Fisheries Research 156 (2014) 1–5

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Letter to the Editor

Experimental methods fail to address the questions posed in studies of surgical techniques

Keywords:
Surgery
Aseptic technique
Implantation
Infections
Transmitters

Recently, Jepsen et al. (2013) questioned the necessity for aseptic surgical techniques for implanting "tags" in fish. Their article joins others that are clearly intended to establish a basis to exempt fish surgeries from the rigor of surgical techniques used in other animals (e.g., Chomyshyn et al., 2011; Cooke et al., 2011; Walker et al., 2013a,b). These papers challenge the doctrines of aseptic surgery that were first published by Joseph Lister almost 150 years ago (Lister, 1867). Interestingly, some of these papers, like Jepsen et al. (2013), continue to state support for aseptic surgical principles, even as they conclude that such techniques are unnecessary. Space limitations will not allow us to deal with all of the problems common to this series of papers; herein we will focus on the paper by Jepsen et al. (2013) with some comments about a similar paper by Chomyshyn et al. (2011) that is referred to three times by Jepsen et al. (2013). We direct our criticisms on two main areas: (1) aseptic technique was not used where it was purported to be used, and (2) the method used to detect infections was not sufficient to identify their presence. To be clear, we do not object to experiments meant to clarify surgical procedures for fish to improve results and speed healing. We do object to overly simplistic attempts to prove that asepsis is unnecessary for fish as done by Jepsen et al. (2013).

Jepsen et al. (2013) purport to test two levels of surgical asepsis in implanting juvenile salmon: aseptic ("clean") and non-aseptic ("dirty"). In reality, they compare one dirty procedure to another dirty procedure, because their aseptic group includes a mixture of sterile and non-sterile techniques. Aseptic technique requires the use of sterile instruments and sterile gloves for each animal. Their surgeon wore non-sterile gloves. Initially, they autoclave-sterilized their transmitters and instruments, but between fish they merely dipped the instruments and scalpel blades in alcohol, which at best is only poor disinfection, not sterilization. The use of the sterile transmitters was the only real difference between groups, as the autoclaved surgical instruments were no longer sterile after the first fish, or even after the surgeon touched them with his non-sterile gloves. Jepsen et al. (2013) did not test what they claim to have tested. Ignorance of essential components of aseptic technique can be found elsewhere in the "anti-asepsis" papers. For example Chomyshyn et al. (2011) attempted to compare different levels of asepsis during fish surgeries and bemoaned the extra time required by the aseptic technique, part of which they blamed on the need to have all of the tools handed to the surgeon by an assistant. We have performed thousands of field surgeries and have never required an assistant to dispense surgical instruments. Being handed instruments is not required as part of aseptic technique. Physician-surgeons often do have assistants hand them instruments but that is to permit them unbroken focus on their surgical site. Mischaracterization of aseptic technique invalidates the claims and conclusions of the authors of these papers that they are comparing aseptic technique to non-aseptic technique.

If biologists wish to test the influence of aseptic technique on the prevalence of post-surgical infections, must they not do so when the opportunities for such infections are known to be present? Jepsen et al. (2013) and others claim that infections do not occur as a result of transmitter implantation surgeries. They then claim to test the effect of surgical technique on the occurrence of infections that they say do not occur. A proper test for whether or not there is a difference between aseptic techniques and "dirty" techniques in the occurrence of post-operative infections would be done in a situation where there is a known occurrence of post-operative infections. Similarly, Chomyshyn et al. (2011) failed to document that there was any chance for an infection to occur by demonstrating that fish pathogens were present in the lake water that they instilled into the coeloms of the fish they were implanting. Instead, they conclude that getting surface water into the surgical incision does not matter, because they did not see signs of infection. Especially when working with otherwise normal fish, it is naïve to think that every, or even most, fish submitted to surgery should die if non-aseptic techniques are used for surgery. Yet that is the basis for the experimental design in all of these papers. In a study of 1010 surgical interventions in dogs and cats, surgical site infections occurred in only 1.5% of otherwise healthy animals, but in 5.6% of severely debilitated animals (Eugster et al., 2004). These clinical cases were naturally conducted under aseptic conditions, but if prevalence of surgical site infections is low, ability to detect statistically significant differences among treatment groups with sample sizes of only 25, as employed in Jepsen et al. (2013), is poor even if proper techniques for detecting effect were used. A difference between six (dirty) and four (clean) mortalities, or seven (trailing antenna) and three (no trailing antenna) mortalities, in conjunction with a water quality problem, could not be statistically significant with these small sample sizes. Even though those differences may appear marginal at first glance, if they carried through with larger sample sizes, they could be statistically significant, financially significant, data quality significant, and animal use and welfare significant, differences long since considered unacceptable in other animal taxa and human surgeries.

The anti-asepsis group will say that, in their experience, infections following transmitter implantation surgeries do not occur. Our reply is three-fold. First, they are mainly concerned with

http://dx.doi.org/10.1016/j.fishres.2014.02.028
0165-7836/Published by Elsevier B.V.

lethal infections. There is far too much emphasis in telemetry papers on death as an endpoint to test quality or success, as it ignores the greater likelihood of non-lethal effects ("morbidity"), including some infections. Fish suffering from non-lethal infections may yield erroneous data; dead fish do not (Mulcahy, 2013). Infected fish may behave differently, for example, by seeking warmer water to boost their inflammatory, immune responses to pathogens (Boltaña et al., 2013; Gräns et al., 2012; Reynolds, 1977; Reynolds et al., 1978). Fish with sub-lethal infections may incur greater energetic costs (Muchlinski, 1985) or suffer increased susceptibility to predation (Johnson et al., 2006; Mesa et al., 1998). Second, the methods used to detect infections are inadequate. Jepsen et al. (2013) used no microbiological techniques, no histopathological techniques, no white blood cell counts, no arrays of serum chemistries that could have indicated the presence of infection. You cannot find what you do not seek (using the proper techniques). In their Discussion, even Jepsen et al. (2013) admit "However, no bacterial cultures were collected from the fish so we cannot exclude the possibility of a bacterial infection." Third, it is very possible that other researchers have seen large-scale post-surgical mortality of their fish but did not recognize or characterize it, subsequently failed to report what they perhaps perceived as a failed experiment, a process referred to as the "file drawer effect" (Bauchau, 1997; Csada et al., 1996).

There are many books and journals devoted in whole or in part to diseases of fish. Clearly, infectious diseases occur in fish and it is inconceivable that infections of surgical wounds do not occur at some level. Researchers who implant transmitters very rarely test their fish for subsequent infections. If they make any effort at all, most, like Jepsen et al. (2013), merely visually examine the outside and inside of their fish and make judgments based on what they see. While the visual inspection method is inadequate, if Jepsen et al. (2013) accept that method for their own work, then they must also accept the evidence of infections in transmitter-implanted fish observed by other researchers using the same visual inspection method (Bauer, 2005; Caputo et al., 2009; Chisholm and Hubert, 1985; Daniel et al., 2009; Isely et al., 2002; Knights and Lasee, 1996; Martin et al., 1995; Matheney and Rabini, 1995; Mortensen, 1990; Schulz, 2003; Skov et al., 2005; Stakènas et al., 2009; Swanberg et al., 1999; Walsh et al., 2000). Walsh et al. (2000) explored variations in implantation technique using hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) at two water temperatures. They found that peritoneal infections occurred in 46–51% of the fish held at high water temperature and 3–5% of the fish held at low water temperature. They used "the presence of viscous pale-pink fluid in a sack around the transmitter or in the body cavity." as an indicator of infection (Walsh et al., 2000). Their descriptions sound similar to the encapsulation of transmitters described by Jepsen et al. (2013) in two-thirds of their implanted fish. In an action rarely done in the fish implantation literature, Walsh et al. (2000) submitted the fluid from several fish to a laboratory and reported the isolation of the bacterium *Escherichia coli* from the fluid. No mention was made of the numbers of bacteria isolated or the isolation of other species of bacteria and no additional laboratory results characterizing the fluid were given. Knights and Lasee (1996) reported that bacteria of several genera (*Proteus*, *Citrobacter*, and *Acinetobacter*) were cultured from skin and organs of both implanted and control fish. In fish held at 6 °C, *Saprolegnia* and bacteria of the genus *Pseudomonas* were dominant; *Saprolegnia* was not isolated from fish at 20 °C. Histopathology showed granulation tissue around implanted transmitters as well as the presence of exudate, hemorrhage, mucus cells, and muscle necrosis. Boone et al. (2013a) used a high level of aseptic technique to implant Siberian sturgeons (*Acipenser baeri*). Upon necropsy, there was no histopathologic evidence of infection, although apparently no culturing for bacteria was done

The use of antibiotics provides some interesting, albeit indirect, information about bacterial infections in implanted fish. Antibiotic treatment of hybrid striped bass implanted with transmitters with trailing antennas delayed the onset of mortality for an average of two weeks, but ultimately, cumulative mortality matched that of fish that were not treated with an antibiotic (Isely et al., 2002). No necropsies, microbiology, histopathology, or clinical pathology was done. The apparent initial effectiveness of the antibiotic in delaying the onset of mortality suggests that bacteria were involved in the deaths of the fish. Presumably, when the effective concentration of the antibiotic in the fish was reduced over time through metabolism and excretion, bacterial infection was no longer suppressed, eventually killing the fish. Two other studies that treated fish undergoing surgery with an antibiotic and that had an experimental group that did not receive antibiotics experienced no differences in mortality rate (Bart and Dunham, 1990; Lucas, 1989). In these cases, antibiotic use failed to affect mortality, possibly because there were no fish pathogens in the experimental set-up, or there was insufficient contamination of the surgical site to reach the threshold numbers of bacteria required to initiate the infection, or the choice, dosage, or route of administration of the antibiotic was wrong, or single doses of the antibiotics were not effective, or the fish died from non-infectious causes before infections could kill them, or the fish were infected but the infection was not lethal. The absence of proper microbiological and other testing of fish in reports such as Jepsen et al. (2013) ensures that post-surgical infections will not be detected and characterized.

Sizable mortalities of fish occurred, amounting to 10% of the experimental group (and an unstated proportion of fish in other groups) in Jepsen et al. (2013) and 40–54% (from Fig. 2; note that the survival rates in Fig. 2 and the mortality rates given in the text do not correspond) of all groups of fish in Chomyshyn et al. (2011). In neither case is the mortality event characterized. Chomyshyn et al. (2011) say nothing about the cause of the deaths, apparently content that it was very roughly equal between their groups, and Jepsen et al. (2013) dismiss it as "...a problem with water quality...". Neither group of researchers considered that such mortality could have interfered with their results, being content that statistical tests found no difference in mortality rates between the groups. We suggest that, when trying to measure mortality rates between experimental groups, if up to half of all of your fish die from some unknown cause, you no longer have a valid experiment.

Jepsen et al. (2013) used multifilament suture material to close incisions, despite ample evidence that this suture material is highly reactive in a variety of fish species (Boone et al., 2013b; Deters et al., 2010; Hurty et al., 2002; Ivasauskas et al., 2012; Wagner et al., 2000). Other reports have described little visual difference in tissue reaction between monofilament and multifilament suture, possibly reflecting variation among fish species (Jepsen et al., 2008) but these reports are in the minority. The simple interrupted pattern used by Jepsen et al. (2013) itself can contribute to the inflammatory response judged both visually and histopathologically, even when monofilament suture material is used (Nematollahi et al., 2010). The use of a type of suture material and a suture pattern that are documented as producing inflammation means that the use of a visual inspection method for detecting infection is compromised.

A survey of biologists and veterinarians who do fish surgeries revealed that 73% of them believed that using only sterile equipment between fish was important and that 78% felt that water should be kept out of the incision because of the possibility of transferring pathogens to the fish (Wagner and Cooke, 2005). Many transmitter implantations in fish have been accompanied by the use of a variety of antibiotics applied in different ways (Mulcahy, 2011).