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RESEARCH PAPER

Evaluation of water entry into the coelom and different levels of aseptic technique during surgical implantation of electronic tags in freshwater fish

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Abstract We reviewed the literature in an attempt to determine the importance of aseptic technique when implanting electronic tags in fish. Given that there was negligible information on this topic we embarked on a study where bluegill (*Lepomis macrochirus*) were used as a model to investigate the effects of different aseptic surgical techniques for the intracoelomic implantation of electronic tags in fish. First we tested the effects of water entry into the incision using five treatments: lake, distilled and saline water introduced into the incision, water-free controls, and non-surgery controls. For fish in the water treatments, 1 mL of the sample was introduced into their coelom prior to incision closure. Fish were held for 10 days to monitor survival and at the end of the study, the survivors were blood sampled and euthanized to evaluate condition and health using the health assessment index. In a second experiment, four aseptic treatments were used: non-sterile, field-based, high-grade sterility, and non-surgery controls and fish were monitored as in the first experiment. For both experiments, no differences in physiological status, health or mortality were noted among treatment groups. However, in the aseptic techniques experiment, surgical times were approximately twice as long for fish in the sterile treatment as compared to other groups and the costs of surgical supplies was greater than that of the less-sterile treatments. Although we failed to document any benefit of keeping water out of the incision or using aseptic technique for bluegill, in other situations and for other species, such approaches may be important. As such, we encourage fish surgeons practicing intracoelomic implants to attempt to prevent water entry into the coelom. We also encourage, at least some level of infection control (e.g., non-sterile gloves, clean tags and surgical tools) consistent with good veterinary practices to maintain the welfare status of tagged fish and ensure that the data from tagged fish representative of untagged conspecifics. However, the most prudent and ethical approach would be to work with veterinarians to incorporate formal sterilization procedures, equipment (e.g., sterile gloves) and aseptic technique into field surgical techniques.

Keywords Surgery · Aseptic technique · Transmitters · Implantation · Telemetry

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Introduction

Biotelemetry (e.g., radio, acoustic and passive integrated transponder technology) and biologging devices (herein collectively called "electronic tags") are increasingly being used to study the spatial

ecology of fish (Lucas and Baras 2000; Cooke et al. 2004; Block 2005; Cooke 2008). Currently there are three different approaches to the attachment of electronic tags on fish (i.e., gastric, external, and intracoelomic). Surgical intracoelomic approaches are the most common tagging approach for fish because the implanted tags cannot be regurgitated by the fish (like gastric tags), do not produce drag (like external tags), and although a small expulsion rate is expected, they are considered present for the lifetime of the fish which is important given the long battery life now being attained with many tags (Bridger and Booth 2003). Surgical implantation of tags is routinely conducted by fish biologists, often without formal training or guidance of veterinarians (Cooke and Wagner 2004). Although the techniques for surgical implantation are relatively simple, fish surgeries for the implantation of electronic tags have generally not been done to the same standards as would be expected in a clinical veterinary setting (Mulcahy 2003). This can be a problem in a research context, as it is necessary to minimize behavioural alterations, physiological disturbances, health impairments and mortality arising from surgery such that data obtained from tagged fish is reflective of untagged conspecifics (Wagner et al. 1999). However, there is little research to clarify which surgical protocols are necessary in field conditions to maintain this standard.

One of the aspects of surgical implantation that is poorly studied is the role of maintaining sterility during procedures. Indeed, an early goal of this study was to synthesize existing information on the topic. However, there is only a single study (i.e., Wagner et al. 1999) that has evaluated any aspect of aseptic technique. In that study, the authors compared the incision healing of rainbow trout for incision sites that were swabbed with iodine compared to those that were not and the results were inconclusive. Based on a survey of practicing fish surgeons, Wagner and Cooke (2005) determined that the majority of the respondents (73%) believed that maintaining sterile equipment between surgeries was important for the preservation of fish health. In addition, 78% of respondents suggested similarly that water should be kept from entering the incision site given that it can carry pathogens (Wagner and Cooke 2005). Although veterinary principles would suggest that keeping water out of the incision site and maintaining some level of sterility during procedures is important, these questions have never been evaluated in a scientific study. Clearly there is need for additional research on this topic.

To address the lack of studies regarding aseptic technique and water in the incision, we conducted two experiments related to infection control during the intracoelomic surgical implantation of electronic tags in fish. In the first experiment we evaluated the consequences of water entering the incision during tag implantation. Water can enter the incision in various ways; while cleaning the incision with water to wash away blood, water being splashed up from the gills when supplying the fish with oxygen, or by being present on surgical tools. The exposure of the incision to water can potentially increase the chance of introducing pathogens into the body, thereby increasing the fish's chance of infection. On the other hand, a surgical flush may be beneficial for the fish by removing blood and debris from the incision and possibly decreasing chance of infection, but this has not been investigated. We predicted that we would observe increased mortality, physiological disturbance, and health impairments in those fish injected with lake water relative to other treatments. By exploring these ideas we aimed to determine whether or not water entering the incision site was beneficial or detrimental to a fish's overall recovery and health. The second experiment examined aseptic technique and its influence on surgical outcome and the overall health of fish. Our approach involved comparing different degrees of aseptic techniques to determine what level is necessary to ensure successful implantations. We predicted that the degree of mortality and health impairments would be negatively correlated with the level of aseptic technique used for surgery. For both experiments we used bluegill (*Lepomis machrochirus*), a small bodied warmwater fish, as a model given their abundance, ease of holding in experimental tanks, and the fact that they have previously been the focal point of surgical tag implantation research (e.g., Gallepp and Magnuson 1972; Knights and Lasee 1996; Paukert et al. 2001) and implanted with electronic tags for studies of spatial ecology (e.g., Prince and Maughan 1978; Paukert and Willis 2002; Paukert et al. 2004).

Methods

Study site

All experiments took place at the Queen's University Biological Station on Lake Opinicon in eastern Ontario, Canada. Bluegill were collected from Lake Opinicon in June 2009 by rod and reel using barbless hooks. We retained all bluegill that were shallowly hooked and were at least 160 mm L_T. Fish were temporarily held in 50 L coolers supplied with fresh water during transport by boat to the research station. Fish were then placed into 100 L flow-through outdoor holding tanks and fasted for 24 h to ensure that fish were in a post-absorptive state prior to surgery. Following surgery, the fish were held for 10 days and fed a maintenance ration of fish pellets mixed with blood worms twice daily. The tanks were monitored regularly for the duration of the 10 day holding period. When a fish was observed to exhibit loss of equilibrium and reactivity during the holding period the fish was removed from the tank. After 10 days, a blood sample was obtained (see below) prior to euthanizing the fish via cerebral percussion to enable a post-mortem health assessment (see below). Two different experiments were performed, using new fish for each.

Experiment 1: Entry of water into incision

This experiment included five test groups ($N = 15$ per group); a control group that was anesthetised but not surgically implanted, a "water-free" group that received an implant but no water injection, as well as lake water (collected from Lake Opinicon), distilled, and saline (physiological saline) treatment groups that received an implant and were injected with 1 mL of water through the incision. The water-free test group surgery used a surgical drape to aid in keeping water out of the incision site.

All surgeries were performed on 26 June 2009. The fish were anesthetized individually in a 4 L receptacle containing a concentration 25 mg L^{-1} 3-aminobenzoic acid-ethyl-estermethanesulfate (MS-222) and 50 mg L^{-1} sodium bicarbonate ($NaHCO_3$). The fish were removed when the reactivity and reflexes of the fish were absent and opercular movements were slow (Wagner et al. 2000). Once sedated, all fish except controls were tagged with numbered anchor tags near the posterior aspect of the dorsal fin. The number of the tag, mass (to the nearest 0.01 g) and total length (to the nearest mm) of the fish were recorded. There was no significant difference in the size of fish among treatments (overall mean 174 ± 3 mm L_T; Table 1).

The fish were placed supine on a foam surgical board. During surgery, gills were continuously irrigated with re-circulating water containing a maintenance dose of MS-222 and $NaHCO_3$ at half the initial anaesthetic concentration. The surgeon wore non-sterile latex gloves that had been rinsed in a betadine solution and then rinsed again in distilled water. All surgical tools, dummy tags and sutures were cleaned with betadine and rinsed with distilled water before use. An incision (~1.5 cm) was made along the ventral midline. A 1 mL sample of the water treatment being tested was introduced directly into the peritoneal cavity via the incision site using a 1 mL syringe (except for fish in the water free-control group). A small dummy tag (4 mm × 6 mm; mass in air of 0.4 g) was then inserted into the coelom before closure. The incision was closed with 3 independent sutures using PDS II (3/0 with FSL needle), a synthetic absorbable monofilament, and tied with surgeon's knots. After the incision was closed the subject was immediately placed in a small recovery tank for approximately 30 min. When fish regained equilibrium they were placed into a single 1,000 L common garden tank for 10 days so survival could be evaluated. The same surgeon completed all of the surgeries.

On day 10 (or if fish died prior to day 10) a 1 mL blood sample was taken via caudal puncture from the caudal vessels using a 25 g, 1 mL heparinized syringe. The syringe was temporarily placed in a water–ice slurry prior to completing assays. Glucose samples were measured on whole blood using an Accu-chek portable field meter (Roche Diagnostics, Indianapolis, IN, USA) that has previously been calibrated for use on fish (Cooke et al. 2008). Leukocrit and hematocrit were quantified using a hematocrit centrifuge for 120 s. The buffy layer was observed under a microscope and measured under the 10× objective using an eyepiece micrometer and values were converted into μm and percent volume of white blood cells (WBC) to whole blood was calculated (i.e., leukocrit). The proportion of red blood cells as measured with a ruler was used to determine hematocrit. The remaining blood was

Table 1 Characteristics of bluegill in Experiment 1 (water wound study) relative to treatment group

Variables	Treatment Means ± SE (*N*)					Statistic values	
	Control	No H_2O	Distilled	Saline	Lake water	*P*	*F*
L_T (mm)	171 ± 3 (15)	174 ± 3 (14)	171 ± 3 (15)	171 ± 3 (15)	176 ± 3 (15)	0.637	0.638
Blood glucose (mmol L^{-1})	2.0 ± 0.1 (12)	2.1 ± 0.2 (11)	2.2 ± 0.1 (12)	2.2 ± 0.1 (12)	2.3 ± 0.1 (12)	0.425	0.983
Plasma protein (mmol L^{-1})	5.9 ± 0.3 (12)	5.8 ± 0.3 (11)	5.9 ± 0.3 (12)	5.9 ± 0.3 (12)	6.0 ± 0.3 (12)	0.998	0.033
Incision score	N/A	2.7 ± 0.3 (11)	2.9 ± 0.3 (12)	2.5 ± 0.3 (12)	2.8 ± 0.3 (11)	0.788	0.351
HAI score	99 ± 9 (12)	110 ± 9 (11)	110 ± 9 (12)	98 ± 9 (12)	128 ± 94 (12)	0.197	1.563
Leukocrit (% white blood cells)	1.7 ± 0.1^{AB} (12)	1.9 ± 0.1^{A} (11)	1.7 ± 0.1^{AB} (12)	1.0 ± 0.1^{B} (12)	1.6 ± 0.1^{AB} (12)	0.048	2.579
Hematocrit (% red blood cells)	26 ± 2 (12)	26 ± 2 (11)	30 ± 2 (12)	25 ± 2 (12)	28 ± 2 (12)	0.284	1.295
I_H (% body mass)	1.5 ± 0.2 (12)	1.6 ± 0.2 (11)	1.2 ± 0.2 (12)	1.4 ± 0.2 (12)	1.6 ± 0.2 (12)	0.543	0.780
Lysozyme (μg mL^{-1})	12 ± 3 (10)	10 ± 4 (6)	10 ± 4 (8)	18 ± 4 (7)	14 ± 4 (8)	0.437	0.781

Significant differences (as determined by ANOVA and Tukey Post Hoc Test; $\alpha = 0.05$) are noted by dissimilar letters. All parameters indicated as percentages were analyzed as (ArcSin Square Root(value)). HAI and lysozyme were analyzed using log values

centrifuged and plasma protein was quantified using a refractometer. Plasma was frozen in liquid nitrogen and stored at -80°C until a lysozyme assay was performed using Litwacks method (1995), modified by Maule et al. (1996). Briefly, a 0.3 mg mL^{-1} suspension of freeze-dried *Micrococcus lysodeikticus* (Sigma) was prepared in 0.1 M sodium phosphate (Na_2HPO_4) buffer. One hundred microliters of plasma was added to 100 μL of the bacterial suspension for a final volume of 0.5 mg mL^{-1} for the cells and an initial reading was taken from the microplate reader. Absorbencies were measured every 30 s for 20 min, and the rate of each reaction was determined using the linear portion of each reaction plot. Values were compared to a standard hem egg-white lysozyme (HEWL, Sigma) curve to determine lysozyme activity (μL mL^{-1}). The fish were euthanized immediately after blood sampling by cerebral percussion. The incision site was visually analyzed post-mortem for inflammation. An incision score was assigned to each fish based on a scale of 0–6 developed by Wagner et al. (2000): 0, incision completely closed, no inflammation; 1, incision closed, some inflammation along incision site (up to 10%); 2, incision closed, little to moderate inflammation (10–50%); 3, incision held in proximity, but edges slide if fish moves, moderate inflammation (up to 50%); 4, incision partially opened at one end or middle, moderate to high inflammation (up to 100%); 5, More than 50% of incision open, moderate to high inflammation along incision edges (up to 100%); 6, completely open incision, high to severe inflammation along edges (up to 100%). After the incision assessment, the fish were necropsied using the health assessment index (HAI; Adams et al. 1993). The HAI is a simple and quick means of assessing fish health and condition (Barton et al. 2002) in which multiple variables are assigned a numerical value. The fish were examined externally as well as internally for abnormalities and an overall health score, including macroscopically visible parasite density, was tabulated for each fish. Hepatosomatic index (I_H) was calculated after measuring the liver mass to the nearest 0.01 g and expressing it as a proportion of total body mass as an indication of energy reserves in each fish.

Experiment 2: Variation in surgical sterility

The general materials and methods for the second experiment were similar to the first experiment except that water was not introduced into the incision. However, the degree of sterility was manipulated. All

surgeries were performed on 27 June 2009 and involved four different test groups ($N = 15$ per group). The first test group had the highest grade of sterility (herein called "sterile"). The foam surgical board was soaked with gluteraldehyde for a 24 h prior surgery and rinsed with distilled water. In between surgeries the foam board was cleaned with a betadine solution. A fresh pair of sterile gloves were used for each successive surgery and placed on the hands of the surgeon by an assistant so that they were not contaminated. The dummy tags were sterilized 24 h prior to surgery using gluteraldehyde, and the surgical tools were sterilized via autoclave for at least 1 h. A fresh (sterile) suture package was used for each fish and no contact between the fish and the surgeon was allowed at all. All surgical tools remained in autoclave packets until used for surgery; the assistant would peel back the packet, and the surgeon would grasp the tool to maintain sterility. A surgical drape (new drape for each fish) with a small window over the incision site was present during surgery to aid in preventing water from entering the incision site. The second test group received aseptic techniques reminiscent to those used in the field (herein called "field"). Non-sterile latex gloves were used and rinsed with betadine between surgeries. The dummy tags, surgical tools, and sutures were cleaned in betadine and then rinsed with distilled water before surgical use. In this treatment, contact between the surgeon and the fish was allowed as well as contact between the suture and the fish. The same sutures were reused on multiple fish after cleaning with betadine. The third test group was subjected to the unsterile conditions (herein called "non-sterile"). No gloves were used and the dummy tags were not sterilized. The surgical tools were left unsterilized from previous treatments surgeries and reused for each consecutive surgery. Fleshy pieces and mucus left from the previous subjects was removed from the surgical tools with a lake water rinse. Leftover sutures from previous fish were not sterilized and reused multiple times. The final test group acted as a control and was anaesthetized but underwent no surgery. The duration of the surgery time was measured to the nearest second. Unlike experiment 1, fish were held in three separate smaller tanks (150 L each). Five fish from each treatment were introduced into each tank. Fish were held for 10 days and sampled as above.

Statistical analysis

We tested whether the physiological status, condition (e.g., HAI, I_H), and size (e.g., L_T) of fish varied among treatments for both experiment 1 and experiment 2 using a one way ANOVA. When significant differences were noted a Tukey HSD test was used to identify where differences occurred. Mortality patterns for each experiment was analysed using the Cox, Log-Rank Survival Analysis. All analyses were conducted with JMP V7 (Carey, NC) and significance was evaluated at an alpha of 0.05. All data presented are means ± SE unless otherwise stated.

Results

Experiment 1: Entry of water into incision

Sixteen of the 75 fish in the experiment died during the 10 day holding period (Fig. 1). The majority of the mortalities occurred 3–4 days post surgery, including control fish, and stabilized in all groups at approximately 20% mortality. There was no significant difference in mortality among treatments groups ($\lambda^2 = 0.729$, $P = 0.948$; Fig. 1). Similarly, none of the physiological or condition-based metrics varied among treatments (Table 1). However, leukocrit, an

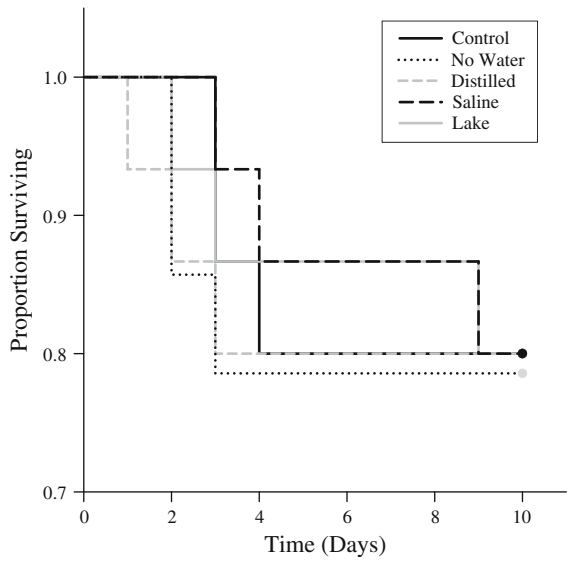

Fig. 1 Survival patterns depicting the loss within each treatment group throughout the 10 days holding period for Experiment 1 (water wound study)

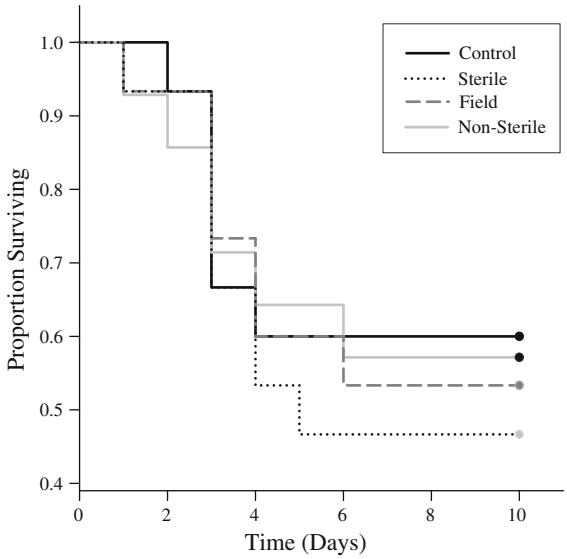

Fig. 2 Survival patterns depicting the loss within each treatment group throughout the 10 day holding period for Experiment 2 (variation in surgical sterility)

indicator of fish health, was lowest for fish in the saline treatment (overall ANOVA model $P = 0.05$; Table 1). Overall, the HAI scores did not reveal variation in fish health among treatment groups. Incision scores for fish with incisions (i.e., excluding non-surgical controls) tended to be level "3" (Table 1) indicating that the incision is held in proximity but the edges are able to slide (Wagner et al. 2000).

Experiment 2: Variation in surgical sterility

A total of 28 fish (out of 60) in the aseptic experimental group died during the 10 days holding period (Fig. 2). As in experiment 1, the majority of the mortality occurred between 3 or 4 days post-surgery, even for fish in the control treatment. Mortality tended to stabilize between 40 and 50% in the treatment groups and no significant differences were noted ($\lambda^2 = 1.517$, $P = 0.678$; Fig. 2). None of the physiological, condition-based or health metrics varied among treatments (Table 2). The incision scores tended to be level "2" (Table 2) indicating that the incisions were closed with little to moderate inflammation (Wagner et al. 2000). The sterile surgeries took significantly longer ($\sim$60 s longer, not including surgical preparation time) than both the field and non-sterile test groups (Table 2).

Discussion

To date, there have been relatively few studies that have attempted to evaluate the effects of different levels of sterility for the intracoelomic implantation of electronic tags in fish (Cooke et al. 2011). Here we evaluated the consequences of introducing different types of water (e.g., lake water, distilled water and physiological saline) into a surgical incision. We also evaluated the consequences of using different levels

Table 2 Characteristics of bluegill in Experiment 2 (variation in surgical sterility) relative to treatment group

Variables	Treatment Means ± SE (*N*)				Statistic values	
	Control	Non-Sterile	Field	Sterile	*P*	*F*
L_T (mm)	172 ± 3 (14)	175 ± 3 (15)	176 ± 3 (15)	172 ± 3 (15)	0.616	0.603
Blood Glucose (mmol L^{-1})	3.3 ± 0.6 (9)	2.1 ± 0.6 (8)	2.3 ± 0.6 (8)	2.6 ± 0.6 (7)	0.479	0.848
Plasma protein (mmol L^{-1})	6.1 ± 0.2 (9)	5.7 ± 0.2 (8)	5.8 ± 0.2 (8)	6.5 ± 0.3 (7)	0.084	2.458
Incision score	N/A	2.4 ± 0.4 (8)	2.3 ± 0.4 (8)	2.5 ± 0.4 (7)	0.893	0.114
HAI score	101 ± 10 (9)	121 ± 10 (8)	115 ± 10 (8)	126 ± 11 (7)	0.224	1.550
Leukocrit (% white blood cells)	2.1 ± 0.2 (9)	2.4 ± 0.3 (7)	2.1 ± 0.3 (8)	2.8 ± 0.3 (7)	0.184	1.731
Hematocrit (% red blood cells)	26 ± 2 (9)	27 ± 2 (7)	28 ± 2 (8)	26 ± 2 (7)	0.847	0.269
I_H (% body mass)	1.7 ± 0.3 (9)	1.5 ± 0.3 (8)	1.8 ± 0.3 (8)	2.0 ± 0.3 (7)	0.686	0.499
Lysozyme (μg mL^{-1})	28 ± 6 (8)	16 ± 6 (7)	8 ± 7 (6)	10 ± 6 (7)	0.439	0.728
Surgery time (s)	N/A	127 ± 8^a (15)	137 ± 8^a (14)	200 ± 8^b (15)	<0.001	24.042

Significant differences (as determined by ANOVA and Tukey Post Hoc Test; $\alpha = 0.05$) are noted by dissimilar letters. All parameters indicated as percentages were analyzed as (ArcSin Square Root(value)). HAI and lysozyme were analyzed using log values

of sterility during surgical procedures that ranged from reusing surgical tools with minimal cleaning between surgeries and failing to wear surgical gloves to our best attempt at achieving sterility in a field environment. We relied on a variety of endpoints including mortality and incision healing that are commonly used in the study of tagging effects (Cooke et al. 2011) as well as indicators of fish physiological status (e.g., glucose), condition (e.g., plasma protein, HSI) and health (using an HAI). Despite using a range of endpoints and monitoring fish over a 10 day period, we failed to detect any consistent trends associated with treatments. Indeed, fish exposed to a variety of surgical procedures, including those that would be regarded as being inconsistent with "good" veterinary practice tended to fare similarly to control fish. Here we discuss the findings from the study, provide context for the results, and present recommendations related to water entry in the incision and different levels of sterility for the intracoelomic implantation of electronic tags in fish.

Entry of water into incision

Water can enter the incision either accidentally (e.g., splashing, getting water on surgical tools) or by intentionally flushing the incision to wash away blood. The exposure of the incision to water could potentially increase the chance of introducing pathogens into the body, thereby increasing the fish's chance of infection. On the other hand, a surgical flush may be beneficial for the fish by removing blood and debris from the incision and possibly decreasing chance of infection. With our results we failed to detect any significant benefit or serious negative consequences of the flush procedure. Mortality patterns were similar among control fish (with no surgery), water-free surgeries, and those fish that had various types of water intentionally introduced into the incision.

The only notable endpoint that was significant was leukocrit which was lowest in the saline treatment. Teleost fish possess various components that make up non-specific immunity that act as a first defence to invading pathogens. Leukocrit is a measure of non-specific immune function and represents the volume (by percentage) of leukocytes (white blood cells) in whole blood (Wang et al. 2006). A priori we had predicted that we would observe increased mortality, physiological disturbance, and health impairments in those fish injected with lake water but our data were inconsistent with that prediction. Indeed, the observation that fish treated with physiological saline had significantly lower leukocrit is inconsistent with our prediction and any rational biological explanation. None of the other metrics including the HAI which is regarded as an integrated measure of fish health indicated that fish health was negatively impacted by introducing water into the incision. Unfortunately there is no literature on the topic of introducing different types of water into the body cavity of fish, although veterinary principles suggest that if water is to be used in associated to the incision site (e.g., to swab the area to reduce contamination)it should be sterile physiological saline (Stoskopf 1993; Harms and Lewbart 2000; Murray 2002). Physiological saline is routinely used in laboratory experimentation (e.g., lengthy and invasive surgeries such as gonadectomies; Brown and Richards 1979; Summerfelt and Smith 1990) or clinical surgical procedures (Stoskopf 1993; Harms and Lewbart 2000; Murray 2002; Harms 2005).

Variation in surgical sterility

Throughout the holding period the mortality was similar across treatments (approx. 45%), including non-surgical controls, and there was no evidence that fish survival, physiological condition, or health varied relative to the degree of sterility. During the 10 day holding period each experimental group suffered significant losses, more so than in experiment 1. The experiment took place just after the bluegill spawning period and began 1 day after experiment 1. In experiment 2 fish were held in smaller tanks than in experiment 1 which may have led to crowding stress which could magnify mortality (Barton and Iwama 1991). The smaller tanks were also on the same flow-through system as the larger tanks with complete turnover several times per hour.

The only significant difference that we noted was for the duration of the surgical procedure. The sterile procedure required approximately 1 min longer to complete than the non-sterile and field-sterile techniques. The prolonged surgical time was a result of the surgeon being unable to touch the fish, the need to manage the suture material to prevent contact with

the fish or water, and the need for all tools to be handed to the surgeon by an assistant. While under anaesthesia, fish are affected directly by reduced cardiac output and reduced gill profusion which can lead to hypoxia and even death (Hill et al. 2002). It is generally believed that there is merit in reducing surgical times (Mulcahy 2003; Copeland et al. 2008). Indeed, the duration of surgery has been used as an endpoint or measure of surgical success in a number of studies (e.g., Cooke et al. 2003, 2011). It is likely that with additional training and experience that the surgical time for sterile surgeries could be reduced. However, it is still noteworthy that the sterile technique does take longer to complete and thus could result in more complications and surgeon fatigue.

Although not part of a formal statistical analysis, we did calculate the relative costs of the consumables and capital expenses needed for the various levels of surgical sterility (Table 3). It is clear that the field-sterility and non-sterility treatments have similar costs (both consumables and capital expenditures). However, the sterile technique required nearly five times the cost in consumables (as a result of using sterilant, new suture packages for each fish, new disposable scalpel for each fish, new sterile gloves for each fish, and a surgical drape) and significant (more than $2,000 CDN) capital expenses for multiple sets of tools and an autoclave. The multiple sets of tools are needed such that they can be constantly cycled through the autoclave without having to stop surgeries for extended periods. Although fish welfare should not be compromised as a result of financial decisions, the reality is that fisheries science and management is limited by budgets so justification for different approaches can be useful for obtaining funding. In the case of this experiment, we found limited evidence that there was a significant benefit of using sterile techniques since costs were higher and the surgery time longer. However, in other situations (e.g., species, season, environment, etc.), maintaining sterility may be of critical importance. Furthermore, Mulcahy (2003) has suggested that implanting devices in fish without using sterilization and aseptic technique is an inhumane act.

Conclusions and recommendations

Although we failed to detect differences in surgical outcome for fish that had different types of water introduced into their surgical incision or surgeries that used different levels of sterility, we do not believe that it is prudent or ethical to abandon attempts to maintain sterile conditions even in field settings. For this experiment we used a single warmwater fish species from a one water body over a relatively short period of time. Although we did not explicitly quantify the pathogen burden in the lake water (which was introduced into the incision in some

Table 3 Cost differences between groups showing both the cost per fish as well as the capital costs

Level of sterility	Consumables	Consumable costs (per fish)	Capital needs	Capital costs (per fish)
Non-sterile	Sterile suture material (that was reused)	$1.28	One set of surgical tools	$95.00
Field-based sterility	Non-sterile gloves	$1.39	Two sets of surgical tools	$210.00
	Betadine			
	Sterile disposable scalpels (used on three fish but cleaned between)			
	Sterile suture material (that was reused)			
Sterile	Sterile surgical gloves	$6.30	Autoclave	$2135.00
	Gluteraldehyde		Ten sets of surgical tools	
	Sterile disposable scalpels (new one for each fish)			
	Sterile suture material (new one for each fish)			
	Surgical sterile drape			

Cost estimates are in CDN dollars

cases) or in the fish (pathogens could be transferred among fish in some cases—e.g., non-sterile surgery), it is reasonable to assume that in some situations it may be possible that pathogens could lead to infections that could influence fish health, condition and survival. As such, we suggest that efforts to prevent the introduction of ambient water into the surgical incision should be adopted such as the use of a surgical drape. Surgical drapes are not only useful for keeping the moisture out; they also retain moisture around the fish (Harms 2005). If it is essential to flush an incision, we would encourage the use of small amounts of sterile, physiological saline, given that this is the fluid recommended for use in veterinary and medical practices (Stoskopf 1993; Harms and Lewbart 2000; Murray 2002).

In terms of the level of sterility during surgical procedures, we did note that the duration of surgery was prolonged if attempts were made to use completely sterile and aseptic techniques in field settings. Furthermore, the costs of surgical supplies were higher and there was need for more capital expenditures to enable one to attempt to maintain sterile conditions in a field setting. However, the economic costs are reasonably minimal given the potential greater cost of collecting data on fish movement and survival that are confounded by impairments from infection (Mulcahy 2003). However, slower surgery times could lead to complications as they require that animals be sedated for longer time periods. At present there is insufficient information to determine the relative tradeoffs between attempting to maintain sterile conditions which could lengthen surgery times versus the benefits that could be accrued from preventing introduction of pathogens. As such, we encourage practicing fish surgeons to work with veterinarians to develop surgical protocols that attempt to balance logistic and ethical concerns. It is worth noting that the use of semi-sterile (termed "field" here) techniques such as non-sterile surgical gloves and cleaning tools with betadine is relatively inexpensive and can be easily adopted in all field settings. In reality, these techniques are not sterile per se, but they do incorporate methods of disinfection. As such, that level of cleanliness should serve as a minimum and where possible techniques should be adapted to achieve a higher level of sterility (i.e., true sterility and aseptic technique) recognizing that complete sterility is difficult in field settings on wild fish. Given that this study represents one of the first to examine the role of sterility in surgical procedures for fish, we echo the recommendations of Cooke et al. (2011) and encourage additional research on this topic using a range of species and environmental conditions. Future studies should consider prolonging the holding time and quantifying or experimentally manipulating pathogen burden in water, in or on fish, and on surgical equipment.

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References

Adams SM, Brown AM, Goede RW (1993) A quantitative health assessment index for rapid evaluation of fish condition in the field. Trans Am Fish Soc 122:63–73

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26

Barton BA, Morgan JD, Vijayan MM (2002) Physiological and condition-related indicators of environmental stress in fish. In: Adams SM (ed) Biological indicators of stress in fish. American Fisheries Society, Bethesda, pp 111–148

Block BA (2005) Physiological ecology in the 21st century: advancements in biologging science. Integr Comp Biol 45:305–320

Bridger CJ, Booth RK (2003) The effects of biotelemetry transmitter presence and attachment procedures on fish physiology and behaviour. Rev Fish Sci 11:13–34

Brown LA, Richards RH (1979) Surgical gonadectomy of fish: a technique for veterinary surgeons. Vet Rec 104:215

Cooke SJ (2008) Biotelemetry and biologging in endangered species research and animal conservation: relevance to regional, national, and IUCN Red List threat assessments. Endanger Species Res 4:165–185

Cooke SJ, Wagner GN (2004) Training, experience, and opinions of researchers who use surgical techniques to implant telemetry devices into fish. Fisheries 12:10–18

Cooke SJ, Graeb BDS, Suski CD, Ostrand KG (2003) Effects of suture material on incision healing, growth and survival of juvenile largemouth bass implanted with miniature radio transmitters: case study of a novice and experienced fish surgeon. J Fish Biol 62:1360–1388

Cooke SJ, Hinch SG, Wikelski M, Andrews RD, Wolcott TG, Butler PJ (2004) Biotelemetry: a mechanistic approach to ecology. Trends Ecol Evol 19:334–343

Cooke SJ, Suski CD, Danylchuk SE, Danylchuk AJ, Donaldson MR, Pullen C, Bulte G, O'Toole A, Murchie KJ, Goldberg TL (2008) Effects of capture techniques on the physiological condition of bonefish (*Albula vulpes*) evaluated using field physiology diagnostic tools. J Fish Biol 73:1351–1375

Cooke SJ, Woodley CM, Eppard MB, Brown RS, Nielsen JL (2011) Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Rev Fish Biol Fisheries. doi:10.1007/s11160-010-9193-3

Copeland SE, Ladd LA, Gu XQ, Mather LE (2008) The effects of general anesthesia on the central nervous and cardiovascular system toxicity of local anesthetics. Anesth Analg 106:1429–1439

Gallepp GW, Magnuson JJ (1972) Effects of negative buoyancy on the behavior of the bluegill, *Lepomis macrochirus Rafinesque*. Trans Am Fish Soc 101:507–512

Harms CA (2005) Surgery in fish research: common procedures and postoperative care. Lab Anim 34:28–34

Harms CA, Lewbart GA (2000) Surgery in fish. In: Bennett RA (ed) Veterinary clinics of North America: exotic animal practice. Saunders Co., New York, pp 759–774

Hill JV, Davison W, Forster ME (2002) The effects of fish anaesthetics (MS222, metomidate and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*). Fish Physiol Biochem 27:19–28

Knights BC, Lasee BA (1996) Effects of implanted transmitters on adult bluegills at two temperatures. Trans Am Fish Soc 125:440–449

Litwack G (1955) Photometric determination of lysozyme activity. Proc Soc Exp Biol Med 89:401–403

Lucas MC, Baras E (2000) Methods for studying the spatial behaviour of freshwater fishes in the natural environment. Fish Fish 1:283–316

Maule AC, Schrock R, Slater C, Fitzpatrick MC, Schreck CB (1996) Immune and endocrine responses of adult chinook slamon during freshwater immigration and sexual maturation. Fish Shellfish Immunol 6:221–233

Mulcahy DM (2003) Surgical implantation of transmitters into fish. ILAR J 44:295–304

Murray MJ (2002) Fish surgery. Sem Avian Exotic Pet Med 11:246–257

Paukert CP, Willis DW (2002) Seasonal and diel habitat selection of bluegills in a shallow natural lake. Trans Am Fish Soc 131:1131–1139

Paukert CP, Chvala PJ, Heikes BL, Brown ML (2001) Effects of implanted transmitter size and surgery on survival, growth, and wound healing of bluegill. Trans Am Fish Soc 130:975–980

Paukert CP, Willis DW, Bouchard MA (2004) Movement, home range, and site fidelity of bluegills in a Great Plains lake. N Am J Fish Manage 24:154–161

Prince ED, Maughan OE (1978) Ultrasonic telemetry technique for monitoring bluegill movement. Prog Fish Cult 40:90–93

Stoskopf MK (1993) Surgery. In: Stoskopf MK (ed) Fish medicine. Saunders, Philadelphia, pp 91–97

Summerfelt RC, Smith LS (1990) Anesthesia, surgery, and related techniques. In: Schreck CB, Moyle PB (eds) Methods in fish biology. American Fisheries Society, Bethesda, pp 213–272

Wagner GN, Cooke SJ (2005) Methodological approaches and opinions of researchers involved in the surgical implantation of telemetry transmitters in fish. J Aquat Anim Health 17:160–169

Wagner GN, Stevens D, Harvey-Clark C (1999) Wound healing in rainbow trout following surgical site preparation with a povidone-iodine antiseptic. J Aquat Anim Health 11:373–382

Wagner GN, Stevens D, Byrne P (2000) Effects of suture type and patterns on surgical wound healing in rainbow trout. Trans Am Fish Soc 129:1196–1205

Wang WB, Wang YP, Hu W, Li AH, Cai TZ, Zhu ZY, Wang JG (2006) Effects of the "all-fish" growth hormone transgene expression on non-specific immune functions of common carp, *Cyprinus carpio* L. Aquaculture 259:81–87