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1 **Comparison of vegetable shortening and cocoa butter as vehicles for cortisol manipulation**
2 **in *Salmo trutta***

3

4 *In press in Journal of Fish Biology*

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6 K. Birnie-Gauvin^{1†§}, K. S. Peiman¹, M. H. Larsen³, K. Aarestrup⁴, K. M. Gilmour⁵, S. J. Cooke¹

7

8 ¹ Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of
9 Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S

10 5B6

11 ³ Danish Centre for Wild Salmon, Brusgårdsvej 15, 8960 Randers, Denmark.

12 ⁴ DTU AQUA, National Institute of Aquatic Resources, Section for Freshwater Fisheries and
13 Ecology, Technical University of Denmark, Vejlsøvej 39, 8600 Silkeborg, Denmark

14 ⁵ Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, ON, Canada K1N 6N5

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16

17 † Author to whom correspondence should be addressed:

18 Email: kbir@aquu.dtu.dk, +45 35 88 31 14

19 § Change of address: K. Birnie-Gauvin, DTU Aqua, National Institute of Aquatic Resources,

20 Section for Freshwater Fisheries and Ecology, Technical University of Denmark, Vejlsøvej 39,

21 8600 Silkeborg, Denmark

22

23 **Running headline:** cortisol manipulation in fishes

24 **ABSTRACT**

25 Vegetable shortening and cocoa butter have been used as vehicles for cortisol implants in
26 a wide range of organisms, though no studies have compared the effects of these vehicles on
27 plasma cortisol and glucose, or change in mass. This study demonstrates that vegetable
28 shortening and cocoa butter are two effective vehicles for intraperitoneal cortisol implants in
29 juvenile teleost fish (brown trout, *Salmo trutta*) residing in north temperate freshwater
30 environments. Each vehicle showed a different pattern of cortisol elevation. Vegetable
31 shortening was found to be a more suitable vehicle for long-term cortisol elevation (elevated at
32 days 3, 6 and 9 post-treatment), while cocoa butter may be better suited for short-term cortisol
33 elevation (only elevated at 3 days post-treatment). Additionally, plasma cortisol levels were
34 higher with cortisol-vegetable shortening than with cortisol-cocoa butter implants. Plasma
35 glucose levels were elevated 6 and 9 days post-treatment for fishes injected with cortisol-
36 vegetable shortening, but did not change relative to controls and shams in cortisol-cocoa butter
37 fishes. In conclusion, vegetable shortening and cocoa butter are both viable techniques for
38 cortisol manipulation in fishes in temperate climates, providing researchers with different options
39 depending on study objectives.

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41 **KEY WORDS:** cocoa butter, cortisol implants, teleost fish, vegetable shortening

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47 INTRODUCTION

48 Cortisol is the primary glucocorticoid stress hormone in fish (Mommsen *et al.*, 1989;
49 Wendelaar Bonga, 1997; Barton, 2002). Not surprisingly, there are hundreds of papers that have
50 measured cortisol in fishes to understand the consequences of different stressors (reviewed in
51 Mommsen *et al.*, 1999). Beyond using cortisol as a biomarker of exposure to a stressor,
52 physiologists started manipulating cortisol in fishes in the 1960s to explore the mechanistic role
53 of cortisol (e.g., Slusher, 1966). This allowed researchers to move past simply observing
54 variation in cortisol levels among individuals to performing cause-and-effect studies. However,
55 despite its potential ecological relevance (Sopinka *et al.*, 2015; Crossin *et al.*, 2016), this
56 technique has been mainly used in the lab (reviewed in Gamperl *et al.*, 1994). Additionally, the
57 best vehicle in which to suspend the cortisol for manipulation remains unclear. Past studies have
58 used saline oil (e.g. coconut oil), cocoa butter, and vegetable shortening to manipulate hormone
59 levels (Pottinger & Pickering, 1985; Gamperl *et al.*, 1994; Eriksen *et al.*, 2006; Doyon *et al.*,
60 2006). Studies have also used mini osmotic pumps going back several decades (Theeuwes &
61 Yum, 1976). However, these are less suitable for field studies owing to expense, as fish may not
62 be recovered to retrieve the pumps, and their higher invasiveness compared to injections. The
63 main advantage of cocoa butter and vegetable shortening is that they allow for prolonged,
64 continuous release of cortisol. They are injected as liquids, and solidify once inside the fish.
65 However, cocoa butter requires high temperatures to remain in liquid form (approx. 40°C),
66 potentially resulting in the scalding of organs when injected into a fish, and becomes very hard at
67 ambient temperatures in the north temperate regions which may lead to damage to the gonads
68 (personal observation; McConnachie *et al.*, 2012). In contrast, vegetable shortening remains in
69 liquid form at a lower temperature (approx. 30°C), and remains soft, even in cold water (5°C,

70 personal observation). Gamperl *et al.* (1994) originally suggested that vegetable shortening was
71 better than cocoa butter at lower temperatures as the hardness of cocoa butter may reduce the
72 absorption of cortisol.

73 This study is the first comparative study of vegetable shortening and cocoa butter as
74 vehicles for cortisol manipulation in the wild. Both vehicles are particularly suitable for field
75 studies (see Sopinka *et al.*, 2015) owing to their low cost and ease of administration. A wild
76 population of juvenile brown trout *Salmo trutta* L. 1758 was used to compare the temporal
77 patterns of circulating cortisol and glucose concentrations resulting from implants of cortisol
78 suspended in vehicles of cocoa butter versus vegetable shortening. Treatment effects were
79 compared to their corresponding sham (vehicle alone) and control (no implant) groups.

80 Additionally, effects on body mass were measured treatment. It was predicted that vegetable
81 shortening implants would result in cortisol being released over a longer period of time and in
82 higher levels, resulting in higher levels of glucose and more mass loss than cocoa butter
83 implants. It was also predicted that sham treatments would not elevate cortisol or glucose
84 concentrations or cause a change in mass compared to control fishes.

85

86 **MATERIALS AND METHODS**

87 The Villestrup stream is located in north-central Jutland, Denmark. The stream runs for several
88 kilometers across agricultural land, where a number of tributaries join in before reaching the
89 Mariager Fjord. The stream is home to a large population of semi-anadromous *S. trutta* (del
90 Villar-Guerra *et al.*, 2014). Three different sites (1 to 2km apart) within the same stream were
91 used. It is unlikely that there are genetic differences among populations so close (Hansen *et al.*,
92 2002), but even if there are, they are unlikely to have any biological significance especially when

93 comparing responses to treatments within a site. Fishes were captured via backpack
94 electrofishing (ELT 60 II GI; 300 volts; Scubla, Remanzacco, Italy) on three separate days in
95 2016: 125 fishes at Site 1 on March 3rd (25 fishes per group), 125 fishes at Site 2 on March 4th
96 (25 fishes per group) and 150 fishes at Site 3 on March 5th (30 fishes per group). During this
97 period, the temperature of the water in Villestrup was between 6 and 7°C.

98 Captured fishes were held in a 60 l bin filled with oxygenated fresh stream water. Fishes
99 were anesthetized in a solution of benzocaine (0.03 g l⁻¹ ethyl-*p*-aminobenzoate; Sigma,
100 www.sigmaaldrich.com) in stream water, then weighed (± 0.1 g), measured for total length
101 (± 0.1 cm), and tagged using a 23mm PIT tag (Passive Integrated Transponder tag, Texas
102 Instruments, RI-TRP-RRHP, 134Hz, 0.6g mass in air, Plano, Texas, USA). Tags were inserted
103 through a 5mm incision in the left side of the body, posterior to the pelvic fin. Only trout that
104 were 12-21cm in length (large enough for the PIT tag, but likely still juveniles; Larsen et al.
105 2013) were used in this study. Fishes were randomly assigned to one of the following treatment
106 groups: (1) control, (2) sham-vegetable shortening (sham-veg), (3) cortisol-vegetable shortening
107 (cort-veg), (4) sham-cocoa butter (sham-cocoa), (5) cortisol-cocoa butter (cort-cocoa). Cortisol-
108 treated fishes received an intracoelomic injection (1.5inch 18-gauge needle) of a suspension of
109 vegetable shortening (100% vegetable shortening, Crisco, OH, USA) or cocoa butter (100% pure
110 cocoa butter, NOW Foods, IL, USA) mixed with hydrocortisone 21-hemisuccinate (Sigma-
111 Aldrich, St. Louis, MO, USA, Product #H2882-1G), using a dosage of 0.01 ml vehicle (with a
112 concentration of 0.01g cortisol per ml) per 1 g of fish (equivalent to a cortisol dosage of 100mg
113 kg⁻¹). Sham fishes were injected with only 0.01 ml g⁻¹ fish vegetable shortening or cocoa butter.
114 The vegetable shortening and cocoa butter were heated using hot water to a temperature of 37°C
115 and 40 °C, respectively. All fishes were recovered (i.e., until full equilibrium was reached) in a

116 60 l tank of benzocaine-free fresh stream water following tagging. Cortisol-treated fishes were
117 recovered separately from sham and control fishes to prevent any cross-treatment contamination
118 of cortisol, and all fishes were then released at the site of capture. The tagging, weighing,
119 measuring and injecting process took less than one minute per fish. Overall, fishes were held in
120 tanks for approximately 60 minutes.

121 Fishes were recaptured via backpack electrofishing after 3, 6 and 9 days post-treatment,
122 at Site 3, Site 2 and Site 1, respectively. Immediately after shocking, we collected a blood sample
123 (<0.3 ml) from the caudal vasculature using a heparinized 1.5-inch 25-gauge needle and a 1 ml
124 syringe. All samples were collected within 3 minutes of capture. Fishes were then weighed.
125 Following recovery, fishes were returned to the river, and not recaptured. Blood samples were
126 held in a water-ice slurry until centrifuged at 2000 g for 2 minutes to separate plasma from red
127 blood cells. Plasma samples were kept at -80°C until analyzed. Environmental conditions should
128 not be a confounding factor here, as the 3 day sampling was within the 6 day sampling, and both
129 were within the 9 day sampling period. Hence, all fishes were exposed to the same conditions,
130 with day 9 fishes potentially experiencing a greater variation. However, this does not affect
131 treatment effects within a single time point, which is the focus of this study.

132 Plasma cortisol concentration was determined using a commercial radioimmunoassay kit
133 (ImmunoChem Cortisol ¹²⁵I RIA kit; MP Biomedicals, www.mpbio.com). This assay was
134 previously validated for use with teleost fish plasma samples (Gamperl *et al.*, 1994). All plasma
135 samples were measured in a single assay. Intra-assay variability (%CV) was 7.9%. Plasma
136 glucose levels were determined using an AccuCheck Compact Plus meter system (Roche, Basel,
137 Switzerland), a point-of-care device previously validated for use in teleost fishes (Stoot *et al.*,
138 2014).

139 Statistical analyses were conducted using JMP v12.0.1 (SAS Institute Inc.,
140 Buckinghamshire, UK). Cortisol and glucose values were log-transformed to achieve normality
141 of residuals. Two-way ANOVAs were used to evaluate differences in cortisol, glucose and
142 change in mass among treatment groups over the three sampling times. A Tukey-Kramer *post-*
143 *hoc* test was used to determine which groups differed, which is conservative with unequal
144 sample sizes as is the case here. Spearman correlations (to reduce the effect of outliers) were
145 used to determine whether cortisol levels were related to glucose levels among individuals using
146 within each category of treatment and day.

147

148 **RESULTS**

149 Between 9 and 17 fishes were recaptured per treatment group. Fishes treated with cortisol
150 suspended in vegetable shortening showed significantly higher plasma cortisol concentrations
151 after 3, 6 and 9 days post-treatment than both sham and the control treatments, with values at day
152 3 significantly higher than at day 9 (Fig. 1A; treatment \times time, $F_{8,172} = 3.07$, $P = 0.0029$). Cort-
153 cocoa fishes at day 3 had significantly higher cortisol levels than both sham and the control
154 treatments, but values for fishes sampled at days 6 and 9 did not differ from those for sham or
155 control fishes. At day 3, cort-veg fishes exhibited significantly higher plasma cortisol levels than
156 cort-cocoa fishes. Cortisol concentrations for fishes in the sham treatment were similar to fishes
157 in the control group across all time points. Glucose concentrations in cort-veg fishes were
158 significantly higher than those for sham and control treatments at days 6 and 9 (Fig. 1B;
159 treatment \times time, $F_{8,170} = 2.30$, $P = 0.023$), whereas plasma glucose concentrations in cort-cocoa
160 fishes did not differ from the sham or control groups on any day. On days 6 and 9, cort-veg
161 fishes had significantly higher glucose concentrations than cort-cocoa fishes.

162 Initially, mass for cortisol-treated fishes did not differ from their sham or the control
163 group (all $P > 0.50$). Sham-veg fishes sampled on day 9 gained mass while all other groups lost
164 mass (Fig 1C, treatment \times time, $F_{8,170} = 2.94$, $P = 0.0042$).

165 Plasma cortisol and glucose concentrations were positively related in day 9 cort-veg
166 treatment ($R^2 = 0.60$, $n=15$, $P = 0.037$) No other correlation was significant (all $P > 0.093$).

167

168 **DISCUSSION**

169 Cortisol implants (100mg kg^{-1}) generated a significant elevation in plasma cortisol
170 concentration using either vegetable shortening or cocoa butter as a vehicle. However, the use of
171 vegetable shortening as a vehicle caused a greater elevation of cortisol concentration than cocoa
172 butter after 3 days, and this elevation lasted longer. Moreover, plasma cortisol concentration
173 likely remained high for more than 9 days in fishes that received cortisol-vegetable shortening
174 implants, as found by Pickering & Duston (1983). In contrast, cocoa butter implants had short-
175 lasting effects on plasma cortisol levels, with circulating concentrations returning to control
176 levels by 6 days post-treatment. The soft texture of vegetable shortening (Fig. 2), even at low
177 temperatures (solidifies at 20°C , but remains soft at lower temperatures – e.g., it was $6-7^\circ\text{C}$
178 during this study) likely allows for more effective (i.e., faster) release of the cortisol. Cocoa
179 butter, however, becomes very hard even at fairly high temperatures (solidifies at 20°C), which
180 may prevent long-lasting release of cortisol in north temperate fish species, as indicated by the
181 peak cortisol levels 3 days post-treatment. The outer cortisol likely gets released quickly, but the
182 hardness of the cocoa butter prevents the release of the inner cortisol. Alternatively, it is possible
183 that cocoa butter releases cortisol more readily than vegetable shortening, leading to the implant
184 being depleted of cortisol more rapidly and the cortisol values in cocoa butter-treated fishes

185 peaking earlier than the first sampling time (3 days). Unfortunately, there is no way to
186 distinguish between the two possibilities with our data. The conclusion however, remains the
187 same: vegetable shortening appears to be a more appropriate vehicle for studies seeking long-
188 term cortisol elevation, while cocoa butter may be better suited for short-term cortisol elevation,
189 at least in north temperate regions.

190 Cortisol increases the rate of gluconeogenesis (reviewed by Mommsen *et al.*, 1999). An
191 increase in plasma glucose following treatment with cortisol implants therefore would be
192 consistent with the known physiological effects of cortisol. Plasma glucose concentrations were
193 found to be higher than those of sham and control treatments at both day 6 and 9 in cort-veg
194 fishes. In contrast, plasma glucose was never elevated above sham or control treatment fishes in
195 cort-cocoa fishes, in agreement with the shorter-lasting physiological effect of cocoa butter than
196 vegetable shortening on cortisol levels. Additionally, cortisol caused an increase in glucose
197 levels earlier in the cort-cocoa treatment (day 3) than in the cort-veg treatment (day 9), further
198 supporting the hypothesis that the cocoa butter vehicle generates a shorter and faster response
199 than vegetable shortening.

200 Increased conversion of stored energy reserves to glucose during gluconeogenesis may
201 also lead to a loss in mass. Additionally, cortisol tends to suppress appetite leading to a reduction
202 in food intake, and this would also be expected to result in mass loss (Madison *et al.*, 2015). The
203 9 days of the cortisol treatment examined in the present study did not have a significant effect on
204 change in mass relative to that observed in control or sham-treated fishes, suggesting that the
205 physiological effects of elevated cortisol take more time to manifest as changes in mass.
206 Previous studies in similar systems have reported decreased growth rates of cortisol-treated
207 fishes over two weeks and longer (Madison *et al.*, 2015; Midwood *et al.*, 2015; Midwood *et al.*,

208 2016; Birnie-Gauvin *et al.*, 2017; Peiman *et al.*, 2017). Sham-veg fishes at day 9 showed a
209 significant increase in mass, which may have resulted from the vegetable shortening itself
210 starting to be absorbed internally, while in the cort-veg fishes this effect may have been offset by
211 glucose metabolized by cortisol. Indeed, it was only in this latter group that cortisol and glucose
212 were positively related. The mechanism by which this occurred is unknown and its biological
213 significance remains evasive.

214 The present study showed that vegetable shortening and cocoa butter are two effective
215 vehicles for cortisol implants in north temperate regions, and that sham treatments with the
216 vehicle alone do not result in growth impairments compared to controls over the short-term, as
217 previously observed in reproductive female *S. trutta* following cocoa butter sham implants
218 (Hoogenboom *et al.*, 2011). However, it was noticed that cocoa butter implants had sharp edges,
219 which could result in internal organ damage, a potentially deleterious effect which has not
220 previously been noted. Cortisol levels peaked 3 days post-treatment for both vegetable
221 shortening and cocoa butter implants, and cortisol levels remained elevated for 9 days with the
222 vegetable shortening implant. Maximum cortisol levels achieved in this experiment are beyond
223 the physiological range for salmonids (Donaldson, 1981; Gamperl *et al.*, 1994). If the goal of the
224 study requires cortisol levels within the normal physiological range, a lower dosage of cortisol
225 may be appropriate. Glucose levels were affected by cortisol in fishes that received vegetable
226 shortening but not cocoa butter implants. Thus, in north temperate regions, vegetable shortening
227 is a more appropriate vehicle for studies seeking longer-term cortisol elevation, while cocoa
228 butter may be better suited for studies looking for short-term cortisol elevation, providing
229 researchers with different options depending on study objectives.

230

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237

238 REFERENCES

239

240 Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to
241 changes in circulating corticosteroids. *Integrative and Comparative Biology* **42**, 517-525. doi:
242 10.1093/icb/42.3.517

243

244 Birnie-Gauvin, K., Peiman, K. S., Larsen, M. H., Aarestrup, K., Willmore, W. G., Cooke, S. J.
245 (2017). Short-term and long-term effects of transient exogenous cortisol manipulation on
246 oxidative stress in juvenile brown trout. *Journal of Experimental Biology* **220**, 1693-1700. doi:
247 10.1242/jeb.155465

248

249 Crossin, G. T., Love, O. P., Cooke, S. J., & Williams, T. D. (2016). Glucocorticoid
250 manipulations in free-living animals: considerations of dose delivery, life-history context and
251 reproductive state. *Functional Ecology* **30**, 116-125. doi: 10.1111/1365-2435.12482

252

253 del Villar-Guerra, D., Aarestrup, K., Skov, C., Koed, A. (2014). Marine migrations in
254 anadromous brown trout (*Salmo trutta*). *Ecology of Freshwater Fish* **23**, 594-603. doi:
255 10.1111/eff.12110
256
257 Donaldson, E. M. (1981). Pituitary-interrenal axis as an indicator of stress in fish. In *Stress and*
258 *Fish* (Pickering, A. D., eds), pp. 11-47. London and New York: Academic Press.
259
260 Doyon, C., Leclair, J., Trudeau, V. L., Moon, T. (2006). Corticotropin-releasing factor and
261 neuropeptide Y mRNA levels are modified by glucocorticoids in rainbow trout, *Oncorhynchus*
262 *mykiss*. *General and Comparative Endocrinology* **146**, 126-135. doi:
263 10.1016/j.ygcen.2005.10.003
264
265 Eriksen, M. S., Bakken, M., Espmark, Å., Braastad, B. O., Salte, R. (2006). Prespawning stress
266 in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during
267 embryonic development affect offspring survival, growth and incidence of malformations.
268 *Journal of Fish Biology* **69**, 114-129. doi: 10.1111/j.1095-8649.2006.01071.x
269
270 Gamperl, A. K., Vijayan, M. M., Boutilier, R. G. (1994). Experimental control of stress hormone
271 levels in fishes: techniques and applications. *Reviews of Fish Biology and Fisheries* **4**, 215-255.
272 doi: 10.1007/BF00044129
273
274 Hansen, M. M., Ruzzante, D. E., Nielsen, E. E., Bekkevold, D., Mensberg, K. L. D. (2002).
275 Long-term effective population sizes, temporal stability of genetic composition and potential for

276 local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology* **11**,
277 2523-2535. doi: 10.1046/j.1365-294X.2002.01634.x
278
279 Hoogenboom, M. O., Armstrong, J. D., Miles, M. S., Burton, T., Groothuis, T. G., Metcalfe, N.
280 B. (2011). Implantation of cocoa butter reduces egg and hatchling size in *Salmo trutta*. *Journal*
281 *of Fish Biology* **79**, 587-596. doi: 10.1111/j.1095-8649.2011.03039.x
282
283 Larsen, M. H., Thorn, A. N., Skov, C., Aarestrup, K. (2013). Effects of passive integrated
284 transponder tags on survival and growth of juvenile Atlantic salmon, *Salmo salar*. *Animal*
285 *Biotelemetry* **1**, 19-25. doi: 10.1186/2050-3385-1-19
286
287 Madison, B. N., Tavakoli, S., Kramer, S., Bernier, N. J. (2015). Chronic cortisol and the
288 regulation of food intake and the endocrine growth axis in rainbow trout. *Journal of*
289 *Endocrinology* **226**, 103-119. doi: 10.1530/JOE-15-0186
290
291 McConnachie, S. H., Cook, K. V., Patterson, D. A., Gilmour, K. M., Hinch, S. G., Farrell, A. P.,
292 Cooke, S. J. (2012). Consequences of acute stress and cortisol manipulation on the physiology,
293 behavior and reproductive outcome of female Pacific salmon on spawning grounds. *Hormones*
294 *and Behaviour* **62**, 67-76. doi: 10.1016/j.yhbeh.2012.05.001
295
296 Midwood, J. D., Larsen, M. H., Boel, M., Aarestrup, K., Cooke, S. J. (2015). An experimental
297 field evaluation of winter carryover effects in semi-anadromous brown trout (*Salmo trutta*).
298 *Journal of Experimental Zoology* **323**, 645-654. doi: 10.1002/jez.1955

299

300 Midwood, J. D., Larsen, M. H., Aarestrup, K., Cooke, S. J. (2016). Stress and food deprivation:
301 linking physiological state to migration success in a teleost fish. *Journal of Experimental Biology*
302 **219**, 3712-3718. doi: 10.1242/jeb.140665

303

304 Mommsen, T. P., Vijayan, M. M., Moon, T. W. (1999). Cortisol in teleosts: dynamics,
305 mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* **9**, 211-
306 268. doi: 10.1023/A:1008924418720

307

308 Peiman, K. S., Birnie-Gauvin, K., Midwood, J. D., Larsen, M. H., Wilson, A. D., Aarestrup, K.,
309 Cooke, S. J. (2017). If and when: intrinsic differences and environmental stressors influence
310 migration in brown trout (*Salmo trutta*). *Oecologia* **184**, 374-384. doi: 10.1007/s00442-017-
311 3873-9

312

313 Pickering, A. D., Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L.,
314 and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *Journal of Fish*
315 *Biology* **23**, 163-175. doi: 10.1111/j.1095-8649.1983.tb02891.x

316

317 Pottinger, T. G., Pickering, A. D. (1985). The effects of 11-ketotestosterone and testosterone on
318 the skin structure of brown trout, *Salmo trutta* L. *General and Comparative Endocrinology* **59**,
319 335-342. doi: 10.1016/0016-6480(85)90389-2

320

321 Slusher, M. A. (1966). Effects of cortisol implants in the brainstem and ventral hippocampus on
322 diurnal corticosteroid levels. *Experimental Brain Research* **1**, 184-194. doi:

323 10.1007/BF00236870

324

325 Sopinka, N. M., Patterson, L. D., Redfern, J. C., Pleizier, N. K., Belanger, C. B., Midwood, J. D.,

326 Crossin, G. T., Cooke, S. J. (2015). Manipulating glucocorticoids in wild animals: basic and

327 applied perspectives. *Conservation Physiology* **3**, cov031. doi:10.1093/conphys/cov031

328

329 Stoot, L. R., Cairns, N. A., Cull, F., Taylor, J. J., Jeffrey, J. D., Morin, F., Mandelman, J. W.,

330 Clark, T. D., Cooke, S. J. (2014). Use of portable blood physiology point-of-care devices for

331 basic and applied research on vertebrates: a review. *Conservation Physiology* **2**, cou011.

332 doi: 10.1093/conphys/cou011

333

334 Theeuwes, F., Yum, S. I. (1976). Principles of the design and operation of generic osmotic

335 pumps for the delivery of semisolid and liquid drug formulations. *Annals of Biomedical*

336 *Engineering* **4**, 343-353. doi: 10.1007/BF02584524

337

338 Wendelaar Bonga, S. E. W. (1997). The stress response in fish. *Physiological Reviews* **77**, 591-

339 625. doi: 10.0031-9333/97

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343

344 **FIGURE CAPTIONS**

345

346 **Fig 1.** PIT-tagged brown trout (*Salmo trutta*) were subjected to one of 5 treatments; control (no
347 implant), sham-veg (given a vegetable shortening implant), sham-cocoa (given a cocoa butter
348 implant), cort-veg (given 100mg kg⁻¹ of cortisol suspended in a vegetable shortening implant)
349 and cort-cocoa (given 100mg kg⁻¹ of cortisol suspended in a cocoa butter implant), and were re-
350 captured at 3 (black bars), 6 (grey bars) or 9 (white bars) days post-treatment. (A) Plasma
351 cortisol concentration, (B) plasma glucose concentration, and (C) change in mass are presented
352 as a function of treatment group and sampling day. Values are means + SEM, *N* = 9 to 17.
353 Groups that share a letter are not significantly different from one another (see text for details).

354

355 **Fig 2.** Representative images of the dissection of brown trout (*Salmo trutta*) post-treatment to
356 illustrate the different implant vehicles; (A) control, (B) vegetable shortening implant, and (C)
357 cocoa butter implant. Arrows point to the implants. Vegetable shortening remained soft at 3, 6
358 and 9 days post-treatment. Cocoa butter implants were hard to the touch at 3, 6 and 9 days post-
359 treatment, with some implants showing sharp edges.

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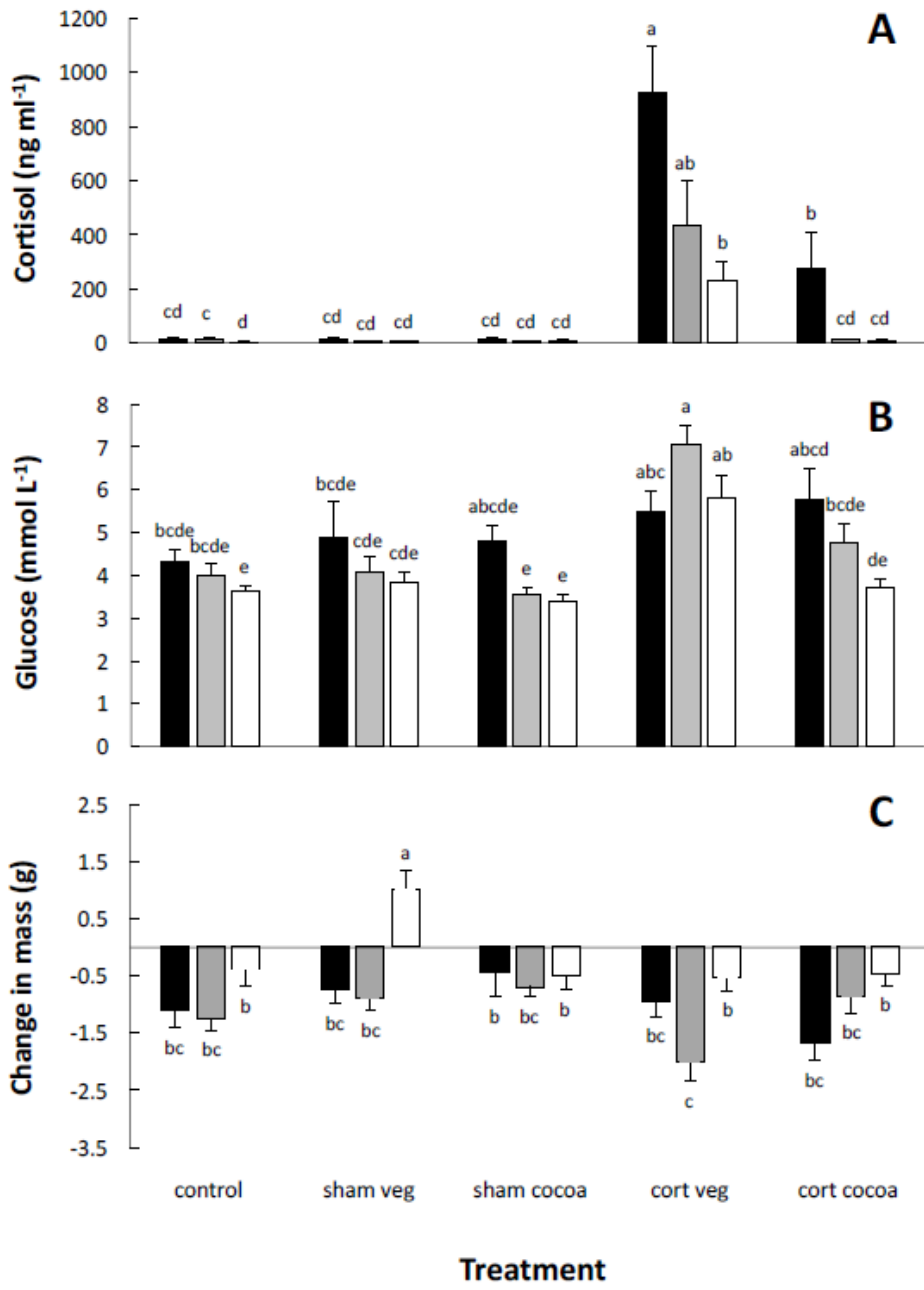
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367 **Figure 1.**



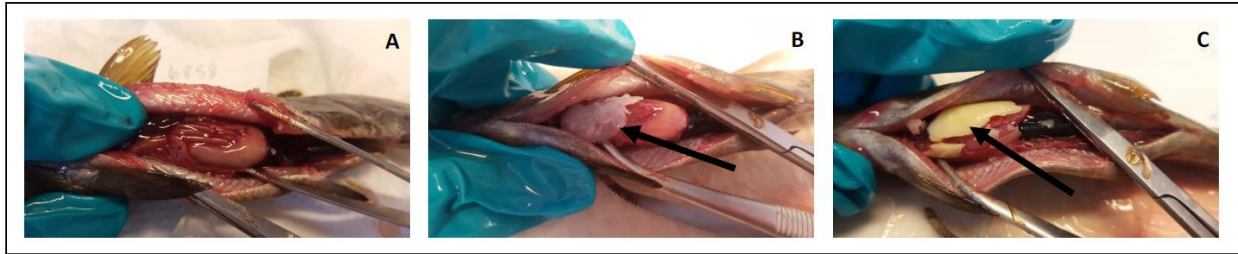
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372 **Figure 2.**



373