Journal of Aquatic Animal Health
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uahh20

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Version of record first published: 09 Jan 2011.

To cite this article: Glenn N. Wagner, E. Don Stevens & Chris Harvey-Clark (1999): Wound Healing in Rainbow Trout following Surgical Site Preparation with a Povidone-Iodine Antiseptic, Journal of Aquatic Animal Health, 11:4, 373-382

To link to this article: http://dx.doi.org/10.1577/1548-8667(1999)011<0373:WHIRTF>2.0.CO;2

Journal of Aquatic Animal Health 11:373–382, 1999

Wound Healing in Rainbow Trout following Surgical Site Preparation with a Povidone–Iodine Antiseptic

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Abstract.—We investigated the effects of preparing surgical incision sites with a topical antiseptic on wound healing and hematological response in rainbow trout *Oncorhynchus mykiss*. A povidone–iodine solution was applied both pre- and postsurgery to the incision sites on treated fish. Three-centimeter incisions in both treated ($N = 9$) and control (nontreated, $N = 9$) fish were closed with four nonabsorbable sutures sewn in a simple interrupted pattern. During the 42-d period of wound healing, there were no statistically significant changes in total erythrocyte counts (1.28×10^6/mm^3 $\pm$ 0.05 SE), in percentage of dividing erythrocytes (0.76% $\pm$ 0.07 SE), or in differential leukocyte counts. Postmortem, pathogenic bacterial infections in the kidney or spleen were not detected in any of the fish. There was no histological difference between control and treated incisions to show either beneficial or adverse tissue reactions to the topical antiseptic treatments. Blinded histological analysis revealed both treated and untreated incision sites healed within 42 d at the same rate. Therefore, preparation of the incision sites with a povidone–iodine antiseptic did not improve wound healing nor alter healing rate in rainbow trout under the conditions of this study.

Much research has been performed during the last 20 years on the processes involved in wound healing in fish (Phromsuthirak 1977; Bullock et al. 1978; Iger and Abraham 1989; Marty and Summerfelt 1990). Both diet (Lim and Lovell 1978; Nirgiotis et al. 1991) and temperature (Bullock et al. 1978; Knights and Lasee 1996) have been shown to affect wound healing in fish. Few studies have evaluated the importance of aseptic conditions or the use of different antiseptics, surgical materials, or surgical techniques.

Surgical aseptic technique is standard practice in mammalian surgery and has been suggested for fish (Summerfelt and Smith 1990; Stoskopf 1993) even though earlier studies dismissed such a need (Krayukhin 1964; Pegel 1964). However, aseptic technique still is not universally practiced for fish (Carmichael 1991). Many surgical procedures are performed on fish (e.g., cannulation and transmitter implantation), but there is little knowledge regarding the effects of surgical asepsis, materials or techniques. Animal care agencies such as the Canadian Council on Animal Care (CCAC) and the National Research Council (NRC) have not established protocols for surgical procedures with fish (CCAC 1993; NRC 1996). This lack of information has undoubtedly had a negative impact on the survival and health of fish used in experimental work (Stoskopf 1993). It is important to develop proper surgery techniques because faster healing presumably increases the survival rate of fish being released back to the wild and limits data loss due to transmitter expulsion through an incision.

Topical antiseptic preparations are an invariable component of preparation for surgery on mammalian species. A topical antiseptic inhibits the growth or development of microorganisms, and may kill them, by interfering with their internal chemical processes or the cell membranes (Ascenzi 1996). One detailed report on the effects of using topical medications on wound healing in garter snakes *Thamnophis sirtalis* (Smith et al. 1988) showed that some topical treatments actually inhibited wound healing by inducing dermal granulomas; other treatments accelerated epithelialization. Although topical antiseptics have been used to pretreat incision sites on fish (Petering and Johnson 1991), no studies have been performed on their efficacy. Hart and Summerfelt (1975) noted that the topical use of benzalkonium chloride (an antiseptic) irritated the skin of flathead catfish *Pylodictis olivaris,* and Briggs (1995) found that

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Received December 28, 1998; accepted July 28, 1999

alcohol used as a surgical disinfectant seemed to interfere with the cutaneous mucus layer of rainbow trout *Oncorhynchus mykiss,* allowing easier penetration by pathogenic organisms. Stoskopf (1993) recommended against the use of alcohol on fish skin and suggested that iodine-based topical antiseptics might be a viable option for some species. For these reasons, we hypothesized that a povidone-iodine treatment should improve healing in rainbow trout by decreasing bacterial entry into the wound site. This decrease should lower the probability of an infection of the wounded tissues, which should lower inflammation and speed healing. We chose a topical iodophor antiseptic because these antiseptics have been used previously on fish (Petering and Johnson 1991) and they are the most commonly used antiseptics in mammalian surgery (D. Holmberg, Ontario Veterinary College, University of Guelph, Guelph, Ontario, personal communication).

Methods

Experimental animals.—Eighteen rainbow trout (length = 28.5 cm ± 0.4 SE; weight = 300.2 g ± 14.1) with a 1:1 sex ratio were obtained from the Alma Aquaculture Facility, Alma, Ontario. They were placed in a round 1,000-L tank at the Hagen Aqualab, University of Guelph, provided with filtered, recirculated well water at 9.3°C ± 0.1, and fed standard five point trout food (Martin Mills, Inc., Elmira, Ontario). The effects of temperature and diet were controlled by maintaining the same daily temperature and feeding the same daily amount of food (2% of body weight). We held the fish for 2 weeks prior to surgery to allow them to adjust to their new environment. The fish were starved for 48 h prior to surgery, as recommended by Summerfelt and Smith (1990), to minimize regurgitation of food and defecation in the water circulating through the surgery table.

Surgical procedures.—Prior to surgery, we anesthetized all fish individually with a 70-mg/L solution of tricaine methanesulfonate (MS-222) until stage five of anesthesia was reached. As defined by Summerfelt and Smith (1990), stage five of anesthesia occurs when reactivity and reflexes are absent and opercular movements are slow and irregular. We tagged all fish for identification with visible implant alphanumeric (VI alpha) tags injected into the transparent adipose tissue of the eyelid with a syringe-like injector (Northwest Marine Technology, Inc, Shaw Island, Washington). A fish was placed on a plexiglass V-board in the surgery table and its gills were continuously perfused with recirculating 10°C ± 0.1 water, also containing MS-222 (70 mg/L), through two soft rubber tubes. Water was changed in the table reservoir every 5–8 surgeries.

The surgeon's hands were scrubbed with a 10% povidone-iodine detergent and covered with clean, nonsterile medical examination gloves. All surgery tools were autoclaved at 121°C for 25 min prior to use. Between surgeries, the single-needle driver was cold-sterilized for 30 s in the disinfectant Conflikt (active ingredient, ammonium chloride), then rinsed with sterile saline to maintain low bacterial loads within the time constraints of each surgery.

Once a fish was anesthetized and placed on the surgery table, one of two ventral incision sites was treated with antiseptic by swiping 10 times in the direction of the scales with a Q-tip dipped in 10% povidone–iodine detergent (0.75% free iodine; Rougier, Inc., Chambly, Quebec), to remove debris, followed by 10 more swipes with a second Q-tip impregnated with 10% providone–iodine solution (1% free iodine; Rougier). This incision area was blotted dry with a sterile swab to remove excess antiseptic. The control incision area on the same fish was not wiped with a Q-tip or blotted. We randomly assigned control and treated incision sites to either the right or left side of the abdomen then alternated for each successive surgery, always beginning with the treated incision.

The two parallel, 3-cm incisions were made on the ventral surface of each trout's abdomen, anterior to the pelvic girdle (Figure 1), with a number 11 scalpel blade. Blunt forceps were used to retract the body wall and depress the viscera to prevent internal damage while the incision was deepened through to the peritoneal cavity. We closed each incision with four simple interrupted stitches approximately 6 mm apart, tied with surgeon's knots. An individual sterile pack of 4–0 Surgipro (USSC, Norwalk, Connecticut), a nonabsorbable polypropylene monofilament suture with a swaged on 3/8-circle reverse cutting needle, was used for each fish.

After the incision was closed, the treated site was again swiped 10 times with 10% povidone–iodine solution. The control incision area was not treated before or after surgery. After surgery, we placed fish individually in darkened recovery aquaria for 24 h then returned them to the communal tank.

Tissue sampling.—Two fish were euthanatized immediately after surgery (time zero), at 1, 3, and 7 d after surgery, then once every 7 d for the five

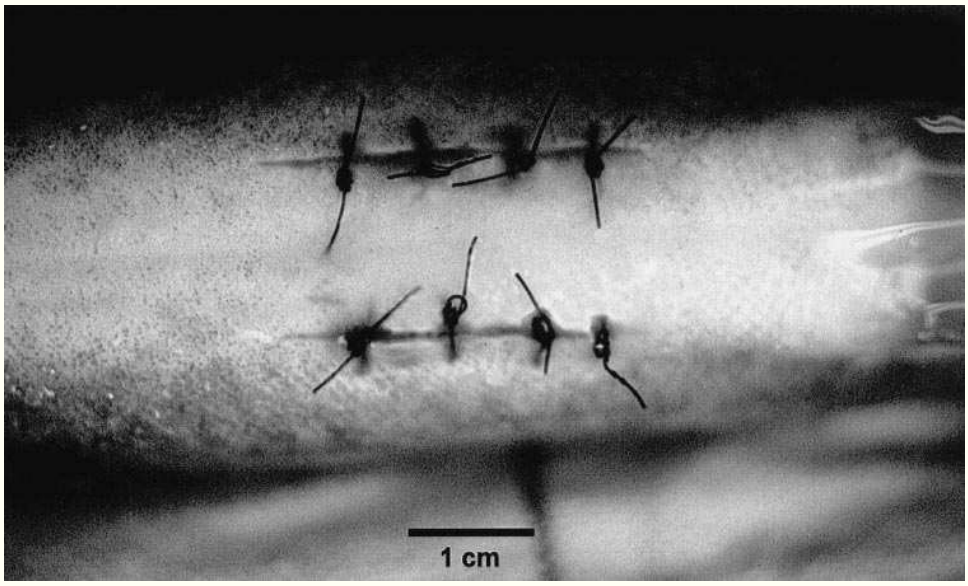

FIGURE 1.—Rainbow trout abdominal surface 7 d after surgery, showing an incision treated with povidone–iodine (upper) and a control (untreated) incision (lower).

following weeks. To measure the response to surgery and determine a time course for healing, blood and incision site tissue samples were collected from each fish.

We first anaesthetized the fish with MS-222 (70 mg/L) and extracted blood samples from the dorsal aorta in a heparinized syringe with a 23-gauge needle. We measured total erythrocyte (red blood cells, RBC), number of dividing erythrocytes, and differential leukocyte (white blood cells) ratios. Total erythrocytes were counted with a Neubauer hemacytometer and Hendrick's diluting solution. Dividing erythrocytes also were counted as defined by Murad et al. (1990) and Houston and Murad (1992). We fixed blood smears with Leukostat (Fisher Scientific) for differential leukocyte ratios and counted at least 200 cells per sample at 1,000 × magnification under oil immersion. All solutions were made according to Houston (1990), and differential counting methods followed those of Filledes (1969). Cells were identified from photographs by Pickering et al. (1982) and Yasutake and Wales (1983).

Following blood sampling, fish were euthanatized by submergence in a receptacle containing a lethal dose of MS-222. We used a dosage of 140 mg/L for at least 5 min, which is well above the lethal dosage of 80 mg/L recommended by Iwama and Ackerman (1994). Incision areas on each fish (approximately 4 × 2 cm) were then excised separately and placed in a tissue cassette into 10% buffered formalin. The tissue blocks were dehydrated in a graded ethanol series and embedded in paraffin. Sections (6–7 μm) of the incision site were mounted on slides, stained with hematoxylin–eosin, and examined by light microscopy.

Histological criteria.—Criteria used to quantify wound healing (Table 1) were similar to those used by Marty and Summerfelt (1988) and Christiansen et al. (1991). Ratings of cell types were based on numbers of cells per high-power field. Accurate alignment of wound margins enhances wound healing. Alignment was assessed qualitatively with five ratings: perfect alignment (all tissue layers in apposition without displacement), discontinuous alignment (opposing tissue layers partially offset), misalignment (opposing tissue layers not aligned anatomically), misaligned with nonanatomical tissues (foreign body such as scales present), open incision (partial or full thickness deficit in wound). We numbered each tissue sample nonsequentially and performed single-blind analysis in which observers were unaware of treatment or postsurgery sampling day. Each sample was categorized as acute, subacute, or chronic based on the observer's histological analysis of each tissue sample.

Bacteriology.—The spleen and a section of the mid-kidney were excised from each fish after euthanasia and placed in sterile twirl bags. Each tis-

TABLE 1.—Histological criteria used to quantify post-surgical wound healing on rainbow trout.

Measure	Rating	Description[a]
Epidermal cell thickness	1–20	Actual number of cells
Blood clots	1	Small zone of hemorrhage
	2–3	Minor hemorrhage involving one tissue layer
	4–5	Major hemorrhage involving multiple tissue layers
Neutrophils	1	1–2 present
	2	3–5 present
Lymphocytes	3	6–10 present
	4	11–20 present
	5	20+ present
Melanomacrophages	1	1–2 present
	2	3–5 present
	3	6–8 present
	4	9–12 present
	5	13+ present
Fibroblasts	1	1–19 active, disorganized
	2	20–40 active, disorganized
	3	>40 actively proliferating, disorganized
	4	>40 organized in whorls, decreasing cytoplasmic volume
	5	>40 organized in cords, anatomical congruity with surrounding tissue
Myoepithelial cells	1	1–5 present
	2	>5 in process of bridging wound gap
	3	>5 aligned, disorganized, completely covering wound bed
	4	Noncontracted wound, organized continuous layer of birefringent staining
	5	Contracted wound, organized continuous layer of birefringent staining
Capillary proliferation	1	1–5 buds visible in periphery of wound bed
	2	6–10 capillaries more extensively branching in wound bed
	3	11–20 capillaries found throughout wound bed
	4	21+ capillaries anastomosing and tortuous, branching throughout wound bed
	5	21+ extensive capillary network, parallel, organized, completely bridging wound bed
Giant or syncytial cells	1	1 present
	2	2–3 present
	3	4–5 present
	4	6–10 present
	5	11+ present
Collagen organization	1	Early synthesis in actively dividing and bridging fibroblasts
	2	Deposition along capillary beds and in early granulation tissue
	3	Organization as whorls
	4	Linear organisation, resembling surrounding tissue
	5	Linear organisation, indistinguishable from surrounding tissue

[a] Numbers of cells present are those per high-power microscopic field.

sue sample was weighed and crushed, and the crushed sample was diluted 10:1 by volume with sterile saline. We then transferred 0.1 mL of each suspension onto trypticase soy agar (TSA) plates by streaking them with a flame-sterilized loop. Isolated bacterial colonies were identified by Gram staining after 2 and 3 d incubation at 20°C. Water samples also were analyzed at each sample period by the same dilution technique.

Statistics.—Each fish had one control and one treated incision site, so blood and bacterial results were analyzed only to detect temporal changes after surgery. We analyzed all the blood variables by analysis of variance (ANOVA) to determine if significant variations occurred with respect to time. Variables were compared with each other by linear correlation analysis. Bacterial results were also analyzed by ANOVA to determine time differences. We analyzed histological characters by analysis of covariance (ANCOVA) with time post-surgery as the covariate, each criterion as a variable, and use of antiseptic as the treatment. All analyses were performed according to Steel et al. (1997).

Results

All of the fish recovered from the anesthetic within 5 min and swam normally within the communal recovery tank after the 24 h isolation period. One fish, meant for euthanasia 28 d postsurgery, died within 24 h of surgery. A postmortem examination of this fish revealed extensive internal bleeding, the cause of which was not determined.

Temporal Hematological and Bacterial Changes

Total erythrocyte numbers did not change significantly with time postsurgery ($P = 0.361$) and averaged 1.28×10^6 RBC/mm^3 $\pm$ 0.05 (Figure 2;

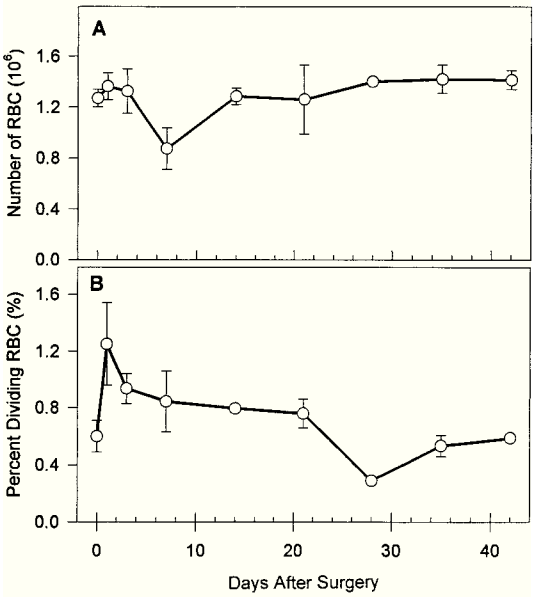

FIGURE 2.—**(A)** Total erythrocyte (red blood cell, RBC) numbers and **(B)** percentages of dividing erythrocytes in rainbow trout blood sampled over 42 d postsurgery. Each datum is the mean ± SE of two fish.

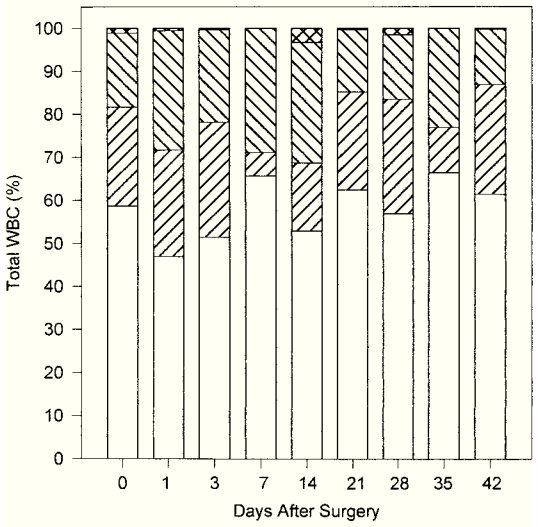

FIGURE 3.—Mean differential leukocyte (white blood cell, WBC) ratios in rainbow trout blood sampled over 42 d postsurgery. Sample-day values are means of two fish. Lymphocytes are open bars (SE = 2.2), thrombocytes are forward-hatched bars (SE = 2.6), granulocytes are backward-hatched bars (SE = 2.1), and monocytes are cross-hatched bars (SE = 0.3).

all error terms are SEs). Likewise, the percentage of dividing erythrocytes did not change significantly with time ($P = 0.083$); the peak of 1.54% 1 d postsurgery was not significantly different from the overall mean (0.76% ± 0.07). We also found no significant changes in differential leukocyte ratios postsurgery except for monocytes, which reached a high of 3.5% on day 14. This percentage was significantly greater than at any other time point ($P = 0.012$) (Figure 3).

The bacteria found in the kidney and spleen plates were three types of Gram-negative, yellow-pigmented cocci in short chains, and likely were *Micrococcus* spp. These bacteria, along with lesser abundances of *Flavobacterium* spp., *Flexibacter* spp., and *Pseudomonas* spp., were identified in water from both holding tanks on all sampling days and in quantities too numerous to count. No significant difference in bacterial numbers occurred between the kidney and spleen ($P = 0.25$). Bacteria were often low in number, but 39% of the plates grew bacterial colonies that were too numerous to count. There was no temporal pattern associated with bacterial counts (Table 2). On the three sample days bacteria were present in healing wounds (Table 2), bacterial numbers were very low or not present in the kidney and spleen.

Antiseptic Treatment Effects

Adhesions of coelomic viscera to the incisions sites occurred in 86% of the fish by 21 d postsurgery. There were no significant differences between histological responses for treated and nontreated wounds within any of the time periods (Table 3). As well, no differences were seen between treated and nontreated incisions with respect to wound apposition. Finally, antiseptic treatment did

TABLE 2.—Numbers of yellow-pigmented bacteria found in the kidney and spleen of postsurgery rainbow trout, expressed as colony-forming units per gram of tissue (average of two fish); +++ represents colonies too numerous to count.

Sample day	Number of colony-forming units from:	
	Kidney	Spleen
0	+++	+++
1[a]	2	0
3[a]	0	0
7	53	+++
14[a]	3	0
21	2	3
28	+++	+++
35	37	14
42	+++	+++

[a] Days on which bacteria were identified in wounded tissue of at least one of the two fish.

TABLE 3.—Healing of control (C) and treated (T) incisions in rainbow trout over acute (days 0–7, eight fish), subacute (days 14–21, four fish), and chronic (days 28–42, six fish) time periods, after surgery. Zero (0) indicates "not present"; otherwise, histological measures and rating scales are those of Table 1. Asterisks denote measures whose ratings changed significantly among time periods ($P < 0.05$). Within time periods and on particular sample days, ratings did not differ between control and treated incisions ($P > 0.05$).

Histological measure	Rating scale	Mean rating over days: 0–7 C	0–7 T	14–21 C	14–21 T	28–42 C	28–42 T
Epidermal cell thickness*	0–20	2.0	3.2	11.5	11.5	13.0	14.0
Blood clots*	0–5	3.2	3.6	4.2	4.2	0	0
Neutrophils	0–5	2.5	2.5	1.8	2.2	2.0	1.4
Lymphocytes*	0–5	0.9	0.8	2.2	2.2	2.4	1.4
Melanomacrophages*	0–5	0.1	0.1	1.8	1.5	3.6	3.4
Fibroblasts*	0–5	0.2	0.4	2.8	2.8	4.2	3.8
Myoepithelial cells*	0–5	0	0.2	0.8	0.8	1.2	1.6
Capillary proliferation*	0–5	0	0	0.2	0.2	2.6	3.6
Giant or syncytial cells*	0–5	0	0	0.8	0.2	1.8	1.6
Collagen organisation*	0–5	0	0	1.0	1.2	3.8	4.2

not affect bacterial presence in wounds, because bacteria were found equally in treated and nontreated wounds. Because there were no significant differences between treated and control groups, they were combined for temporal evaluations.

Temporal Tissue Changes

Incised tissues generally displayed acute healing responses over days 0 to 7, subacute responses over days 14 to 21, and chronic responses over days 28 to 42 with some overlap between categories (Table 4). Incisions were completely healed by day 42 d, but only one fish in the study had perfect wound alignment. Epidermal covering was significantly less in the first week than later. Blood clots were present (3.7 rating average) over the first 3 weeks of the study, but were absent after day 28. No significant temporal patterns occurred in neutrophil numbers in the wound area during the course of the study. The number of lymphocytes found in the wound region increased significantly after the first week. These results differ from those for blood lymphocytes, which did not change significantly during the course of the study (Figure 3). Melanomacrophage and fibroblast activities, myoepithelial and giant or syncytial cells, capillary proliferation, and collagen organization increased significantly over the course of the study (Table 4).

TABLE 4.—Temporal changes in wound healing of combined control and treated rainbow trout groups (combined due to lack of significant differences between them; $P > 0.05$) over acute (days 0–7, eight fish), subacute (days 14–21, four fish), and chronic (days 28–42, six fish) time periods after surgery. Along a line, ratings without a letter in common are significantly different ($P < 0.05$). A Bonferonni correction was applied to the significance level, ($P_{0.05} = 0.017$) because of the number of tests performed.

Histological measure	Mean rating over days: 0–7	14–21	28–42
Epidermal cell thickness	2.6 z	11.5 y	13.5 y
Blood clots	3.4 z	4.2 z	0.0 y
Neutrophils	2.5 z	2.0 z	1.7 z
Lymphocytes	0.9 z	2.2 y	1.9 y
Melanomacrophages	0.1 z	1.7 y	3.5 x
Fibroblasts	0.3 z	2.8 y	4.0 y
Myoepithelial cells	0.1 z	0.8 zy	1.4 y
Capillary proliferation	0.0 z	0.2 zy	3.1 y
Giant or syncytial cells	0.0 z	0.5 zy	1.7 y
Collagen organisation	0.0 z	1.1 y	4.0 x

Discussion

The only surgical complication was the single postsurgical death. By maintaining the same daily temperature and feeding the same daily amount of food (2% of body weight), we controlled the effects of temperature and diet on wound healing. Visceral adhesions to incision sites have been noted previously (Lucas 1989; Knights and Lasee 1996), but the degree to which they occurred was not reported. We made parallel incisions in individual fish in order to compare adjacent tissue reactions to treatment with the antiseptic and without it. This approach worked well for its intended purpose but created problems with comparisons of tissue adhesions, blood variables, and bacterial contents. Therefore, these measurements were taken over the 42-d time period but were not compared between treated and control incisions.

Blood Variables

Maule and Schreck (1990) recommended against the use of total blood leukocyte counts as a means

of estimating inflammatory response in the tissues of salmonids because rapid, transient changes in leukocyte numbers found in tissues of coho salmon *Oncorhynchus kisutch* were not reflected in the peripheral blood. One alternative measure recommended by Lowe-Jinde and Niimi (1983) was the use of differential white cell counts.

Lymphocytes that infiltrate wound sites without significant change in their percentage of blood cells may reflect their capability of lysing localized foreign cells (Ellis 1988). Some granulocytes are eosinophilic and have the ability to phagocytose antibody–antigen complexes. Monocytes are precursors of macrophages and have the ability to phagocytose foreign particulate matter (Roberts 1978). We expected granulocytes and monocytes to increase after surgery because of these phagocytic abilities, but this did not occur. We expected thrombocyte numbers to increase during the experiment because they are responsible for blood clotting, but this also did not occur.

The ratios of leukocytes can change within hours of a physiological stress on rainbow trout (Casillas and Smith 1977; Spannhof et al. 1979), which may explain the lack of significance among differential counts with time in the present study. Leukocyte levels may have changed but returned to normal even before the day 1 samples were taken. Pickering et al. (1982) found that lymphocyte numbers of brown trout *Salmo trutta* increased significantly immediately after handling, then decreased significantly after 24 h and returned to normal by 3 d poststress; as in our case, none of the other blood variables measured (neutrophils, thrombocytes, erythrocytes) changed over the 28-d sampling period.

There can be large differences in differential leukocyte numbers between and among fish species. The leukocyte percentages we found differed from those for other rainbow trout (Lowe-Jinde and Niimi 1983) and common (mirror) carp *Cyprinus carpio* (Hines and Spira 1973). For the species in these two studies, differential counts averaged 95% for lymphocytes, 3.3% for thrombocytes (trout only), and 0.5–4.5% for granulocytes. Our differentials were 58%, 20%, and 21%, respectively, with monocytes making up the remaining 1%. Our counting methods were similar to those of the previous authors, but they took blood samples immediately after anaesthetizing the fish whereas we took the first blood samples after anesthesia and surgery, which may explain the wide divergence in numbers. Differences in leukocyte percentages within a species has occurred in other studies. For normal channel catfish *Ictalurus punctatus,* Dogden and Sullivan (1969) found 72% lymphocytes, 23% thrombocytes, and 2.5% neutrophilic granulocytes, whereas Scott and Rogers (1981) found respective values of 35%, 54%, and 11%. Scott and Rogers (1981) suggested that such wide intraspecific divergences may be due to the size and physiological condition of the fish sampled. One problem encountered with the use of differential leukocyte counts is that all ratios change even if only one cell type increases or decreases in absolute number. The researcher may incorrectly interpret physiological events with this method. We, therefore, recommend the use of absolute numbers in future studies. Veterinary doctors prefer absolute numbers to percentages for all species (D. Smith, Ontario Veterinary College, University of Guelph, personal communication), and we feel they are easier to compare among studies and better represent physiological changes in the fish.

Murad et al. (1990) and Houston and Murad (1992) found the percentage of dividing erythrocytes to be a valuable estimator of erythrocyte proliferation after blood loss. This mechanism increases the total number of hemoglobin-synthesizing cells for recovery from surgery. Although these percentages did not differ significantly among temporal points, there was a peak 1–3 d after surgery; however, no correlated decrease in erythrocyte numbers was observed. Moore et al. (1990) reported no response in hematocrit and leukocrit values of Atlantic salmon *Salmo salar* 14 d after surgery. Spannhof et al. (1979) observed that rainbow trout erythrocyte numbers decreased in favor of erythroblasts or proerythrocytes only within the first 24 h postsurgery. Either situation could apply to our study. Our percentages of dividing erythrocytes were similar to those found by Houston and Murad (1992) and Houston et al. (1993) in goldfish *Carassius auratus* stressed by temperature and heavy metal exposure. Total erythrocyte numbers were within the normal range for rainbow trout (Barham et al. 1980), which supports our finding that no significant response to surgery occurred.

Bacteria

The two major blood-filtering organs in fish are the kidney and spleen. The yellow-pigmented bacteria (YPB) found in these organs are common in water and soil. Common YPB in these environments include *Flavobacterium* spp., *Flexibacter* spp., and *Pseudomonas* spp., but the colonies we

isolated were most likely *Micrococcus* spp. because they consisted of cocci in chains, while the other genera contain rods. The species of these genera pathogenic to fish tend to be involved with skin and gill diseases. Therefore, it is unlikely they would occur systematically, but may occur transiently as they are cleared by leukocyte activity (R. M. Stevenson, Department of Microbiology, University of Guelph, personal communication). Their considerable abundance in 39% of the samples is of some concern, but we do not believe the bacteria found were systemic; inadvertent contact of the organs with fish skin during their excision may have transferred skin bacteria to the samples, because high numbers of bacteria colonize mucus membranes of fish (Austin and Austin 1987). Alternatively, bacteria might have entered the peritoneal cavity through the sutured incisions and survived on the outer surface of the kidney and spleen. Nemetz and MacMillan (1988) reported bacteria entering the peritoneal cavity through wounds, although their fish died within 48 h of peritonitis and septicemia. The number of bacterial colonies cultured from renal and splenic tissue may not be a good indicator of systemic bacterial infection. Organs should be rinsed thoroughly first to remove bacteria that may have entered through a surgical incision. A test of these possibilities would be to swab the organs and compare their external and internal bacterial contents.

We found bacteria in histological wound sections on three of the five sample days when bacterial numbers in the kidney and spleen were low or absent (Table 2). The wounds of these fish were open or misaligned. In other fish with open wounds, conversely, we found no bacteria in wound tissue but many bacteria in kidney and spleen samples. Bacteria were likely present in the incision areas of all the fish with open wounds, but may not have been present in the individual sections sampled.

Tissues

We found no significant results in our experiment to prove either beneficial or adverse tissue reactions to topical treatment with povidone–iodine solutions. Treated and untreated incision sites healed at the same rate, within 42 d, with little difference in histological attributes (Table 3). The temporal changes in cell activity in the healing tissues were different from those found in other studies. Epithelialization and most of the cells migrating to the healing tissues were up to a week slower in rainbow trout than in threespine sticklebacks *Gasterosteus aculeatus* (Phromsuthirak 1977), common carp (Iger and Abraham 1989), and channel catfish (Marty and Summerfelt 1990). The migrating cells of rainbow trout in our study maintained high levels up to 35 d longer than the other three species. We feel these differences are more likely temperature related (we held our fish at 10°C compared with 20°C) than species related, because temperature has been shown to play a major role in wound healing (Knights and Lasee 1996).

That only one fish in the study had perfect wound alignment is of some concern. Good alignment of wound margins is particularly important in an aquatic environment, where rapid epithelialization of a wound minimizes osmotic disturbance and bacterial penetration.

In most fish experiments in which they were used, antiseptics and antibiotics have been applied via injections or water treatments. Few reports have detailed the effects of these treatments on fish tissues. Ahmed and Tan (1992) reported that water treated with the antibiotic tetracycline helped prevent tissue necrosis in and increased the rate of skin healing in the catfish *Clarias macrocephalus*. Contrary to our study, Nemetz and MacMillan (1988) reported epidermal cell loss at 3 d after application of a topical iodophor to channel catfish incisions. These results were complicated by tissue reaction to the cyanoacrylic adhesive used to close incisions.

Some topical antiseptics have been reported to chemically burn or otherwise damage fish skin (Nemetz and MacMillan 1988; Stoskopf 1993). As well, Kashyap et al. (1995) reported that povidone–iodine decreased wound strength in mice and may be cytotoxic to fibroblasts and keratinocytes. We could not find analogous reports for fish and no such effect was seen with the povidone-iodine used in our study. Epidermal thickness was similar in all treatments and no difference in histological tissue response occurred with treatment. These results are similar to those reported by Moore et al. (1990), who found no adverse effects of a topical Cicatrin antibiotic applied to wounds on Atlantic salmon, but they performed only gross tissue analysis.

Along with creating a physical barrier to pathogenic microorganisms, the mucus of fish contains lysozymes, proteolytic enzymes, and other compounds that inhibit bacterial colonization and infiltration (Alexander and Ingram 1992). The lack of an adverse affect does not exclude the possibility that, in other species of fish, the removal of

the cutaneous layer of mucus with its natural antibiotic properties might allow wound infections and thus slow the healing process. On the other hand, the removal of this mucus layer by antiseptic treatment of the skin surface may be beneficial during surgery by not allowing pathogenic bacteria that reside in the mucus (Austin and Austin 1987) to enter the wound. We were unable to test either of these hypotheses in our study.

Conclusions

Our results show that the use of a povidone–iodine topical antiseptic neither helped nor hindered the full healing of abdominal incisions in rainbow trout. We recommend against the use of this topical antiseptic in surgery because its application has no obvious benefit to healing and may be detrimental. Some authors have reported that topical antiseptics may actually hinder healing in some fish species (Hart and Summerfelt 1975; Stoskopf 1993; Kashyap et al. 1995), but no such reports have been made on povidone–iodine solutions used on fish. Further studies would have to be performed on other fish and antiseptic combinations to verify a beneficial or detrimental effect.

Acknowledgments

We thank Elizabeth Niimi for her assistance with blood staining techniques and differential leukocyte analysis, Chris Jastrebski for his aid during surgery, and Roselynn M. Stevenson for her help analyzing bacterial plates. We also thank David Holmberg and Dale A. Smith for their technical input and editing. This project was supported by a Natural Sciences and Engineering Research Council grant to E. Don Stevens and made possible by the use of the Hagen Aqualab facilities at the University of Guelph.

References

Ahmed, G. U., and E. S. P. Tan. 1992. The responses to tetracycline treatment of the epidermis of injured catfish (*Clarias macrocephalus*) raised under intensive culture conditions. Aquaculture 105:101–106.

Alexander, J. B., and G. A. Ingram. 1992. Noncellular nonspecific defence mechanisms of fish. Annual Review of Fish Diseases 1992:249–279.

Ascenzi, J. M. 1996. Antiseptics and their role in infection control. Pages 63–72 *in* J. M. Ascenzi, editor. Handbook of disinfectants and antiseptics. Marcel Drekker, New York.

Austin, B., and D. A. Austin. 1987. Bacterial fish pathogens: disease in farmed and wild fish. Ellis Horwood, New York.

Barham, W. T., G. L. Smit, and H. J. Schoonbee. 1980. The haematological assessment of bacterial infection in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 17:275–281.

Briggs, C. T. 1995. The metabolic costs of activity of free swimming, adult rainbow trout (*Oncorhynchus mykiss*) estimated by electromyogram telemetry. Master's thesis. University of Calgary, Calgary, Alberta.

Bullock, A. M., R. Marks, and R. J. Roberts. 1978. The cell kinetics of teleost fish epidermis: epidermal mitotic activity in relation to wound healing at varying temperatures in plaice (*Pleuronectes platessa*). Journal of Zoology (London) 185:197–204.

Carmichael, G. J. 1991. Recovery of channel catfish from abdominal surgery. Progressive Fish-Culturist 53:193–195.

Casillas, E., and L. S. Smith. 1977. Effects of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). Journal of Fish Biology 10:481–491.

CCAC (Canadian Council on Animal Care). 1993. Guide to the care and use of experimental animals, volume 1, 2nd edition. CCAC, Bradda Printing Services, Ottawa.

Christiansen, J. S., M. Jobling, A. B. Amin, and H. Ribe. 1991. Histomorphological changes in epidermal structure of juvenile Arctic charr (*Salvelinus alpinus* L.) subjected to sustained exercise. Canadian Journal of Zoology 69:2450–2455.

Dogden, C. L., and S. Sullivan. 1969. Hematological effects of apholate on channel catfish (*Ictalurus punctatus*). Proceedings of the Society for Experimental Biology and Medicine 131:124–126.

Ellis, A. E. 1988. General principles of fish vaccination. Pages 1–19 *in* A. E. Ellis, editor. Fish vaccination. Academic Press, London.

Filledes, T. P. 1969. Experimental physiology and applied biology. Educators Publishing Service, Cambridge, Massachusetts.

Hart, L. G., and R. C. Summerfelt. 1975. Surgical procedures for implanting ultrasonic transmitters into flathead catfish (*Pylodictis olivaris*). Transactions of the American Fisheries Society 104:56–59.

Hines, R. S., and D. T. Spira. 1973. Ichthyophthiriasis in the mirror carp: II, leukocyte response. Journal of Fish Biology 5:527–534.

Houston, A. H. 1990. Blood and circulation. Pages 213–272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Houston, A. H., S. Blahut, A. Murad, and P. Amirtharaj. 1993. Changes in erythron organization during prolonged cadmium exposure: an indicator of heavy metal stress? Canadian Journal of Fisheries and Aquatic Sciences 50:217–222.

Houston, A. H., and A. Murad. 1992. Erythrodynamics in goldfish, *Carassius auratus* L.: temperature effects. Physiological Zoology 65:55–76.

Iger, Y., and M. Abraham. 1989. The process of skin healing in experimentally wounded carp. Journal of Fish Biology 36:421–437.

Iwama, G. K., and P. A. Ackerman. 1994. Anaesthetics.

Pages 1–15 *in* P. W. Hochachka and T. P. Mommsen, editors. Biochemistry and molecular biology of fishes, volume 3. Elsevier, New York.

Kashyap, A., D. Beezhold, J. Wiseman, and W. C. Beck. 1995. Effect of povidone-iodine dermatologic ointment on wound healing. American Surgeon 61:486–491.

Knights, B. C., and B. A. Lasee. 1996. Effects of transmitters on adult bluegills at two temperatures. Transaction of the American Fisheries Society 125: 440–449.

Krayukhin, B. V. 1964. Methods for installing chronic fistulas in the digestive tract of fish. Pages 125–137 *in* E. N. Pavlovskii, editor. Techniques for the investigation of fish physiology. Translated from Russian, 1971: Israel Program for Scientific Translations, Catalog Number 1130, Jerusalem.

Lim, C., and R. T. Lovell. 1978. Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). Journal of Nutrition 108:1137–1146.

Lowe-Jinde, L., and A. J. Niimi. 1983. Influence of sampling on the interpretation of haematological measurements of rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 61:396–402.

Lucas, M. C. 1989. Effects of implanted dummy transmitters on mortality, growth and tissue reaction in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 35:577–587.

Marty, G. D., and R. C. Summerfelt. 1988. Inflammatory response of channel catfish to abdominal implants: a histological and ultrastructural study. Transactions of the American Fisheries Society 117: 401–416.

Marty, G. D., and R. C. Summerfelt. 1990. Wound healing in channel catfish by epithelialization and contraction of granulation tissue. Transactions of the American Fisheries Society 119:145–150.

Maule, A. G., and C. B. Schreck. 1990. Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. Journal of Aquatic Animal Health 2:298–304.

Moore, A., I. C. Russel, and E. C. E. Potter. 1990. The effects of intraperitoneally implanted dummy acoustic transmitters on the behaviour and physiology of juvenile Atlantic salmon, *Salmo salar* L. Journal of Fish Biology 37:713–721.

Murad, A., A. H. Houston, and L. Samson. 1990. Haematological response to reduce oxygen-carrying capacity, increased temperature and hypoxia in goldfish, *Carassius auratus* L. Journal of Fish Biology 36:289–305.

Nemetz, T. G., and J. R. MacMillan. 1988. Wound healing of incisions closed with a cyanoacrylate adhesive. Transactions of the American Fisheries Society 117:190–195.

Nirgiotis, J. G., P. J. Hennessey, C. T. Black, and R. J. Andrassy. 1991. Low-fat, high- carbohydrate diets improve wound healing and increase protein levels in surgically stressed rats. Journal of Pediatric Surgery 26:925–928.

NRC (National Research Council). 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.

Pegel, V. A. 1964. Methods of study of the digestive properties of pancreatic juice and bile in fish in long-term experiments. Pages 141–142 *in* E. N. Pavlovskii, editor. Techniques for the investigation of fish physiology. Translated from Russian 1971: Israel Program for Scientific Translations, Catalog Number 1130, Jerusalem.

Petering, R. W., and D. L. Johnson. 1991. Suitability of a cyanoacrylate adhesive to close incisions in black crappies used in telemetry studies. Transactions of the American Fisheries Society 120:535–537.

Phromsuthirak, P. 1977. Electron microscopy of wound healing in the skin of *Gasterosteus aculeatus*. Journal of Fish Biology 11:193–206.

Pickering, A. D., T. G. Pottinger, and P. Christie. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time course study. Journal of Fish Biology 20:229–244.

Roberts, R. J. 1978. Fish pathology. Baillière Tindall, London.

Scott, A. L., and W. A. Rogers. 1981. Haematological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafinesque). Journal of Fish Biology 18:591–601.

Smith, D. A., I. K. Barker, and O. B. Allen. 1988. The effect of certain topical medications on healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). Canadian Journal of Veterinarian Research 52:129–133.

Spannhof, L., N. Dimeter, and H.-L. Kreutzmann. 1979. Early recognition of metabolic disturbances in trout (*Salmo gairdneri* Rich.) stocks. Aquaculture 18: 317–323.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and procedures of statistics a biometrical approach, 2nd edition. WBC/McGraw-Hill, Boston.

Stoskopf, M. K. 1993. Surgery. Pages 91–97 *in* M. K. Stoskopf, editor. Fish medicine. Saunders, Philadelphia.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213–272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Yasutake, W. T., and J. H. Wales. 1983. Microscopic anatomy of salmonids: an atlas. U.S. Fish and Wildlife Service, Resource Publication 150, Washington, D.C.