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1 **Recovery of gastric evacuation rate in Atlantic cod *Gadus morhua* L surgically**

2 **implanted with a dummy telemetry device**

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12 *Short title* Gastric evacuation in surgically implanted cod

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Abstract

The current study investigated how the gastric evacuation rate (GER) was affected after surgically

introducing dummies of a blood-flow biotelemetry system into the abdominal cavity of Atlantic

cod *Gadus morhua*. Gastric evacuation experiments were performed two and ten days post

surgery on surgically implanted and control *G. morhua* force-fed sandeel *Ammodytes tobianus*.

The results were compared with previously obtained estimates from unstressed conspecifics

voluntarily feeding on a similar diet. After two days, GER was significantly lower in the group of

fish with the dummy implants compared to the control group, but following ten days of recovery

no significant difference was seen between the two groups. The difference between implanted

and control fish observed 2 days post surgery may have arrived either from surgery, post-surgical

stress and/or the presence of the implant. The conclusion is that 10 days of postsurgical recovery

will stabilize GER in *G. morhua*, thus indicating that at this point the implant *per se* did not affect

GER. Both the fish with surgical implants and controls in this study evacuated their stomachs much

slower and with much higher inter-individual variation compared to *G. morhua* feeding voluntarily

on similar prey items.[1] The lower GER and higher inter-individual variation for force-fed fish

indicates that handling, anaesthetization and force-feeding impair GER and that individual fish

respond differently to the suppressing effects.

Keywords Post-surgical recovery, gastric evacuation, *G. morhua*, implant, biotelemetry

41 Invasive procedures are often necessary for the physiologist to understand the mechanisms

42 behind various physiological parameters. In fish, measurements of cardiovascular parameters such

43 as blood flow and blood pressure involve handling, anaesthesia and surgical implantation of

44 catheters and flow probes in and around vessels and sutures to close the incisions. In traditional

45 laboratory experiments, standard bench top blood pressure and blood flow measuring units are

46 used, which means that the animals are 'hardwired' to the equipment during the experiments.

47 Catheters and leads from flow probes penetrate the body wall and increase the risk of infection.

48 Furthermore, to prevent tangling and ripping of wires the animals are most often confined in small

49 containers, which severely limits the range over which they can move with the risk of introducing

50 confinement stress. The animals are usually left for 18 to 48 hr before experimentation is initiated,

51 the main reason for such shorts periods being the high risk of infection after an extensive surgery.[2]

52 In cases where catheters are used, limits on the time where these will remain open may also be

53 decisive. However, unless appropriate recovery time is ensured following surgical procedures and

54 instrumentation major concerns are the validity of data and ethical considerations. In a study on

55 rainbow trout *Oncorhynchus mykiss* using bioelectric potential recordings to measure heart rate,

56 f_H, (i.e. a non-invasive method) the fish were handled and then left to recover for at least 3 days

57 where after their basal heart rates were much lower than those presented in previous studies

58 using traditional methods involving surgical procedures.[3] A lower heart rate usually is indicative of

59 lower stress levels pointing to that these animals were not as stressed due to confinement and the

60 use of 'hardwired' techniques. Obviously, any misinterpretation of data may have important

61 consequences for our understanding of the physiology of fishes and researchers should be

62 encouraged to seek refinements of techniques to ensure more valid results as well as better

63 animal welfare.

64 The rapid development of telemetric devices opens new research areas that have not been

65 possible with hardwired animals and during the last few decades there have been great

66 advancements made in applications of biotelemetry in fish physiology. Biotelemetry allows data

67 collection from specimens that move around freely and can behave in a more natural way. In the

68 telemetrically instrumented animal the risk of infections is lower since there are no skin-

69 penetrating wires. Furthermore long term recovery- and recording periods are possible reducing

70 stress from handling, making this an attractive alternative. Altogether, this provides a number of

71 welfare advantages and the opportunity to obtain higher quality of the measured variables and

72 more reliable routine values. A recent study on white sturgeon (*Acipenser transmontanus*) showed

73 that routine heart rate of freely moving biotelemetric instrumented fish was lower compared with

74 hardwired, confined conspecifics as well as with previous values reported for white sturgeon.[4, 5]

75 The downside is that the animals are carrying the entire recording/transmitting unit, increasing

76 their body mass and possibly impairing the capacity to generate thrust by altering the ability to

77 bend the body. It may furthermore interfere with the internal organs if placed in the body cavity.

78 Nevertheless, a fully implantable system maximizes the likelihood that the studied fish will be

79 treated normally by surrounding fish[6] and minimizes the risk of infection and expulsion.[7] Even the

80 presence of a small, protruding antenna has been shown to cause adverse tissue reaction at the

81 antenna exit[8, 9] and to elicit aggressive attacks from other individuals of Atlantic salmon smolts.[6]

82 We are looking to use a fully implantable biotelemetry system [5,10,] to study blood flow distribution

83 in Atlantic cod *Gadus morhua* L. voluntarily feeding and moving between different habitats. Before

84 employment of such a system it is, however, imperative to evaluate appropriate post-surgery

85 recovery time and possible adverse effect of the telemetric implant. Using a dummy version of the

86 dual blood flow biotelemetric system used by Gräns and co-workers,[5, 10] the aim of the present

87 study was to examine the effects on the gastric evacuation rate (GER) of handling, surgery and

88 instrumentation of *G. morhua* with a dual channel blood flow telemetric system following short

89 term (two days post surgery) as well as prolonged (ten days post surgery) recovery. Both the

90 surgically implanted and control fish were force-fed in order to standardize the amount of food

91 and the time of feeding. The ability to evacuate the force-fed meal was determined and compared

92 with estimates obtained from unstressed conspecifics that fed voluntarily.

93 **Materials and methods**

94 **Fish**

95 Wild *G. morhua* ranging in total body mass from 290 to 815 g were caught in late 2009 by fyke net

96 in the vicinity of the Marine Biological Laboratory in the northern part of Øresund, Denmark. The

97 fish were transferred to the laboratory where they were kept in 9-10 °C re-circulated, aerated

98 seawater. The tank was covered by tarpaulin to avoid unnecessary disturbance of the fish.

99 Light/dark conditions were 16h: 8h. All fish were acclimated to laboratory conditions for a

100 minimum of 12 weeks before experiments were initiated and fed *ad libitum* three times a week

101 with chopped herring *Clupea harengus* L. or Raitt's sandeel *Ammodytes marinus* Raitt. Before the

102 experiment the fish were starved for five days to ensure that their stomachs were empty.

103

104 **Preoperative care**

105 Individual *G. morhua* was anaesthetized in 15 l of water containing 0.15 g l^{-1} 3-aminobenzoicacid

106 ethyl ester (MS-222) until gill ventilation ceased. Each fish was weighed and length measured

107 before it was positioned with the ventral side facing up on an operating table covered with wet

108 sponges. Anaesthesia was maintained during the surgery by pumping oxygenated sea water

109 containing MS-222 (0.075 g l^{-1}) over the gills. All surgical instruments, the dummy implant and

110 associated leads were sterilized by the use of a cold steriliant (Cidex, Johnson & Johnson Company,

111 USA).

112

113 **Dummy implant**

114 Our long-term goal is to implement the fully implantable biotelemetry system used by Gräns et

115 al.[5, 10] This system weighs 35g (total mass of implant and battery) in air and we aim for a

116 transmitter:fish mass ratio of 3-4%. Consequently, the dummy implant was constructed so that its

117 total mass equaled 19 g in air, thus, corresponding to an average of 3.7 (0.3) % of the mean body

118 mass of the present experimental *G. morhua*. It was made of silicon-coated stainless steel (L = 35

119 mm, H = 5 mm, W = 16 mm) with an attached silicon-coated dummy battery made of plastic tubes

120 (L = 18 mm, D = 11 mm) and two silicon leads with a probe cuff each, and the implant fitted easily

121 within the abdominal cavity of the fish.

122

123 **Surgery**

124 The splanchnic circulation in *G. morhua* is derived from the celiacomesenteric artery (CoMe). The

125 CoMe is the largest single branch of the dorsal aorta and branches to form the celiac artery and

126 mesenteric artery. The celiac artery supplies parts of the cardiac stomach, the pyloric stomach, the

127 proximal intestine and parts of the distal intestine, while the mesenteric artery supplies the

128 remainder of the gastrointestinal system, the gallbladder and the spleen.[11] The surgical

129 procedures were performed on 10 fish [body mass 550 (144) g and length 38.7 (3.2) cm] and done

130 in accordance with the guidelines described in permission 2010/561-1812 from the Danish

131 Ministry of Justice. For placement of the first dummy probe, a 2-cm-long incision was made

132 alongside the basibranchiale bone left of the midline, and the liver carefully retracted, where after

133 the CoMe artery was exposed and cleared. The dummy implant and battery were then carefully

134 placed in the abdominal cavity and the retracted organs restored to their places of origin.

135 Furthermore, an individual PIT tag (12×2 mm, ISO standard 11784/11785, FDX-B) was placed in the

136 body cavity for later identification of the fish. To access the ventral aorta for placing the second

137 dummy probe, a 0.5-cm ventral incision was made posterior to the gill juncture and carefully,

138 without disrupting the pericardium or damaging any vessels, the dermal and sub-dermal

139 musculature and connective tissue were separated using blunt dissection tools to expose the

140 ventral aorta. The probe was tunneled under the skin from the abdominal cavity to the incision

141 above the ventral aorta (see Figure 1 for schematic overview of positioning of the system). The

142 wires from the probes were anchored with a single stitch of 3/0 silk suture in the sub-dermal

143 muscle tissue and the two incisions were closed using sterile monofilament prolene 3-0 suture.

144 Subsequently the cod was given a subcutaneous injection of 0.4 mg/kg [1 M] butorphanol

145 (Torbugesic , Fort Dodge, Iowa, USA) for postoperative pain relief in addition to an injection with

146 10 mg/kg [1 M] Enrofloxacin (Baytrilo, Bayer, USA) antibiotics, to minimize the risk of infection.

147 Finally, to reduce incidence of oomycete infections povidone iodine powder was applied to the

148 closed incision before returning the fish to recovery water. The surgical procedure including

149 anaesthesia and awakening took approximately 20 min for each fish and when ventilation and

150 locomotion had been reestablished, the fish were returned to their home tank. All but one fish

151 survived the surgery.

152 The control fish [n=8, body mass 518 (155) g and length 37.1 (2.7) cm] were anaesthetized as

153 described above, and subsequently weighed, length measured and individually PIT tagged. The

154 tags were placed in the abdominal cavity, using a syringe implanter, taking care not to harm any

155 organs. The control fish were likewise left to recover before being returned to their home tank.

156

157 **Food**

158 *A. marinus* constitutes a natural and large part of the diet of wild *G. morhua* and one specimen per

159 cod was used as the experimental meal. To minimize variation in size and condition, specimens of

160 *A. marinus* from a single commercial batch (TripleNine, Hvide Sande, Denmark) were used for

161 force-feeding. The energy density of these prey fish was determined by bomb calorimetry [IKA C-

162 7000 bomb calorimeter (www.ika.net)] on a representative sample after drying it at 60° C until

163 constant mass according to the procedure described in Pedersen & Hislop.[12]

164

165 **Gastric evacuation experiments**

166 The first experiment was initiated 48 hr post surgery. On the day of experiment, *A. marinus* were

167 quickly thawed in running water, and those of similar total lengths were selected. These were

168 subsequently dabbed dry with paper towel and weighed, and individuals within a mass range of

169 9.4-15.7 g were chosen (corresponding to 2-3% of the body mass of the *G. morhua*). This food

170 ration was chosen from the largest daily feeding rates observed in wild *G. morhua*.[13] In random

171 order individual cod were anaesthetized by placing them in 15 l of water containing 0.07 g l^{-1} MS-

172 222. They were then force-fed by gently pushing a whole *A. marinus*, bend on the middle, through

173 the esophagus and into the stomach. Immediately thereafter the fish were returned first to

174 recovery water and subsequently to their home tank. The exact time of force-feeding was noted

175 for each fish, as was the length and mass of the prey.

176 Stomach contents were recovered 22 to 26 hr post force-feeding by stomach flushing following

177 anaesthetization (0.07 g l^{-1} MS-222). The stomach content from each fish was collected on a filter

178 with mesh-size 200 µm, and gently patted with a moist paper towel and weighed. The exact time

179 for recovery of stomach content was noted for each cod.

180 The fish were subsequently left to recover for 7 days in their home tank where after the above

181 described protocol was repeated in a second trial.

182

183 **Calculations and statistical analyses**

184 In accordance with the cylinder model of Andersen & Beyer,[14] the gastric evacuation rate of

185 stomach contents in *G. morhua* (as well as in a variety of other piscivorous fishes) can be described

186 independently of meal size by the current mass of stomach contents S_t (g) together with the rate

187 parameter ρ:

188

189 $$\frac{dS_t}{dt} = -\rho \sqrt{S_t} \tag{1}$$

190 Integrated from time 0 of force-feeding to time t (h) of recovery of stomach contents, the solution

191 to equation (1) is *G. morhua* evacuating its stomach contents according to the relationship

192

193 $$\sqrt{S_t} = \sqrt{S_0} - \frac{1}{2}\rho t \; ; \qquad 0 \le t \le t_{end} = 2\rho^{-1}\sqrt{S_0} \tag{2}$$

194

195 where t_{end} is the time for complete evacuation of the prey.

196

197   The ability to evacuate the stomach contents is then described by ρ, which can be calculated for

198   each individual *G. morhua* by reorganization of equation (2):

199

200    $$\rho = 2(\sqrt{S_0} - \sqrt{S_t})t^{-1}$$ (3)

201

202   The rate parameter ρ depends on predator (total) length L (cm), temperature T (°C) and prey

203   energy density E (kJ g^{-1}). The effects of these variables were estimated by Andersen (2001) for

204   different gadoids fed fish prey, and the following relationship was obtained:

205

206    $$\rho = \rho_{LTE}L^{1.44}e^{0.078T}E^{-0.86}$$ (4)

207

208   The basic rate parameter ρ_{LTE} specifies the general ability of the predator to digest and evacuate a

209   specific prey type from the stomach (or reversed: it indicates the resistance of the specific prey to

210   the digestive processes in the stomach). The value 0.00142 ± 0.00011 (estimate $\pm$ S.D.) of ρ_{LTE} was

211   obtained from an experiment by Andersen[1] on *G. morhua* that fed voluntarily on lesser sandeel

212   *Ammodytes tobianus* L. The temperature (10.3° C) and mean body size of *G. morhua* (41 cm; 664

213   g) were similar to those of the present study. This value of ρ_{LTE} was used to decide the evacuation

214   time t of c. 24 h employed in this study, which is shorter than t_{end} and yet long enough to ensure

215   that a significant part of the meal was evacuated from the stomach prior to recovery of the

216   remains.

217   *G. morhua* used in the present study varied to some extent in size (Table 1). ρ_{LTE} was therefore

218   used to represent the ability of individual *G. morhua* to evacuate their force-fed meals of *A.*

219   *marinus*. By use of this parameter, the results could further be compared directly with the above

220   estimate obtained from unstressed *G. morhua* that fed voluntarily. Combining equations (3) and

221   (4), the value of ρ_{LTE} for each individual *G. morhua* was so calculated by

222

223    $$\rho_{LTE} = 2(\sqrt{S_0} - \sqrt{S_t})\, t^{-1} L^{-1.44}\, e^{-0.078\,T}\, E^{0.86} \qquad (5)$$

224

225   Assuming a *G. morhua* to operate with its individual value, the basic rate parameter ρ_{LTE} can be

226   considered the outcome of a normally distributed stochastic variable.[14] Then, the performance of

227   two groups of *G. morhua* with regard to gastric evacuation rate can be compared statistically by

228   use of a *t*-test to the values of this parameter calculated for each *G. morhua* according to equation

229   (5). Before *t*-tests were performed (SigmaStat version 3.5) the assumptions of normality

230   (Kolmogorov-Smirnov test) and homogeneous variance of data (Bartlett's test) were tested.

231   Significance was accepted at $P < 0.05$.

232

233   CV of ρ_{LTE} is constant and does not depend on the value of this parameter[15]. According to Sokal &

234   Rohlf[16], this implies that the variances of two values of ln ρ_{LTE} are identical, and that a test of

235   equality of two values of CV is equivalent to *H*-test of equality of the variances of the logarithmic

236   transforms of ρ_{LTE}. The latter was therefore used here to test for equality of CV obtained from the

237   different treatments of fish. Significance was accepted at $P < 0.05$.

238

239   **Results**

240   **Short term recovery (two days)**

241   Two days post surgery, evacuation of force-fed sandeel was considerable slower in fish with

242   surgical implants compared with control fish. The calculated gastric evacuation rate parameter ρ_{LTE}

243 was thus significantly lower ($P < 0.001$) in implanted *G. morhua* compared to the control, being

244 respectively 4.2×10^{-4} (1.2×10^{-4}) and 9.3×10^{-4} (1.9×10^{-4}) (mean [SD]) (Figure 2). Because the effects

245 of fish length were accounted for by ρ_{LTE} (eq. 5), while temperature end energy density of the prey

246 were similar for all animals (Table I), the observed difference in gastric evacuation rates can be

247 attributed to pure physiological causes. When comparing our data with the value of $\rho_{LTE} = 14.2 \times 10^{-}$

248 ^{4} (1.1×10^{-4}) obtained in the study by Andersen[1] on *G. morhua* feeding voluntarily on *A. tobianus*

249 (i.e. involving no handling or anaesthesia priory to recovery of the stomach contents) the present

250 gastric evacuation rates only constituted approximately one third (fish with implants) and two

251 thirds (control) of this rate (Figure 2), i.e. both being significantly lower ($P < 0.001$). This difference

252 was also reflected in that on average only 21 and 42% (instrumented and control fish, respectively)

253 of the meals had been evacuated 25 h post force-feeding, which is inefficient compared to the

254 prediction[1] that 62% of the meal should be evacuated.

255 According to Andersen & Beyer[15] the coefficient of variation (CV) of the basic rate parameter ρ_{LTE}

256 is constant (i.e. independent of the estimated value of this rate parameter). CV should therefore

257 be used when comparing the *variability* of ρ_{LTE} in the different groups of *G. morhua*. For the two

258 groups of force-fed *G. morhua*, CVs of 0.21 and 0.29 (controls and operated, respectively) were

259 significantly ly higher ($P<0.002$ and $P<0.001$, respectively) than the value of 0.08 obtained from *G.*

260 *morhua* that fed voluntarily[1] (Table II).

261

Prolonged recovery (ten days)

263 Ten days post surgery, there was no significant difference ($P = 0.878$) between the rate at which

264 animals with implants evacuated their meal compared to control fish (Figure 2). These estimates

265 of ρ_{LTE} were however still significantly lower (7.7×10^{-4} [1.9×10^{-4}] for implanted and 7.9×10^{-4}

266   [2.8×10⁻⁴] for controls) than the value of 14.2×10^{-4} (1.1×10^{-4}) obtained from *G. morhua* feeding

267   voluntarily on *Ammodytes spp.* ($P< 0.001$).[1] Again, as described above for *G. morhua* 2 days post

268   recovery, the coefficients of variation in both of our groups of fish were significantly higher

269   ($P<0.001$) being 0.24 in surgically implanted fish and 0.35 in the control group (Table II) compared

270   to the value of 0.08 obtained from *G. morhua* that fed voluntarily. In contrast to the improved

271   evacuation ability of the surgically implanted *G. morhua* following prolonged recovery, the

272   percentage of food that had been evacuated by control *G. morhua* at *c.* 23h post force-feeding

273   (36%) was comparable both to the week earlier (43% *c.* 25 h post force-feeding after two days of

274   recovery) and to the value for the surgically instrumented fish (36% *c.* 23 h post force-feeding).

275

276   **Discussion** The present study demonstrates that following two days of post-surgical recovery

277   after introduction of a dummy implant into the body cavity, *G. morhua* is not capable of

278   evacuating a meal at a rate similar to non-operated fish. By using ρ_{LTE} (eq. 5) to compare gastric

279   performance between groups of fish we have accounted for the effects of fish length, while

280   temperature and energy density of the prey were similar for all animals (Table I). Our results thus

281   suggest that the observed difference in GER can be attributed to pure physiological causes derived

282   either from surgery, post-surgical stress and/or the presence of the implant. But following ten

283   days of recovery the suppressing effects on gastric performance caused by surgical implantation

284   was gone. Thus at this stage the presence of neither the implant *per se* nor the surgery was

285   limiting the GER. Reduced post-surgical gastric performance has been reported previously.

286   Twenty-four to thirty-six hr subsequent to invasive surgery sea bass *Dicentrarchus labrax* L.

287   showed a significantly higher gastric evacuation time (GET) [the relationship between GET and GER

288   can be deduced from equation (3)] compared to controls, with stomach contents still above 60%

13

289 of the initial 24 hr post force-feeding, whereas control animals at this time had less than one third

290 of the meal remaining in the stomach.[17] This is thus in accordance with our results showing that

291 GER had not stabilized in *G morhua* two days post surgery.

292

293 A factor that may affect the recovery and even long term function is the effect on buoyancy. A fish

294 with an implant has to counteract the downward force exerted by the added mass which can be

295 done by secretion of gasses into the swimbladder.[18, 19] With the present implant in the body

296 cavity, a fish, irrespectively of its size, would have to increase its swimbladder volume (5% of the

297 total volume in *G. morhua*[18]) with 14.2 cm^3 of air to become neutrally buoyant. In the smallest *G.*

298 *morhua* this implies an almost doubling of swimbladder volume, which may approach the limit for

299 their capabilities.[20] Swimbladder adjustment is a low cost solution but is slow (up to a day or

300 two)[21, 22] and meanwhile compensation must occur through active swimming which is

301 energetically expensive.[19, 23] Such excess energy use will reduce the aerobic scope available for

302 other processes[24] and this may be part of the explanation for the lower gastric performance

303 observed two days post surgery. Furthermore, active swimming may result in blood being shunted

304 away from the stomach region to prioritize oxygen delivery to the working muscle. [25, 26]

305

306 The use of biotelemetry devices allows experimenters to provide their animals with long

307 recoveryperiods to facilitate complete recovery from instrumentation procedures. Nonetheless,

308 for biotelemetry systems to hold their true/full potential it is imperative that the fish is capable of

309 dealing with not only the effects arising from anaesthesia, handling and surgical intervention but

310 also any potential disadvantages related to carrying the entire recording/transmitting unit. Taken

311 together, our results show that a potential for returning to pre-surgical levels indeed exists, as GER

312 was stabilized 10 days post surgery in fish carrying the implant in the body cavity. Thus the

313 presence of the implant *per se* did not seem to affect the average gastric performance at this

314 point. The fish in the present study weighed between 290 and 815g resulting in transmitter:fish

315 mass ratios from 2.3 to 6.5%. It has been suggested that the mass of telemetry tags should not

316 exceed 2% of body mass[24, 27]. Several experiments (including experiments on cod) using tags larger

317 than this have however found no significant effect on the swimming performance[29, 30]. We found

318 no correlation between GER and body mass indicating that cod can recover from carrying tags that

319 represent up to 6.5% of the body mass. Along the same lines results from salmonids on recovery

320 of swimming performance following surgical implantation of telemetry devices have shown that

321 Juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) had a significantly lower critical

322 swimming speed 1 day post surgery compared to controls, whereas full recovery was

323 accomplished after 21 days (in-between evaluations are lacking);[21] Although full recovery was not

324 established, the swimming performance of tagged juvenile *Salmo salar* L. had improved 7 days

325 post tagging at termination of the experiment.[30] Suggestively the fish may either have recovered

326 in the long term, or the tags, representing on average 8.5% of the fishmass, may have been too

327 heavy or have reduced the mobility of the fish.

328

329 When comparing the variability in gastric performance between different groups of *G. morhua* the

330 coefficient of variation, CV, of the basic rate parameter ρ_{LTE} should be used (for further details on

331 this see Materials and methods). The value 0.080 of CV estimated from the voluntarily feeding fish

332 by Andersen[1] (Table II) compared well with the estimate of 0.098 obtained from a variety of

333 predatory gadoids and their fish prey.[15] This variation probably reflects the inter-individual

334 variation in gastric performance. Substantially higher values of CV were obtained from both

groups of force-fed *G. morhua*, which may then be explained by an additional variability due to the suppressing effects of handling, anaesthetization and force-feeding on GER to which the individual fish responds differently. An alike large variability has been observed in *O. mykiss*; the lag phase from force feeding until the stomach started to empty varied between zero and 5h.[31] As anticipated, for the present *G. morhua* this furthermore resulted in on average 30% lower values of ρ_{LTE} from control fish compared to the voluntarily feeding *G. morhua* in the study by Andersen[1]. Studies on cod using intragastric transmitters have shown that if the fish voluntarily ingest the baited transmitter (in this case a transmitter wrapped in a fillet of herring) high food consumption rates are maintained in the days subsequent to tagging, in contrast to fish tagged by forced insertion (involving handling and anaesthesia) where food intake was notably lower for up to 15 days post tagging.[32] This indicate that appetite prevails despite the presence of a transmitter as long as handling and anaesthesia is avoided.

To omit interference from anaesthesia and handling in future studies, one important unanswered question to investigate is thus how long it takes for instrumented fish to commerce voluntarily feeding following the surgical procedure.

Although the primary intention of this study was not to focus on wound healing, this is an important matter, not only for ethical considerations but also because biotelemetric methods enable long-term measurements where open incisions may facilitate internal infections and/or cause changed behavior and performance thus inflicting data invalidity. Two days post surgery the surgically implanted fish had no or only slight signs of inflammatory reaction around the incisions. The exception was two fish, one in which the larger wound had opened, and another where it gapped at one end. These animals were instantly euthanized and data omitted from the analysis.

358 Ten days post surgery an inflammatory response (redness and slight swelling) was noted around

359 all the larger incision made alongside the basibranchiale bone. These observations are in

360 accordance with a previous study in which *G. morhua* (kept at comparable temperatures of 9.5-

361 14° C) were surgically implanted with dummy transmitters into the body cavity via incision along

362 linea alba .[33] In these animals inflammatory responses begun 5-7 days post surgery and subsided

363 for 4-8 days with complete wound healing after a total of 24-34 days. Obviously, the time course

364 of, and how to secure proper wound healing following surgical implantation demands further

365 attention.

366 In summary, two days of post-surgical recovery will not stabilize GER in *G. morhua*, but ten days

367 will, when using a standard force feeding protocol. The results indicate that at ten days post

368 surgery the presence of a dual channel dummy telemetric implant *per se* did not affect GER but

369 that the effects observed 2 days after instrumentation are due to surgery, post-surgical stress

370 and/or the presence of the implant. Biotelemetry has the welfare advantages of allowing long

371 recovery periods and avoiding the unnecessary stress arising from handling and confinement,

372 altogether improving quality of data and we believe our results to leave a promising future for

373 implementation of fully implantable biotelemetry systems in fish.

374

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493   Figure 1.

494   Schematic drawing of a *Gadus morhua* showing the placement of the dummy implant and battery

495   (both drawn to scale) in the abdominal cavity and the flow probes belonging to it. The

496   celiacomesenteric artery branches off the dorsal aorta (DAo) and subsequently divides into the

497   celiac (ACo) and the mesenteric artery (AMe). VAo, ventral aorta; STO, stomach

498

499   Figure 2.

500   Comparison of the basic rate parameter rho (ρ_{LTE}) two and ten days post surgery for force-fed

501   surgically implanted (hatched, n=9) and control (grey, n=8) *Gadus morhua* and with estimates

502   from non-operated voluntarily feeding conspecifics (white).[1] Data are presented as mean (SD), *

503   indicates a significant difference ($P<0.05$) between the force-fed groups two days post surgery, †

504   indicate significant difference ($P<0.05$) between non-operated voluntarily feeding fish and all

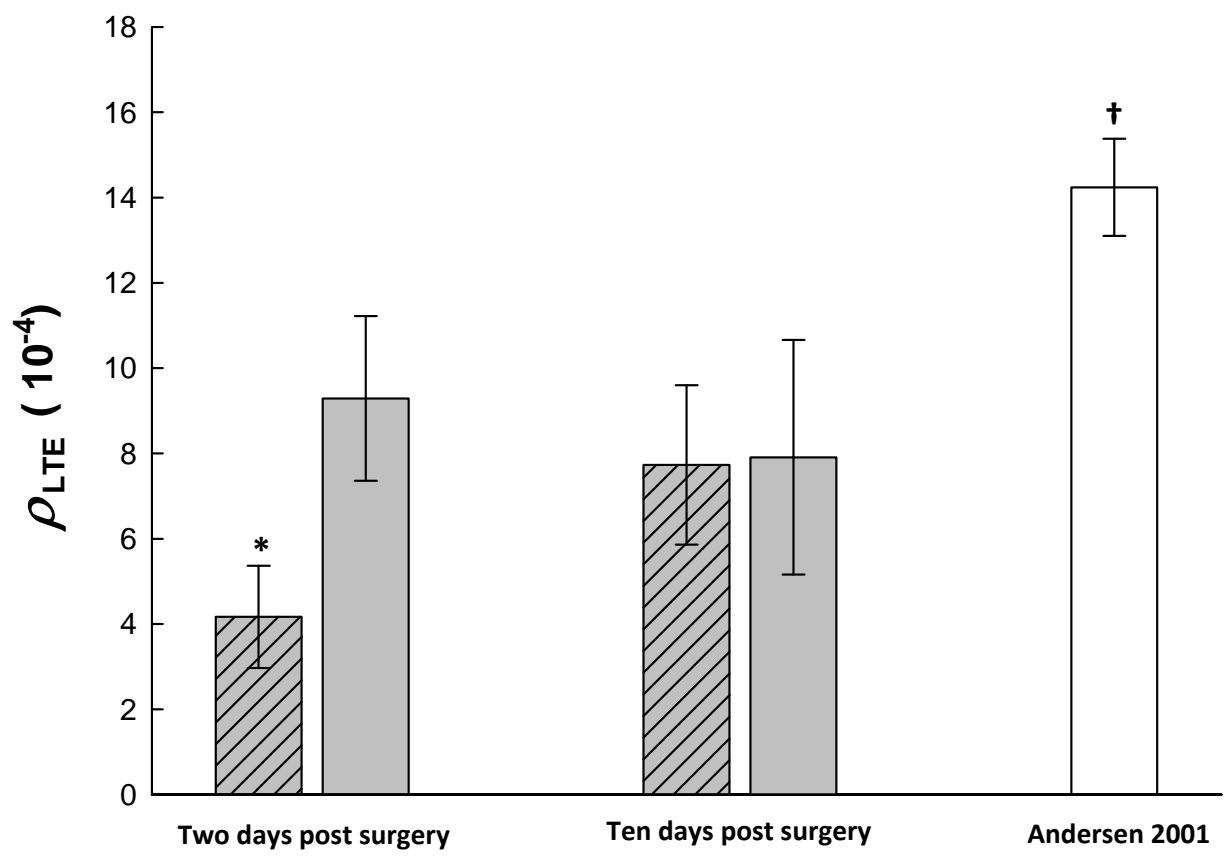505   force-feeding trials (i.e. both two and ten days post surgery).
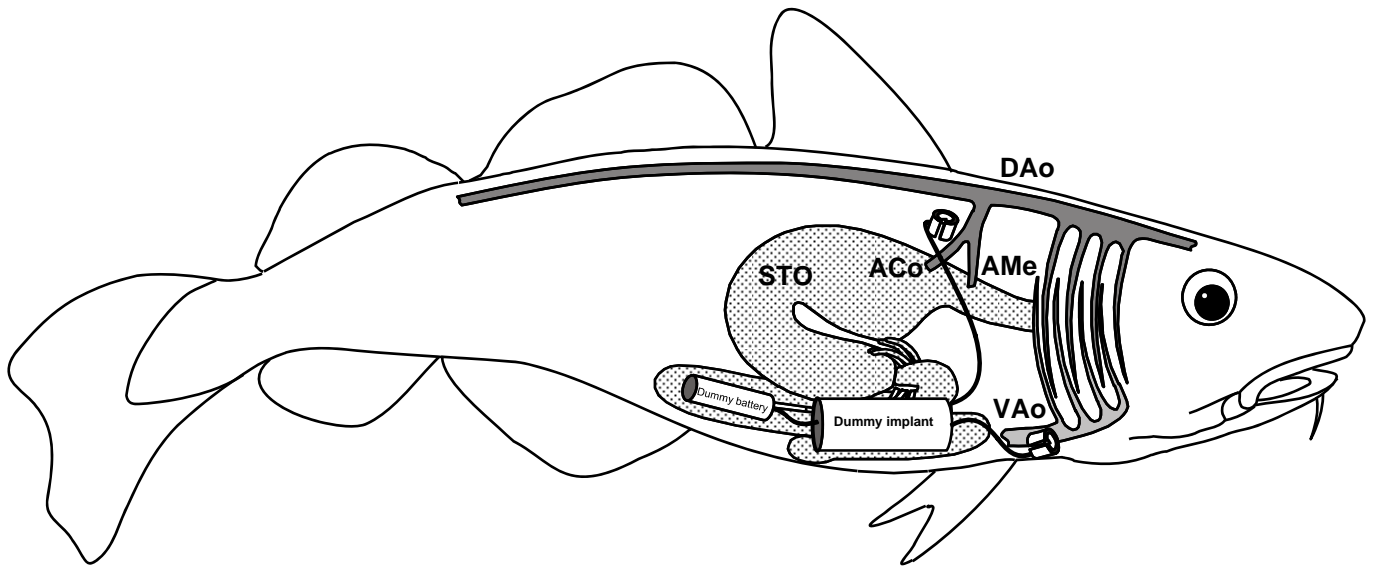
Table I. Basic data on gastric evacuation experiments (mean [SD]) on *Gadus morhua*. All experiments were carried out at 9-10 °C and all meals consisted of a single prey.

Group and experiment	Predator *Gadus morhua*				Prey *Ammodytes marinus*			
	L (cm)	Body mass (g)	Time *t* (h) for recovery of force-fed meal		Original body mass (g)	Recovered body mass at time *t*	Energy *E* (kJ g^{-1})	Observations (*n*)
Surgically implanted fish two days post surgery	38.7 (3.2)	550 (144)	25.3 (0.5)		12.9 (1.3)	10.1 (0.8)	6.83	9
Control fish two days post surgery	37.1 (2.7)	518 (155)	25.2 (1.4)		12.3 (1.8)	7.0 (0.9)	6.83	8
Surgically implanted fish ten days post surgery		545 (146)	23.6 (0.8)		12.9 (1.8)	8.2 (1.3)	6.83	9
Control fish ten days post surgery		503 (151)	23.5 (1.3)		11.5 (1.4)	7.2 (0.9)	6.83	8

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Table II. Coefficient of variation (CV) of the basic rate parameter ρ_{LTE} obtained from *Gadus morhua* fed an *Ammodytes marinus*. Different letters indicate a significant difference.

	Surgically implanted *G. morhua* (*n*=9)	Control *G. morhua* (*n*=8)	*G. morhua* feeding voluntarily on *Ammodytes tobianus* (n=20) (from Andersen[1])
Two days post surgery	0.287^a	0.207^a	0.080^b
Ten days post surgery	0. 242^a	0. 348^a	