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Does the level of asepsis impact the success of surgically implanting tags in Atlantic salmon?

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ABSTRACT. It is generally recommended that a high level of asepsis be maintained during surgical implantation of electronic tags into fish. However, documentation of a positive effect of asepsis in fish surgery is lacking. To compare the effects of surgical implantation performed under different sanitary conditions, 100 hatchery salmon smolts (*Salmo salar*) were surgically implanted with tags with and without trailing antenna and were kept in a hatchery facility. After 34 days, the surviving smolts were euthanized and survival, growth and healing were compared between fish tagged under aseptic conditions and fish tagged without regard to aseptic technique. The results demonstrated that there was no detectable difference in survival, growth and healing between the treatments. Thus, this study could not provide evidence supporting the general recommendation of achieving a high level of asepsis during fish surgery.

Keywords: Tagging effects, Asepsis, Wound healing, Suture Infections;

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11 **Running headline:** Asepsis in fish surgery?

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38 **Abstract**

39 It is generally recommended that a high level of asepsis be maintained during surgical implantation
40 of electronic tags into fish. However, documentation of a positive effect of asepsis in fish surgery is
41 lacking. To compare the effects of surgical implantation performed under different sanitary
42 conditions, 100 hatchery salmon smolts (*Salmo salar*) were surgically implanted with tags with and
43 without trailing antenna and were kept in a hatchery facility. After 34 days, the surviving smolts
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45 conditions and fish tagged without regard to aseptic technique. The results demonstrated that there
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47 study could not provide evidence supporting the general recommendation of achieving a high level
48 of asepsis during fish surgery.

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55 **Key words:** tagging effects, asepsis, wound healing, suture, infections
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Introduction

58

59 Many important management decisions are based upon results from telemetry studies. Numerous
60 studies have demonstrated that electronic tags can be surgically implanted and carried by some fish
61 for long periods without significant effect on mortality (Reviewed in: Cooke et al., 2011); however,
62 results may still be compromised by sub-lethal effects brought about by the treatment. Reported
63 sub-lethal effects of surgical implants include decreased swimming capacity (e.g. McCleave and
64 Stred, 1975; Arnold and Holford, 1978), reduced growth (e.g. Greenstreet and Morgan, 1989;
65 Jepsen et al., 2008) and tag expulsion (e.g. Chisholm and Hubert, 1985; Marty and Summerfelt,
66 1986; Jepsen et al., 2008). Partitioning the extent to which these sub-lethal effects are caused by the
67 surgery rather than the presence of the tag itself can be challenging, yet is essential for
68 interpretation of telemetry results as well as for improving tagging protocols.

69

70 In addition to concerns over the validity of the results, the ethics of the methodology attract
71 attention because researchers are obligated to refine techniques to ensure better animal welfare. To
72 limit potential sub-lethal effects from surgery, it is important for both veterinarians and biologists to
73 reduce infection in relation to surgical implants in fish. For example, although there is no published
74 evidence that pathogen transmission has occurred during transmitter implant surgeries, there is
75 evidence that transmission of *Renibacterium salmoninarum*, (the cause of BKD) increased among
76 juvenile Chinook salmon (*Oncorhynchus tshawytscha*) by coded-wire tagging procedures (Elliott
77 and Pascho 2001).

78

79 It is clearly of the utmost importance to assure sterile or aseptic conditions when performing
80 surgery in the body-cavity of mammals or birds, but how important is this for fish? In theory, it
81 would be beneficial to perform fish surgery under totally aseptic conditions to minimize the risk of

82 infection and disease. However, procedures used for mammals (drying, rinsing the skin with
83 chemicals) are generally harmful for fish. Any complication that prolongs the time of surgery may
84 also have negative effects on the fish. It is clear there are pros and cons, but little documentation on
85 this issue; Chomyshyn et al. (2011) addressed this and acknowledged that the few studies that have
86 investigated have failed to document a positive effect of aseptic techniques, prophylactic or
87 postoperative treatments, but still recommend aiming for a high level of asepsis. Furthermore,
88 Mulcahy (2003) states:

89 *“The surgical implantation of a non-sterile transmitter into any animal is an inhumane act, which*
90 *should not be performed. Fish, like mammals, are susceptible to infections from contaminated*
91 *implants.”*

92 This highlights the assumption that fish surgery is comparable to mammalian surgery and therefore
93 aseptic conditions are equally critical. Mulcahy (2011) further argues that instead of using
94 antibiotics, more care should be taken in attaining asepsis, even under field conditions. Harms and
95 Lewbart (2011) are in agreement suggesting that while “sterile surgery” is likely impossible in the
96 field, most aseptic techniques can be adopted without making surgeries unnecessarily long. It
97 should be noted however, that despite the wise adoption of asepsis in terms of the precautionary
98 principle, neither Mulcahy (2003) nor Harms and Lewbart (2011) directly evaluate whether aseptic
99 conditions are necessary. Therefore despite recommendations from veterinarians, there is no clear
100 evidence of a need for asepsis.

101
102 Such recommendations are indeed an integral part of most standard operating procedures for
103 surgical fish tagging (e.g. Brown et al., 2010; Liedtke et al., 2012) as well as in the guidelines from
104 the Canadian Council on Animal Care (2005). However, many experienced fish surgeons and
105 researchers have attained a clear perception that for standard fish surgery under normal conditions

106 (good water quality, moderate water temperatures), attempts to decrease the risk of infection by
107 instituting extensive aseptic procedures have no effect (e.g. Wagner and Cooke, 2005). Wagner et
108 al. (1999) tested the effect of prophylactic preparation with a povidone-iodine antiseptic prior to
109 conducting surgery on rainbow trout (*Oncorhynchus mykiss*), but found no clear effect. In a field
110 study where 100% of tagged fish were recaptured after one year in a reservoir, no negative effects
111 (on growth, survival, performance) of surgical implants with trailing antenna were found, even
112 though surgery was performed under field conditions with no prophylactic or postoperative
113 treatments (Jepsen et al., 1999; 2000). Similarly, Koed and Thorstad (2001) found no long-term
114 effect of radio tagging on the swimming performance of fish more than one year after non aseptic
115 tagging. Chomyshyn et al. (2011) tested if the intrusion of lake water into the coelom had negative
116 effects on survival and healing of bluegill (*Lepomis macrochirus*) and results showed no positive
117 effect of avoiding water entry or using sterile/aseptic equipment. Thus, there are currently no
118 published papers documenting a positive effect of reducing the risk of infection. One can therefore
119 argue that until such documentation appears, it can be assumed that infection is not a common
120 problem in fish surgery and as such efforts should be focused on improving capture, handling and
121 holding conditions rather than on keeping clean conditions and using sterilized tools during tagging.

122

123 In a recent review paper, Cooke et al. (2011) highlighted the need for focused research on the topic
124 of asepsis in fish surgery. Therefore, our objectives are to experimentally test whether there are
125 significant differences in growth, survival and healing between fish that have tags surgically
126 implanted under aseptic (clean) and non-aseptic (dirty) conditions. In addition, we investigate
127 whether the presence of an external trailing antenna further impacts post-operative recovery.

128

129

Material and Methods

130 A total of 100 hatchery salmon smolts (*Salmo salar*) were used. They were offspring from wild
131 fish collected in River Skjern, Denmark. We randomly assigned each fish to one of two treatment
132 groups representing two different degrees of asepsis. The “clean” treatment incorporated all aseptic
133 practices that are widely included in many guidelines for fish surgery (e.g., CCAC 2005). In
134 contrast, since ensuring aseptic conditions is often not possible or practical in the field, the non-
135 aseptic treatment represented an extreme example of *in situ* surgery where no attempt was made to
136 limit exposure to pathogens. Specifically for the aseptic treatment, all dummy tags were sterilized in
137 an autoclave and packed separately. All surgical tools were also sterilized in an autoclave prior to
138 the surgical procedure. In between surgeries on separate fish, needle-holders, handles for scalpel
139 blades and hollow needles for antenna placement were rinsed in 97 % ethanol and dried. A new
140 sterile scalpel blade and new sterile suture with needle (Vicryl 5-0 FS-2; Ethicon, Piscataway, NJ,
141 U.S.A.) were used for each new fish. The surgeon wore a new set of standard (non-sterile) surgical
142 gloves for each fish. The surgical table and support pillow were cleaned between each fish by
143 rinsing with ethanol, drying and changing the sheet of laboratory paper. In contrast, for the non-
144 aseptic treatment, dummy tags were not sterilized, nor rinsed in ethanol. All surgical tools including
145 sutures and scalpel blades were previously used at least once (for the clean surgery) and not cleaned
146 between surgeries. No gloves or masks were used and the surgical table was not cleaned between
147 surgeries.

148

149 In addition to separating the fish into aseptic and non-aseptic treatments, we tagged half of each
150 treatment group (25 aseptic and 25 non-aseptic) with dummy tags (6 x 20 mm, 0.5 g weight in air)
151 that had no antenna (NA), and half with tags (6 x 20 mm, 0.7 g weight in air) that had a trailing
152 antenna (A). Antennas were trimmed down to not exceed the caudal fin by more than 5 cm. This
153 second layer of treatment was incorporated to evaluate the potential additional complications from

154 an external antenna. The experimental fish ranged in size from 14.5 – 20.5 cm with weights from 28
155 – 88g. Tag/body mass ratios ranged from 0.7 – 2.4 % (mean A: 1.07 %; NA: 1.71%).

156

157 An experienced fish surgeon performed surgical implants in accordance to the guidelines described
158 in permission (2012-DY-2934-00007) from the Danish Experimental Animal Committee. The
159 surgical procedure followed that described in Jepsen et al. (1998) and the incisions were closed with
160 a single absorbable suture. After treatment and recovery (10-20 min) all fish were released back into
161 their original tank. The dummy tags consisted of simulated tags used in studies of smolt migration
162 (i.e. Jepsen et al., 2000; Hockersmith et al., 2003) and were obtained from Advanced Telemetry
163 Systems (Isanti, MN, USA). The surgeries were performed on 28 July 2011 and the study was
164 terminated on 31 August 2011. After surgery, a total of 16 (4 of each treatment group) tagged
165 smolts were placed in a separate tank for monitoring short-term effects. These were euthanized and
166 examined five days after tagging.

167

168 All fish were measured and weighed within a few hours post euthanization. Each fish was
169 necropsied and a photo was taken to document healing and tissue reaction. The remaining 84 fish
170 were housed for 34 days before ultimately being euthanized. These fish were measured and
171 weighed within a few hours post euthanization and each fish was necropsied to determine the level
172 of inflammation, adhesion, tag encapsulation within the body cavity as well as the position of the
173 tag (e.g., free floating, in a pocket, in the process of being expelled). Healing of the incisions was
174 evaluated using a rating scale that ranged from 1-4 (adapted from Wagner et al. 2000), one being a
175 perfectly healed, healthy incision, and four being an open wound with severe inflammation.

176

177 *Statistics*

178

179 A multinomial regression with healing (1–4) as the dependent factor and degree of asepsis
180 (aseptic/non-aseptic), maturity (yes/no) and antenna type (A/NA) as the independent factors and
181 length as a covariate was performed.

182

183 A General Linear Model (GLM) with specific length growth ($G_L = (\ln L_2 - \ln L_1) * t^{-1}$) as the dependent
184 factor and degree of asepsis (aseptic/non-aseptic), antennas (A/NA) and maturity (mature/non-
185 mature) as fixed factors and length as covariates was performed. All the interaction terms were
186 included in the analysis using a backward elimination approach using IBM SPSS Advanced
187 Statistics 20.0.

188

189

Results

190

191 The whole procedure for each implant in the aseptic procedure took approximately eight minutes,
192 whereas implanting the dummies in the non-aseptic procedure took approximately four minutes.
193 However, the actual time that each fish was handled did not differ between treatments. Instead, the
194 longer time was due to the extra steps required between the aseptic surgeries, i.e. changing gloves,
195 scalpel blades and sutures as well as cleaning and drying equipment.

196

197 Most experimental fish survived and performed well during the study; however, a problem with the
198 water quality in the facility caused some mortality of both the experimental fish and untagged fish
199 in adjacent tanks. This occurred between 24-27 days after the surgery and a total of 10 experimental
200 fish, all mature males, died. Thus, the analyses of growth, healing and survival were based on 100
201 minus 16 (pre-sampled) minus 10 (dead) = 74 individuals. Most fish were observed feeding
202 throughout the study period and there was positive growth for some individuals, whereas others did

203 not eat much and experienced negative growth. Length changes ranged from 0 – 2.9 cm and weight
204 from -7 to 30 grams. The specific growth for each treatment group is shown in Table 1.

205

206 For the 16 fish examined five days after tagging, there were no clear differences in tissue reaction
207 between the two treatments (Table 2). A high degree (13 of 16 fish) of tissue adhesion to the body
208 wall at or close to the incision site was observed. The adhesions were fragile fibrinous adhesions
209 and were seen from the dummy tags (with or without the antenna), and also from internal organs
210 (i.e., spleen, intestine, testes) separately without involvement of the dummy or antenna. For some
211 fish (3 of 16), hemorrhages were apparent as part of the acute inflammatory response to the
212 incision.

213

214 Post-mortem examinations of smolt 34 days after tagging revealed that about half (53 %) of them
215 were sexually mature males (mature male parr). A high degree (64 of 74 fish) of tissue adhesion
216 was also observed at this time (Table 2); the extent of the adhesions was comparable to those seen
217 five days after tagging but at this time the exudates had reorganized into a fibrous tissue.

218 Hemorrhages were only seen in a small number (9 of 74) of fish (Table 2), indicating that some
219 acute inflammatory reactions were still active. In 24 fish, the dummy tag was free in the body
220 cavity; in the other 50 it was either encapsulated by a thick brim of fibrous tissue or resting in a
221 “pocket”. The “pocket” consisted of a very thin, sometimes transparent, membrane arising within
222 the omentum of the intestines around the stomach. The nature of the pocket is unclear; it might be
223 an outpouch on the omentum trying to embrace the foreign body for immobilization, or an
224 inflammatory reaction with a very small amount of exudation, which when uncomplicated, could
225 reorganize into a fine membrane.

226

227 After 34 days, most incisions had healed well, with minimal differences in the average healing
228 score between aseptic (1.84) and non-aseptic (1.74) treatments. However, of the 74 examined fish,
229 47 (64 %) still had some absorbable suture material present. Multinomial regression demonstrated
230 that there were no statistically significant effects of either the degree of asepsis ($p=0.830$), maturity
231 ($p=0.617$), antennas ($p=0.614$), or length ($p=0.694$) on the degree of healing. The general linear
232 model demonstrated that while there was a statistically significant effect of length (GLM; $F_{1,69}=$
233 18.95 ; $p < 0.001$) and maturity (GLM; $F_{1,69}= 7.787$; $p = 0.007$) on specific length growth, there
234 were no effects of degree of asepsis ($p=0.631$) or antennas ($p = 0.353$) and none of the interaction
235 terms were statistically significant ($p>0.05$).

236

237

Discussion

238 This study demonstrated that no apparent benefit was achieved by ensuring a very high degree of
239 asepsis during surgery, compared to a simple field-like procedure. This result is similar to what was
240 reported by Chomyshyn et al. (2011). Obtaining a high degree of asepsis will significantly increase
241 equipment costs and was found to double the total time between surgeries. Both time and cost are of
242 great importance for studies that involve tagging a large number of individuals. The two groups of
243 fish here were tagged under extremely clean and extremely unclean conditions, respectively, and
244 still they performed equally well, highlighting the questionable value of asepsis in fish surgery. The
245 fact that no attempts were made to achieve asepsis does not necessarily mean that there was a
246 significant load of pathogens present during the non-aseptic surgery. However, we deliberately
247 chose hatchery Atlantic salmon in the period after their normal smolt migration, because they are
248 known to be sensitive to diseases (high mortality), when kept in the hatchery after their normal
249 migration period. Still, it is not possible or prudent solely on the basis of the present results to

250 recommend abandoning the use of asepsis in field tagging of fish, but we hope to encourage a
251 discussion of whether it is necessary to require “the highest attainable level of asepsis” as a
252 standard.

253

254 Overall, the fish recovered quickly post-surgery and were observed to have resumed feeding within
255 24 hours. The mortality observed (regardless of treatment) was in the expected range for post-
256 smolts held in freshwater (Jepsen et al., 2001) and was similar to mortality rates among untagged
257 fish in adjacent tanks (data not shown). From the post-mortem examination of the fish, five and 34
258 days after tagging, no significant difference was found between fish tagged under aseptic or non-
259 aseptic conditions.

260

261 Recently, Daniel et al. (2009) described tag expulsion by common carp (*Cyprinus carpio*) and
262 related this to “infection associated with the sutures/incision”. Such observations are not uncommon
263 in the literature and could be seen as evidence that infections are indeed a major problem in fish
264 surgery. In fact, putting fish back into a potentially contaminated environment with a breach in the
265 integument could be more of a problem than the surgery itself. Nonetheless, we still lack
266 documentation that 1) these problems really are caused by “infected incisions” and not just tissue
267 reaction or necrosis in the presence of sutures and the tag and 2) that a high level of asepsis can
268 reduce the risk of such problems. Studies documenting problems associated with infections from
269 tagging as well as studies demonstrating that these issues can be solved by improving the protocol
270 (i.e., aseptic techniques) are greatly needed and should be possible to conduct if the problem really
271 is evident.

272

273 Similar to observations by Bauer et al. (2005), Mesa et al. (2011), Sandstrom et al. (2013) and
274 Wagner et al. (2000), it seems clear that suturing was the main cause for complications in our study
275 (e.g., tissue trauma, oedema and necrosis). Even in well-healed fish, there was typically a “cross” of
276 scar tissue, where the longitudinal line (the original incision) was less pronounced than the line
277 from the suture material. The same was observed in wild trout, recaptured several months after
278 tagging (Jepsen et al., 2008). Thus, optimization of the suturing process and material may be more
279 important than level of asepsis for wound healing and the prevention of infection. Relevant issues
280 here include how fast the absorbable material dissolves, the diameter and type of material, and how
281 to suture to give the most support to the tissue for optimal healing.

282

283 In this study, there was an unexpectedly high degree of tissue adhesion between the organs and the
284 body wall at the position of the incision (Table 2). The reason for this is unknown, and there were
285 no differences among treatment groups. In a similar post tagging evaluation (Jepsen et al., 2008) of
286 wild trout with similar tag/body mass ratios (0.6 – 3.9 %), very little adhesion was observed. This
287 evaluation was 5-6 months post-surgery, so it is possible that tissue adhesion represents an
288 intermediate state in the healing process.

289

290 In this study, the gross lesion pattern did not indicate the presence of a bacterial infection,
291 specifically where adhesion of the telemetric dummy tag to the abdominal wall could be interpreted
292 as the beginning of expulsion of the dummy. However, no bacterial cultures were collected from the
293 fish so we cannot exclude the possibility of a bacterial infection. Some post tagging infections in
294 fish have been reported but these have been described as being of a secondary nature rather than
295 introduced due to a breach in asepsis during/at the time of the surgical procedure (see Mellas and
296 Haynes, 1985). Indeed, Daniel et al. (2009) state:

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1 **Table 1.** Key information about the experimental fish. Specific growth rates are medians, calculated
2 by the formula $G_L = (\ln L_2 - \ln L_1) * t^{-1}$ (see text). Clean refer to fish tagged under
3 aseptic conditions and dirty to fish tagged under non-aseptic conditions. A = tags with
4 trailing antenna and NA= tags without antenna.
5

Treatment	N-tagged	Mortalities	Specific length growth (Var)	Healing (Median)
Clean-A	25	3	0.04 (0.0155)	1.88
Dirty-A	25	4	0.06 (0.0054)	1.76
Clean-NA	25	1	0.09 (0.0065)	1.79
Dirty-NA	25	2	0.07 (0.0139)	1.71

6

7

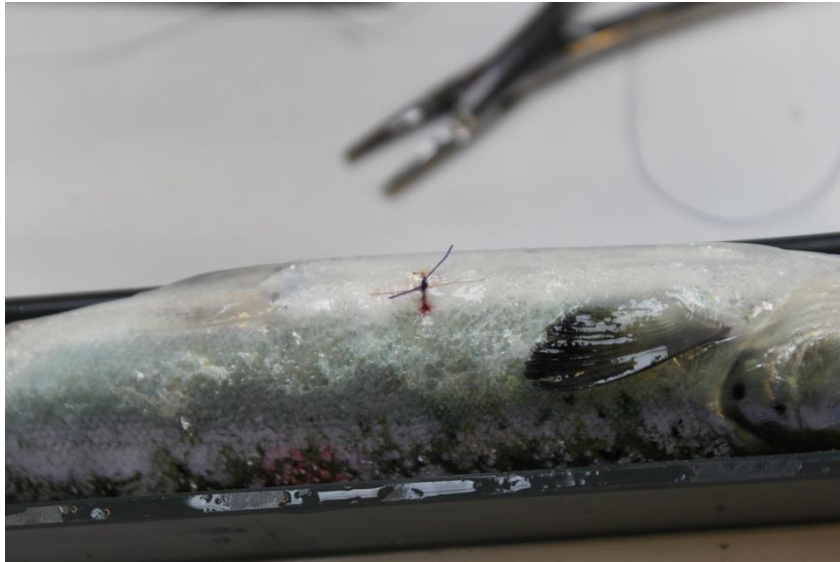
1 **Table 2.** Summarized findings from post-mortem examinations of fish 5 and 34 days after tagging
 2 under aseptic (clean) or non-aseptic (dirty) conditions.
 3
 4

Category	5 Days Post Surgery				34 Days Post Surgery			
	Clean, N=4		Dirty, N=4		Clean, N=20		Dirty, N=19	
	Yes	No	Yes	No	Yes	No	Yes	No
Antenna Present								
Adhesion	4	0	2	2	15	3	16	1
Tag Free in Cavity	0	0	0	0	8	10	10	7
Inflammation/Hemorrhages	4	0	4	0	3	15	3	14
In Pocket	4	0	2	2	10	8	5	12
Encapsulated	0	0	0	0	3	15	4	13
Expulsion Progressing	0	0	0	0	2	16	2	15
	Clean, N=4		Dirty, N=4		Clean, N=20		Dirty, N=19	
No Antenna								
Adhesion	4	0	3	1	19	1	16	3
Tag Free in Cavity	0	0	0	0	6	14	6	13
Inflammation/Hemorrhages	4	0	3	1	0	20	3	16
In Pocket	0	4	0	4	11	9	10	9
Encapsulated	0	0	0	0	9	11	7	12
Expulsion Progressing	0	0	0	0	4	16	4	15

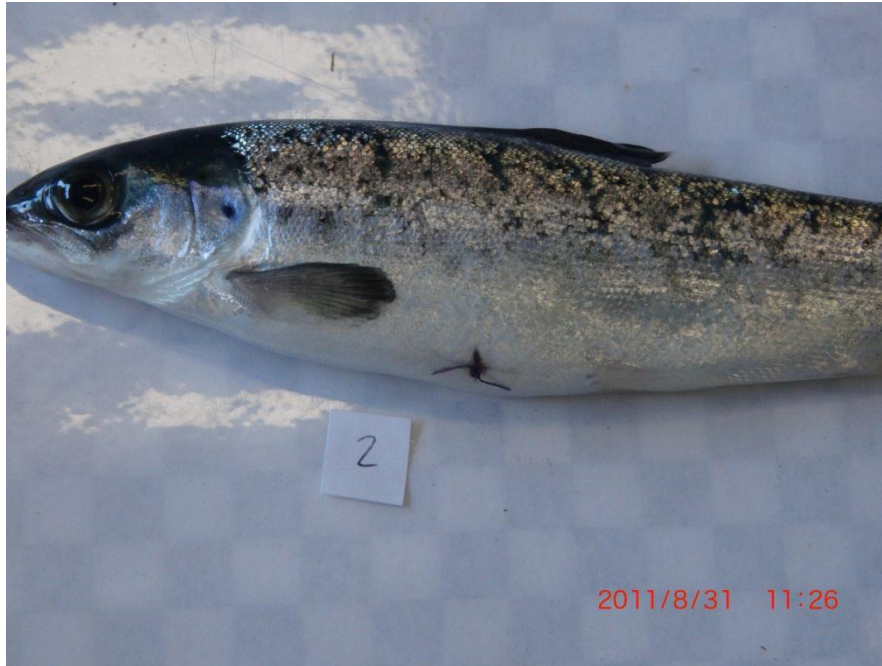
1 **SUPPLEMENTARY MATERIAL:**



2
3 A dummy NA-tag ready to be implanted, note the number for recognition
4



5
6 A freshly tagged smolt, ready for recovery
7
8
9
10



1
2 34 days post surgery, note the suture material is still in place
3



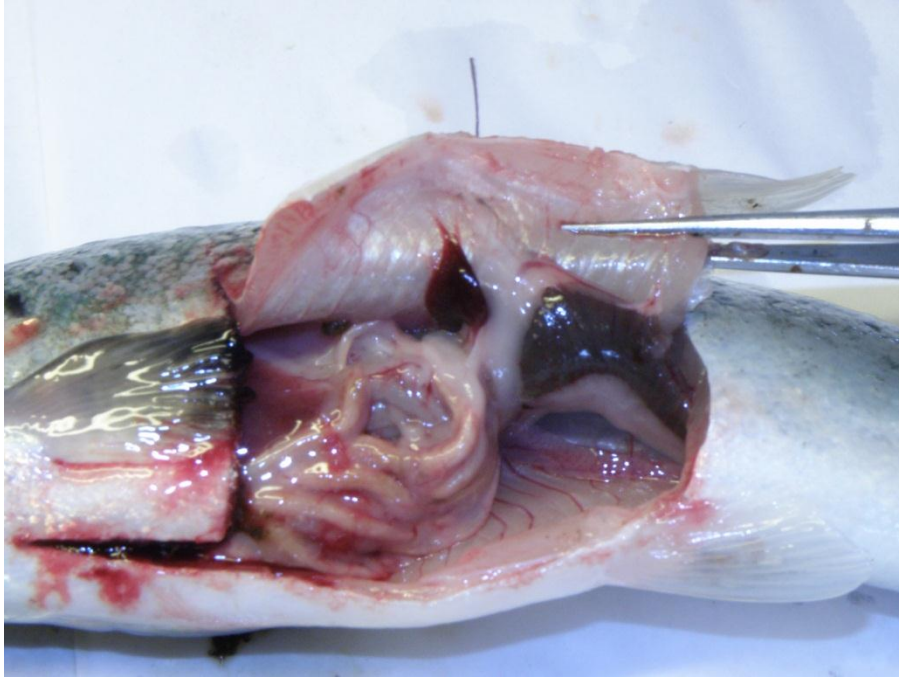
4
5 34 days post surgery, note how the suture has been causing irritation and necrosis



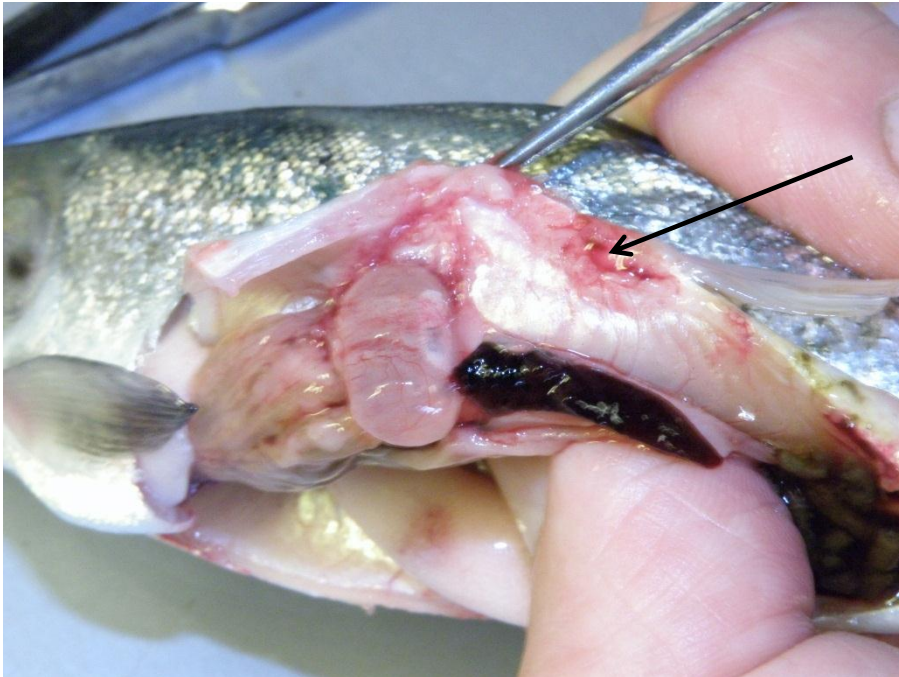
1
2 34 days post surgery, this fish has shed the suture, but it is evident that the skin was ruptured during
3 the process. This is very often seen, despite the use of thin absorbable suture.
4



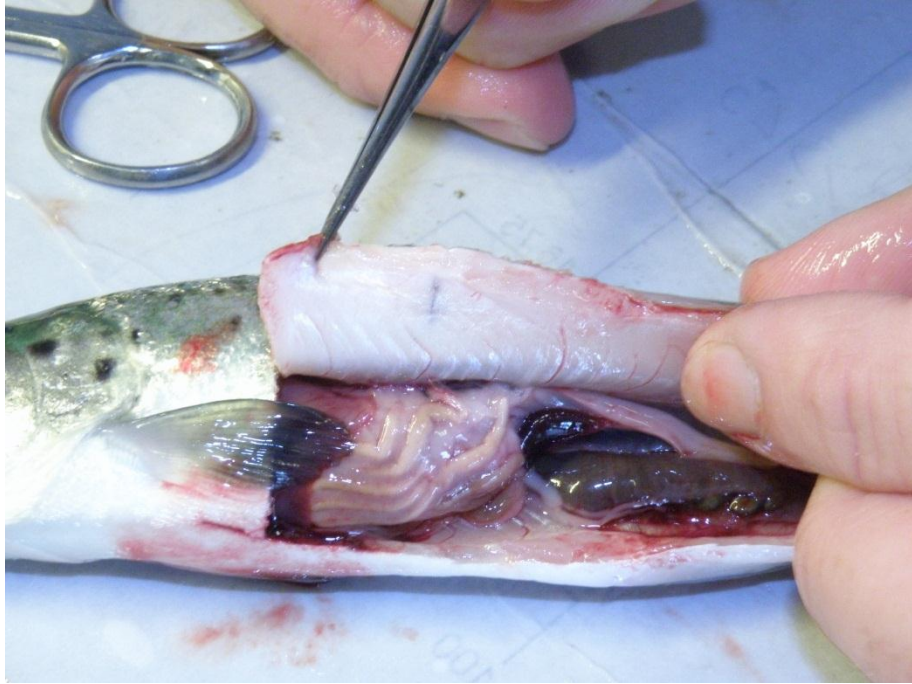
5
6 34 days post surgery, this is a fish with trailing antenna, note again the suture causing irritation,
7 whereas the incision is almost healed.



1
2 34 days post surgery, here we see adhesion, involving the spleen.
3



4
5 34 days post surgery, a NA-dummy has been fully encapsulated and expulsion was in progress,
6 arrow indicating the groove where the muscles were degraded to facilitate expulsion through the
7 side.
8



1
2 34 days post surgery, a well healed fish, without adhesion.