fish lasts for 10 min, V_2 is the current velocity increment (10 cm s^{-1}), t_1 is the swimming time (cm s^{-1}) when the fish swims at maximum flow velocity, and t_2 is the time interval (10 min).

2.3. Data processing

V_{crit} of the 5 fish groups was statistically analyzed using SPSS 25.0 (IBM SPSS Statistics 25) software. The measurement data were described by mean ± SD, 1-way ANOVA was used for multi-group comparison, and ANOVA was used for repeated measurement data. Differences were considered statistically significant at values of $p < 0.05$.

3. RESULTS

3.1. Effect of suturing on the body of *Sebastes schlegelii*

No mortality occurred in any of the groups throughout the 7 d trial. In the needle-sham and -implantation groups, no breakage or dislodgment of the 3 surgical sutures was observed in the abdomen. In the stapler-sham and -implantation groups, the 2 suture staples in the abdomen did not fall out, and the ultrasonic pinger stayed within the fish during the test. In addition, on the 7th day after the operation, healing was observed in all experimental groups.

The mean (±SE) time to scrape off the scales and to make the incision in the abdominal cavity using a scalpel was 30.42 ± 1.75 s, and implanting the ultrasonic pinger into the abdominal cavity took 3.0 ± 0.82 s. Suturing with a surgical needle took on average about 55 s longer (needle-sham: 61.67 s, needle-implantation: 64.67 s) than with a stapler (stapler-sham: 8.67 s, stapler-implantation: 10.67 s; Fig. 6).

3.2. Effect of ultrasonic pinger implantation on the swimming speed of *S. schlegelii*

ANOVA of repeated measurements between V_{crit} in different groups and at different time points showed that the overall data conform to sphericity (Mauchly $W = 0.651$, $p = 0.545$). Comparisons were made between different groups at the same time points. At 0 h, there were significant differences between stapler-sham and control, needle-sham, needle-implantation and stapler-implantation ($p < 0.05$). At 2 h, there were significant differences between control and the other 4 groups ($p < 0.05$), as well as between needle-implantation and stapler-sham and stapler-implantation ($p < 0.05$). At 1 d, we found significant differences between control and needle-implantation and stapler-implantation ($p < 0.05$), and between needle-sham and needle-implantation and stapler-implantation ($p < 0.05$). Stapler-sham was significantly different from needle-implantation and stapler-implantation ($p < 0.05$). At 3 d, significant differences were observed between stapler-sham and needle-implantation and stapler-implantation ($p < 0.05$). On the 7th day, there was a significant difference between stapler-sham and the other 4 groups ($p < 0.05$) (Fig. 7, Tables 1 & 2).

Comparisons were also made between different time points within the same group. In control fish, there were significant differences between 2 h and 3 d and 7 d ($p < 0.05$). In the needle-sham group, we

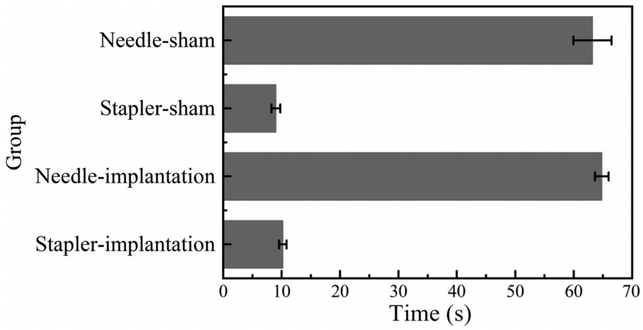

Fig. 6. Time required for different suturing methods (mean ± SE) in the sham and implantation fish groups. See Section 2.2 for definitions of the groups

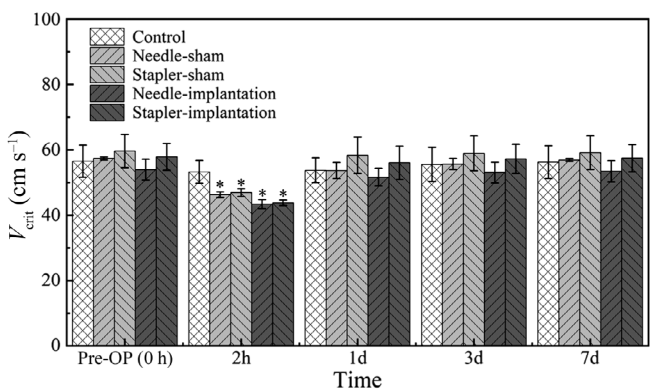

Fig. 7. Critical swimming speed (V_{crit}; mean ± SE) of *Sebastes schlegelii* at different times before and after surgery on the treatment fish; see Section 2.2 for definitions of the groups. (*) Denotes significant difference ($p < 0.05$) compared with control

Table 2. Results from 1-way ANOVA comparing the critical swimming speed of *Sebastes schlegelii* in each experimental group among the 5 different sampling points (see Section 2.2 for details on groups and sampling times)

Group	SS	df	*F*	p
Control	86.055	4, 20	4.604	0.008
Needle-sham	314.809	4, 20	15.343	<0.001
Stapler-sham	43.952	4	4.742	0.007
Needle-implantation	70.174	4	5.941	0.003
Stapler-implantation	59.445	4	4.183	0.013

found significant differences between 0 h and 2 h, 1 d ($p < 0.05$), 2 h and 1 d, 3 d and 7 d ($p < 0.05$), and 1 d and 7 d ($p < 0.05$). In the stapler-sham group, significant differences were recorded between 2 h and 0 h, 1 d, 3 d and 7 d ($p < 0.05$), and between 1 d and 3 d ($p < 0.05$). In needle-implantation fish, there were significant differences between 2 h and 0 h, 1 d, 3 d, and 7 d ($p < 0.05$). In the stapler-implantation group, significant differences occurred between 2 h and 0 h, 1 d, 3 d, and 7 d ($p < 0.05$) (Table 1).

4. DISCUSSION

4.1. Comparison of different suture methods and their effects on *Sebastes schlegelii*

This experiment showed that disposable skin staples were superior to surgical needle sutures in 2 ways. First, suture time was shorter than when using a surgical needle to close, saving approximately 55 s in this step. This time is similar to that saved by Smircich & Kelly (2014), who showed that the disposable skin stapler suture method saved 1 min compared to the steel suture needle method. A shorter suturing time reduces the time a fish is anesthetized and thus also the impacts of the anesthetic on the organism (Li et al. 2019). Secondly, stapler sutures required only 2 staples to keep the incisions closed, whereas the surgical needle suture required 3 stitches. Reducing the number of skin perforations at the incision site minimizes irritation to the fish and thus the risk of infection. In addition, the disposable skin stapler was easier to use during suturing (i.e. more user friendly), and the sutures were as effective as needle sutures, both ensuring that the incisions remained closed and that no suture staples or stitches were lost or broken within the 7 d of the experiment. We observed no infections or other signs of ill health in fish treated with either of the 2 suture methods. These results are consistent with those obtained by L. Yu et al. (2017) in an ultrasonic pinger implantation study on grass carp *Ctenopharyngodon idellus*.

Surgical needle and thread were used to close the wounds, but this suture method is complex to perform, the method takes longer to complete, and the suture (i.e. the silk thread) is prone to rejection if the thread crosses through subcutaneous tissue instead of just the epidermis (Liu & Zhang 2019). The disposable skin stapler has been widely used in human skin suturing (Bridger & Booth 2003). The disposable skin stapler anastomosis is an effective wound closure method (Deters et al. 2010). Swanberg et al. (1999) used surgical needles and thread and metal staples to close wounds in rainbow trout. Similar to the results of the present study, they found that metal staples were a better method than suture stitches because metal staples can suture wounds faster and reduce the risk of infection.

4.2. Recommendations when performing ultrasonic pinger implantation suture surgery

S. schlegelii needed some time to adapt and recover after the ultrasonic pinger was implanted. The results of a study on ultrasonic pinger implantation of juvenile Masou salmon *Oncorhynchus masou* showed that salmon needed 2 h of adaptation and recovery time (Makiguchi & Ueda 2009). Adequate postoperative recovery and acclimation time are essential for experimental fish (Li et al. 2019): if the recovery time is insufficient, the experimental fish may display stress behavior, resulting in the displacement of ultrasonic pingers in the abdominal cavity and eventually abdominal cavity rupture. This can lead to the death of the experimental fish or increase the risk of wound infection.

Implantation proficiency on the part of the experimenter is also critical, as reducing operational errors during the procedure will effectively reduce the post-operative recovery time of the experimental fish, reduce the risk of wound infection, and improve the survival rate of the fish (Cooke et al. 2003, Wagner et al. 2011). Moreover, the suture needle may cause the suture to tear the tissue, which will affect the speed of wound healing, or even lead to wound rupture or death of the fish (Dunn & Phillips 2004, Panther et al. 2011, Smircich & Kelly 2014). Some types of ultrasonic pingers are not suitable for every kind of fish, and inappropriate size may lead to the loss of tags and an increase in fish mortality, which may distort research results (Bridger & Booth 2003, Hussey et al. 2015). Our current findings help to optimize the surgical protocol for transmitter implantation in fish such that the retention time of transmitters in fish can hopefully be extended and mortality of experimental fish due to operation procedures can be reduced.

Acknowledgements. This study was funded by the Science and Technology Innovation Fund of Dalian, China (2021JJ 11CG001), and the National Natural Science Foundation of China (No. 31672673). All animal work was conducted in conformity with institutional guidelines for the care and use of laboratory animals in Dalian Ocean University, Dalian, China, complying with animal ethics.

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Editorial responsibility: Helmut Segner, Bern, Switzerland
Reviewed by: M. J. Cabrera-Álvarez and 2 anonymous referees

Submitted: June 25, 2022
Accepted: February 28, 2023
Proofs received from author(s): April 15, 2023