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1 # Does juvenile Baltic sturgeon (*Acipenser oxyrinchus*) smell the enemy?

2

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14 **SUMMARY**
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18 Atlantic sturgeon (*Acipenser oxyrinchus*), also known as Baltic sturgeon, is considered extinct
19 in German waters. Fish-rearing for conservation purposes still relies on classical hatchery
20 technology producing fish not well suited for facing life in the wild, lacking behavioural skills
21 such as foraging or anti-predation. Predation is hence a major source of mortality in newly
22 stocked individuals. The aim of this study was to evaluate if naïve Baltic sturgeon juveniles
23 were able to smell and recognize a common predator, sander (*Sander lucioperca).* Over a
24 period of 30 days, three tanks from each group of Baltic sturgeon were attached to a rearing
25 tank with sander (sander unit) and, as a control, carp (carp unit). Morphology of the dorsal
26 scutes, distribution within the tank, stress (glucose, lactate and cortisol) and gene expression
27 of brain plasticity and cognition were studied in comparison to the control group (carp unit).
28 No significant differences were observed in any of the parameters measured. Thus, we
29 conclude that naïve Baltic sturgeon is not able to innately recognize potential predators and
30 future studies should focus on implementing predator odour together with chemical alarm
31 substances.

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34 **Key words:** *Acipenser oxyrinchus, fitness, antipredator behaviour, conservation*
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37 ## 1. Introduction

38 Sturgeons (Acipenseridae) have experienced a drastic decline due to several reasons including
39 overfishing, habitat destruction and pollution over the last decades. Nowadays, sturgeons are
40 among the most endangered fish species worldwide (IUCN, 2011). Baltic sturgeon (*Acipenser*
41 *oxyrinchus)* has been indigenous to the Baltic region, but it is currently considered extinct
42 (Gessner et al., 2006; Langhans et al., 2016). In order to recover Baltic sturgeon populations,
43 restoration programs have been established to reestablish the species in its former distribution
44 range by releases of juveniles with hatchery origin in an attempt to build up self-sustaining
45 populations (Gessner et al., 2011).
46 Fish-rearing for conservation purposes still relies on classical aquaculture hatchery techniques
47 that focus on growth, survival and reproduction within the hatchery, which are known to have
48 shortcomings with regard to the behavioural skills needed in the wild (Ferno & Jarvi, 1998;
49 Olla et al.,1998). One of the pitfalls of these methods is the high mortality of newly stocked
50 individuals (Brown & Smith, 1998; Suboski & Templeton, 1989). In juvenile fishes, predation
51 is a major source of the post-stocking mortality following release (Brown & Day, 2002;
52 Brown & Laland, 2001).
53 Prey animals can determine risk by using a variety of visual, chemical and mechanical cues
54 (lateral line). Regarding chemical cues, fishes heavily rely on chemosensory information,
55 specifically semiochemicals. There are three classes of semiochemicals: kairomones,
56 disturbance cues and damage-released alarm cues. Kairomones are cues emitted by one
57 species and are detected by another, for instance, the scent of predators detected by prey.
58 Kairomones are adaptively favourable to the receiver and help prey to detect and avoid
59 potential predators (Ferrari et al., 2010).
60 The possibility of training captive-bred animals in predator avoidance prior release into the
61 wild has received attention in the conservation context (Olla et al., 1994; Brown & Laland,
62 2001; Griffin et al., 2000; Wallace, 2000). Exposure to various predator-stimuli prior to
63 reintroductions has already been used with mammalian and avian prey (Griffin et al., 2000),
64 but has received much less attention in fishes (Brown & Day, 2002). Antipredator behaviour
65 is often assumed to be strongly defined by genetic components, but fishes can be very flexible
66 in adjusting their responses (Kelley & Magurran, 2003). Studies have shown that the
67 appropriate stimulus is able to improve the avoidance responses of fishes (Berejikian, 1995;
68 Brown & Smith, 1998; Mirza & Chivers, 2000)
69 In this study, the objective is to determine if juvenile Baltic sturgeon is able to recognize a
70 common predator, sander (*Sander lucioperca*) by smell. Therefore, sturgeon juveniles were

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71 kept in the rearing water of sander for 30 days. Distribution within the tank, stress (glucose,
72 lactate and cortisol), morphology of the dorsal scutes and gene expression of brain plasticity
73 and cognition markers were studied in comparison to a control group that was reared in water
74 used to rear carp (Fig. 1).
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76 **2. Materials and Methods**

77 **2.1. Experimental design**

78 A total of 120 juvenile Baltic sturgeon, *Acipenser oxyrinchus,* (23.5 ± 2 cm; 39.9 ± 8.8 g)
79 were randomly distributed to 6 tanks (132 cm* 62 cm* 72 cm; V = 140 l). Three tanks of each
80 group was attached to a rearing tank with sander (sander unit) or, as a control, carp (carp
81 unit). The sander units were maintained in flow through with water of the municipal water
82 supply, the carp rearing water was recirculated through a filter unit. The tanks were stocked at
83 4 kg/m³ corresponding to 10 sander and 13 carp per tank (Fig. 1A). Water turnover was 540
84 l/h and 509 l/h in the sander (T1) and carp tank (T2) respectively. In the sturgeon tanks, water
85 turnover was 172 ± 7.2 l/h. Fish were kept at a temperature of 19.8± 0.4 under a natural
86 photoperiod. Water parameters (T, O_2 9.1 mg/L) were monitored daily, nitrite (< 0.05 mg/L)
87 and TAN (< 0.09 mg/L) were determined every 3 days. All fish (sturgeon, sander and carp)
88 were allowed to acclimate for two weeks prior to the start of the experiment.

89 **2.2.Sampling procedure**

90 Sturgeon juveniles were sampled after 30 days of experiment. Blood samples were collected
91 from the caudal vein with a syringe. Serum was collected after centrifugation (10,000 rpm, 5
92 min, 4° C) and stored at -20° C until use. Scute morphology was assessed by photographs.
93 The increase in length of the protrusions was determined before and after the experiment with
94 the ImageJ program (https://imagej.nih.gov/ij/). At the end of the experiment, the fish were
95 killed by an overdose of MS222 and the brain was removed surgically. Sturgeon brains were
96 dissected and divided into three parts representing the three main brain regions (forebrain,
97 midbrain and hindbrain). Samples were stored in RNA later at -80 °C until gene expression
98 analysis.

99 All experiments were in compliance with EU Directive 2010/63/EU and approved by the
100 national authorities (G0305/15, Landesamt für Gesundheit und Soziales, Berlin, Germany).

101 **2.3.Tank distribution**

102 Distribution of sturgeon in the rearing tank was determined from 7 photos taken every 5 min
103 at 1 day, 5 days, 10 days, 20 days and 30 days of the experimental trial. In order to assess the
104 distribution, the tank was divided into 3 zones (up-, mid-, downstream section of the through).
105 The juveniles recorded in each zone expressed as percentage of the total number of fish in the
106 tank was recorded.

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108 **2.4.Scute morphology**

109 To determine the morphology of the scutes, three scutes anterior to the dorsal fin were
110 analyzed using ImageJ (**https://imagej.nih.gov/ij**). The measurements included diagonal
111 (distance from the basis to the tip of the respective scute) and basal distance. The
112 measurements were calibrated to a scale bar. All measurements were carried out in triplicate.

113 **2.5. Blood parameters (glucose, lactate and cortisol)**

114 For the determination of serum parameters, blood was sampled from the caudal vein with a
115 heparinized syringe. After centrifugation at 5000 g for 5 min, cell-free plasma was
116 immediately shock frozen and stored at -80 °C until further processing.

117 Glucose in the plasma of Baltic sturgeon was measured with a glucose colorimetric GOD-
118 PAP kit (Greiner). In order to initiate the reaction, 5 µl of serum (1:5 dilution) from Baltic
119 sturgeon were mixed with 250 µl of reagent. The mixture was incubated for 10 min at 25° C.
120 Afterwards, the absorbance was measured at 500 nm and concentrations were calculated from
121 a dilution series (0.0625-1 mg/ml).

122 Plasma lactate in the serum of Baltic sturgeon was measured with a lactate colorimetric LOD-
123 PAP kit (Greiner). In brief, 5 µl of serum (1:2 dilution) from Baltic sturgeon was mixed with
124 250 µl of reagent. The mixture was incubated for 10 min at 25° C. Afterwards, the absorbance
125 was measured at 500 nm and concentrations were calculated from a dilution series (0.0375-
126 0.3 mg/ml).

127

128 Cortisol was determined using an ELISA kit (IBL, Germany). In brief, 100 µL plasma was
129 extracted by vigorously shaking for 3 min with 1.9 mL ethanol in 5 mL glass vials. The
130 organic phase was transferred to a new vial and the extraction was consecutively repeated
131 twice as described above. The three fractions were pooled and allowed to evaporate under a
132 constant nitrogen stream. For analysis, the remaining steroid fraction was redissolved in assay
133 buffer (IBL, Germany). Corstisol concentrations were determined in duplicate at 450 nm with
134 an Infinite M200 microplate reader (Tecan, Germany) and calculated from a standard dilution
135 series.

136 **2.6. Gene expression**

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137 Total RNA was extracted with TRIzol as described by Reiser et al., (2011), including a
138 DNase I digestion. Total RNA concentration and purity were determined in duplicates with a
139 Nanodrop® ND-1000 UV–Vis spectrophotometer. Purity was validated by the ratio of the
140 absorbance at 260 and 280 nm (A260/280) ranging between 1.8 to 2.0. Moreover, integrity of
141 the total RNA was checked by gel electrophoresis and, in 10% of all samples, on a RNA 6000
142 Nano chip with an Agilent 2100 Bioanalyzer. To eliminate potential DNA contamination,
143 DNAse I digestion was performed in all samples prior to transcription. Next, mRNA was
144 transcribed with MMLV Affinity reverse transcriptase (Agilent, 200 Units/µl) according to
145 the manufacturer's instruction. In 10% of the samples, the enzyme was substituted by pure
146 H_20, serving as a control (-RT) to monitor DNA contamination.

147 Species-specific primers targeting elongation factor 1α (*ef1a*), brain-derived neurotrophic
148 factor (*bdnf*), neurogenic differentiation factor (*neurod1*) and proliferating cell nuclear antigen
149 (*pcna*) were designed using the sequence information available. Specificity of the assays was
150 confirmed by direct sequencing (SeqLab, Germany). Real-time PCR was carried out with
151 Mx3005p qPCR Cycler (Stratagene), monitoring specificity by melting curve analysis.

152 For the PCR reaction, 2 µL of the diluted samples (40 ng/µL) were used as template in 20 µL
153 PCR mix [SYBR-Green I (Invitrogen), 200 µM of each dNTPs (Qbiogene), 3 mM MgCl 2
154 and 1 U Invitrogen Platinum Taq polymerase]. PCR conditions comprised an initial
155 denaturation at 96 °C for 3 min, followed by 40 cycles of denaturation at 96 °C for 30 s,
156 primer annealing (for Ta, see Table 1) for 30 s and elongation at 72 °C for 30 s. PCR
157 efficiencies were determined experimentally with a dilution series of a calibrator
158 corresponding to 200 ng/µl. PCR assays for all individual samples were run in duplicate.
159 Expression of target genes were calculated by the comparative CT method (ΔΔCT) according
160 to (Pfaffl, 2001), correcting for the assay efficiencies and normalizing to *ef1a* as a
161 housekeeping gene. Expression data are presented as fold increase of the respective calibrator.

162 **2.7. Data analysis and statistical methods**

163 Data are presented as mean ± standard deviation (SD). Prior to statistical analyses, all data
164 were tested for normality of distribution using the Kolmogorov-Smirnov/ Shapiro Wilk test
165 and for homogeneity using Levene’s test. Data on the behavior were scored and analyzed
166 using Dunn’s multiple comparison test. RNA expression, lactate, glucose and cortisol were
167 analysed with a parametric student T-test or non-parametric Mann-Whitney test. The level of

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168 significance used was $P \leq 0.05$. All statistical analyses were performed with GraphPad Prism
169 statistical program.
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171 **3. Results**

172 **3.1. Tank distribution**

173 The distribution of the fish in the tanks did not reveal significant differences for the
174 percentage of Baltic sturgeon recorded in each zone (Fig 2) over time. Approximately, 25-
175 35% of sturgeon stayed in each of the three zones regardless of the source of the inflow water.
176 No significant differences were observed between dates or treatment observed (Dunn's, $P \leq$
177 0.05).

178 **3.2. Scute morphology**

179 Diagonal and basis length of the scutes were correlated (Fig 3). There were no significant
180 differences of the coefficient between the sander and the carp group observed.

181 **3.3. Glucose, lactate and cortisol activities**

182 No significant differences were observed in glucose, lactate or cortisol concentrations in the
183 serum of Baltic sturgeon at the end of the experimental trial (Fig 4). Cortisol concentrations
184 did not reflect any differences between treatments groups.

185 **3.4. Brain plasticity and cognition**

186 Selected genes related to brain plasticity and cognition (*neurod1, bdnf, pcna*) were analyzed
187 in all three brain areas of Baltic sturgeon at the end of the rearing trial. No significant
188 differences were observed in the respective genes for any of the three brain regions (forebrain,
189 midbrain, hindbrain) of Baltic sturgeon (Fig 5).

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192 **Discussion**

193 Stocking programs routinely release hatchery-reared fish into their natural habitat in an
194 attempt to restore or maintain stable populations. One of the pitfalls of these stocking
195 programs is the elevated mortality of newly stocked individuals (Brown & Smith, 1998;
196 Suboski & Templeton, 1989), which in most cases is highest during and immediately after
197 release (Olla et al.,1994). Unfortunately, methods for reducing post-release mortality have not
198 kept the same pace as aquaculture technology. In general, conservationists have major
199 concerns regarding early life experiences in artificial environments and their impact upon the
200 resulting lack of fitness for survival (Johnsson et al., 2014).
201 When hatchery-reared fish are released into the wild, they are immediately placed in a novel
202 and variable environment and are also exposed to predatory risk, which they do not
203 experience during the hatchery period. Most mortality during and right after release is due to
204 predation (Brown & Day, 2002) Conservation managers should focus on improving fish
205 survival through reducing mortality for the periods during and immediately following release
206 (Sproul &Tominaga, 1992), which will help in closing the gap between wild and hatchery-
207 reared fish. The differences might be overcome by enriching the environment in which the
208 fish are reared. Furthermore, there is growing evidence that antipredator behaviour is highly
209 sensitive to artificial rearing (Berejikian, 1995). This process should include anti-predator and
210 foraging training before releasing the fish into the wild.
211 As with other behavior, anti-predator responses have both inherent and learned components.
212 However, most behavior patterns should be viewed as a combination of these two (Kieffer &
213 Colgan, 1992). Learned recognition of novel predators could potentially increase individual's
214 survival during later predator encounters (Gazdewich & Chivers, 2002; Mirza & Chivers,
215 2000). Furthermore, several studies have shown that prey reduce their vulnerability to
216 predation by changing morphology, life history strategy and/or behavior when exposed to
217 substances emitted by a predator (Brönmark & Hansson, 2000).
218 In this study, the objective was to determine if Baltic sturgeon (*A. oxyrinchus*) was able to
219 innately (inherent component) recognize the smell of a common predator, sander (*Sander*
220 *lucioperca*). In the experiments, there was no indication of differences in the morphology of
221 the dorsal scutes, uneven distribution within the tank, stress levels (glucose, lactate and
222 cortisol) or gene expression of brain plasticity. Thus, we conclude that there was no indication
223 that Baltic sturgeon was chronically stressed during the experimental trial.
224 Several options should be considered as underlying causes to contributing to this result. First,
225 the fish might not be able to differentiate the smell of the two species when naïve. Second, at

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226 the size of the fish tested, carps could be considered a potential predator too. For the behavior
227 observations, the time span between introducing the fish and recording their response has
228 already allowed acclimation. The same reason holds true for the blood stress parameters. In
229 contrast to these hypothetical explanations, the results for the plasticity of the brain regions
230 provide clear evidence that either the control was not functional or that the smell recognition
231 is not effective in naïve fish.
232 In conclusion, these results suggest that in order to raise Baltic sturgeon with the necessary
233 anti-predator behavior to survive in the wild, it would be necessary to undergo a more
234 complex training period. Only the exposure to predator smell is not sufficient to stimulate
235 anti-predator behavior. In future trials, the training period should consist in implementing
236 pairing predator odour with conspecifics alarm substances. This would be mostly appropriate
237 as it seems that Baltic sturgeon lack anti-predator inherent components. Also, the type of
238 predator exposure could impact antipredator behavior learning. For example, instead of a
239 continuous exposure (ongoing stress), the exposure of Baltic sturgeon to potential predators
240 could take place only intermittently to avoid habituation. It might be needed to be taken into
241 account that as sturgeon develop morphological defenses, such as the growth of pointed bony
242 scutes, they may rely less upon antipredator behavior to prevent being utilized as prey. If
243 morphological or chemical defenses of prey are effective defense mechanisms, it is possible
244 that other behavioral responses may appear to be weak (DeWitt & Langerhans, 2003).

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252

253 **Competing Interests**

254 I declare that there are no competing interests.

255 **Author contributions**

256 The experiment was conducted by A.M. and C.E.S.. The laboratory analysis was carried out
257 by M.C.R. M.C.R. wrote the first draft of the manuscript. S.W. supervised the project. The
258 manuscript was revised by all co-authors.

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260 **References**

261 Berejikian, B.A. (1995). The effects of hatchery and wild ancestry and experience on the
262 relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator.
263 *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 2476–2482.

264 Brönmark, C., & Hansson, L.A. (2000). Chemical communication in aquatic systems: An
265 introduction. *Oikos*, *88*, 103–109. https://doi.org/10.1034/j.1600-0706.2000.880112

266 Brown, C., Day, R.L. (2002). The future of stock enhancements: Lessons for hatchery
267 practice from conservation biology. *Fish and Fisheries*, *3*, 79–94.
268 https://doi.org/10.1046/j.1467-2979.2002.00077

269 Brown, C., & Laland, K. (2001). Social learning and life skills training for hatchery reared
270 fish. *Journal of Fish Biology*, *59*, 471–493. https://doi.org/10.1006/jfbi.2001.1689

271 Brown, G.E., & Smith, R.J.F. (1998). Acquired predator recognition in juvenile rainbow trout
272 (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of
273 a predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*, 611–617.
274 https://doi.org/10.1139/f97-261

275 DeWitt, T.J., & Langerhans, R.B. (2003). Multiple prey traits, multiple predators: Keys to
276 understanding complex community dynamics. *Journal of Sea Research*, *49*, 143–155.
277 https://doi.org/10.1016/S1385-1101(02)00220-4

278 Ferno, A., & Jarvi, T. (1998). Domestication genetically alters the antipredator behaviour of
279 anadromous brown trout (*Salmo trutta*)- dummy predator experiment. *Nordic Journal of*
280 *Freshwater Research*, *74*, 95–100.

281 Ferrari, M.C.O., Wisenden, B.D., & Chivers, D.P. (2010). Chemical ecology of predator–prey
282 interactions in aquatic ecosystems: a review and prospectus.. *Canadian Journal of*
283 *Zoology*, *88*, 698–724. https://doi.org/10.1139/Z10-029

284 Gazdewich, K.J., & Chivers, D.P. (2002). Acquired predator recognition by fathead minnows:
285 Influence of habitat characteristics on survival. *Journal of Chemical Ecology*, *28*, 439–
286 445.

287 Gessner, J., Arndt, G.M., Tiedemann, R., Bartel, R., & Kirschbaum, F. (2006). Remediation
288 measures for the Baltic sturgeon: Status review and perspectives. *Journal of Applied*
289 *Ichthyology*, *22*, 23–31. https://doi.org/10.1111/j.1439-0426.2007.00925

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290 Gessner, J., Tautenhahn, M., Spratte, S., Arndt, G.M., & von Nordheim, H. (2011).
291 Development of a German Action Plan for the restoration of the European sturgeon
292 *Acipenser sturio* L. - implementing international commitments on a national scale.
293 *Journal of Applied Ichthyology*, *27*, 192–198. https://doi.org/10.1111/j.1439-
294 0426.2011.01697

295 Griffin, A.S., Blumstein, D.T., & Evans, C.S. (2000). Training captive-bred or translocated
296 animals to avoid predators. *Conservation Biology*, *14*, 1317–1326.
297 https://doi.org/10.1046/j.1523-1739.2000.99326

298 IUCN (2018). The IUCN Red List of Threatened Species. Version 2018-1.Johnsson, J.I., &
299 Abrahams, M.V. (1991). Interbreeding with domestic strain increases foraging under
300 threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): An experimental
301 study. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*, 243–247.
302 https://doi.org/https://doi.org/10.1139/f91-033

303 Johnsson, J.I., Brockmark, S., & Näslund, J. (2014). Environmental effects on behavioural
304 development consequences for fitness of captive-reared fishes in the wild. *Journal of*
305 *Fish Biology*, *85*, 1946–1971. https://doi.org/10.1111/jfb.12547

306 Kelley, J.L., & Magurran, A.E. (2003). Learned predator recognition and antipredator
307 responses in fishes. *Fish and Fisheries*, *4*, 216–226. https://doi.org/10.1046/j.1467-
308 2979.2003.00126

309 Kieffer, J.D. & Colgan, P. (1992). The role of learning in fish behavior. *Reviews in Fish*
310 *Biology and Fisheries*, *2*, 125–43.

311 Langhans, S.D., Gessner, J., Hermoso, V., & Wolter, C. (2016). Coupling systematic planning
312 and expert judgement enhances the efficiency of river restoration. *Science of the Total*
313 *Environment*, *560–561*, 266–273.https://doi.org/10.1016/j.scitotenv.2016.03.232

314 Mirza, R.S., & Chivers, D.P. (2000). Predator-recognition training enhances survival of brook
315 trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of*
316 *Zoology*, *78*, 2198–2208. https://doi.org/10.1139/z00-164

317 Olla, B.L., Davis, M.W., & Ryer, C.H. (1998). Understanding how the hatchery environment
318 represses or promotes the development of behavioral survival skills. *Bulletin of Marine*
319 *Sciences*, *62*, 531–550.

bioRxiv preprint doi: https://doi.org/10.1101/476952; this version posted November 30, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

320 Olla, B.L., Davis, M.W. & Ryer, C.H. (1994). Behavioural deficits in hatchery-reared fished:
321 Potential effects on survival following release. *Aquaculture and Fisheries Management*,
322 *25*, 19–34.

323 Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-
324 PCR. N*ucleic Acids Research*, *29*, 45.

325 Reiser, S., Wuertz, S., Schroeder, J.P., Kloas, W., Hanel, R. (2011). Risks of seawater
326 ozonation in recirculation aquaculture - Effects of oxidative stress on animal welfare of
327 juvenile turbot (*Psetta maxima*, L.). *Aquatic Toxicology*, *105*, 508–517.
328 https://doi.org/10.1016/j.aquatox.2011.08.004

329 Sproul, T., & Tominaga, O. (1992). An economic review of the Japanese flounder stock
330 enhancement project in Ishikary Bay. *Bulletin of Marine Science*, *50*, 75–88.

331 Suboski, M.D., & Templeton, J.J. (1989). Life skills training for hatchery fish: Social learning
332 and survival. *Fisheries Research*, *7*, 343–352. https://doi.org/10.1016/0165-
333 7836(89)90066-0

334 Wallace, M.P.(2000). Retaining natural behavior in captivity for reintroduction programmes,
335 In *Behaviour and Conservation* (eds L.M. Gosling and W.J. Sutherland). Cambridge
336 University Press, Cambridge, pp. 300-314-

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340 **Tables**

341 **Table 1.** Specifications of qPCR assays including primer sequences, annealing temperature
342 (Ta), amplicon length [bp], PCR efficiency (Eff) and NCBI accession number of the
343 respective housekeeping (ef) and target genes: ef- elongation factor 1 a, neurod1- neurogenic
344 differentiator factor, bdnf - brain-derived neurotrophic factor, pcna- proliferating cell nuclear
345 antigen-

gene	primer	5'-3' sequence	Ta [°C]	length [bp]	Eff.[1] [%]	GeneBank #
efa	f	TCAgggAgAAgATTgACCgT	65	239	97	
	r	AgACTTggTgACTTTgCCTg				
neuroD	f	TATCATTCCCCTggTCTgCC	65	175	98	
	r	CATTAACgCTCAgTggTggg				
pcna	f	gAAgAAggTTTTggAggCg	65	187	92.5	
	r	CCTgCTCAgATTgACCCC				
bdnf	f	gACggCCgTAgACAAgAAgA	65	188	84.5	
	r	TggTCCgACACTgTgAATTg				

[1]Efficiency was determined from serial dilution series

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350 **Figure legends**

351 **Figure 1**. Experimental design. 1A. Three tanks from each group were attached to a rearing
352 tank with sander (sander unit) or, as a control, carp (carp unit). 1B. Tank distribution.
353 Different zones were established in order to observe the distribution towards the inlet.

354 **Figure 2.Tank distribution.** Percentage of Baltic sturgeon (*A. oxyrinchus*) in each zone of
355 rearing troughs during the exposition to water from Sander (left) or carp (right) rearing
356 systems. No significant differences over time (Dunn's, $p \leq 0.05$) or between treatments
357 (Mann-Whitney test, $p \leq 0.05$) were observed.

358 **Figure 3**. Comparison of the dorsal scutes dimensions (b-basis, d – diagonal) from juvenile
359 Baltic sturgeon *A. oxyrinchus* kept in the rearing water of sander or, as a control, carp for 30
360 days.

361 **Figure 4.** Glucose, lactate and cortisol in the plasma s of juvenile Baltic sturgeon *A.*
362 *oxyrinchus* kept in the rearing water of sander or, as a control, carp for 30 days. No significant
363 differences between treatments were observed (Mann-Whitney test, $p \leq 0.05$).

364 **Figure 5.** Gene expression of neuroplasticity markers (bdnf, neuroD1, pcna) in the fore-, mid
365 and hintbrain of juvenile Baltic sturgeon *A. oxyrinchus* kept in the rearing water of sander or,
366 as a control, carp for 30 days. No significant differences between treatments were observed
367 (Mann-Whitney test, $p \leq 0.05$). Data are expressed as fold relative. bdnf - brain-derived
368 neurotrophic factor (*bdnf*), neurod1 - neurogenic differentiation factor, pcna - proliferating
369 cell nuclear antigen (*pcna*).

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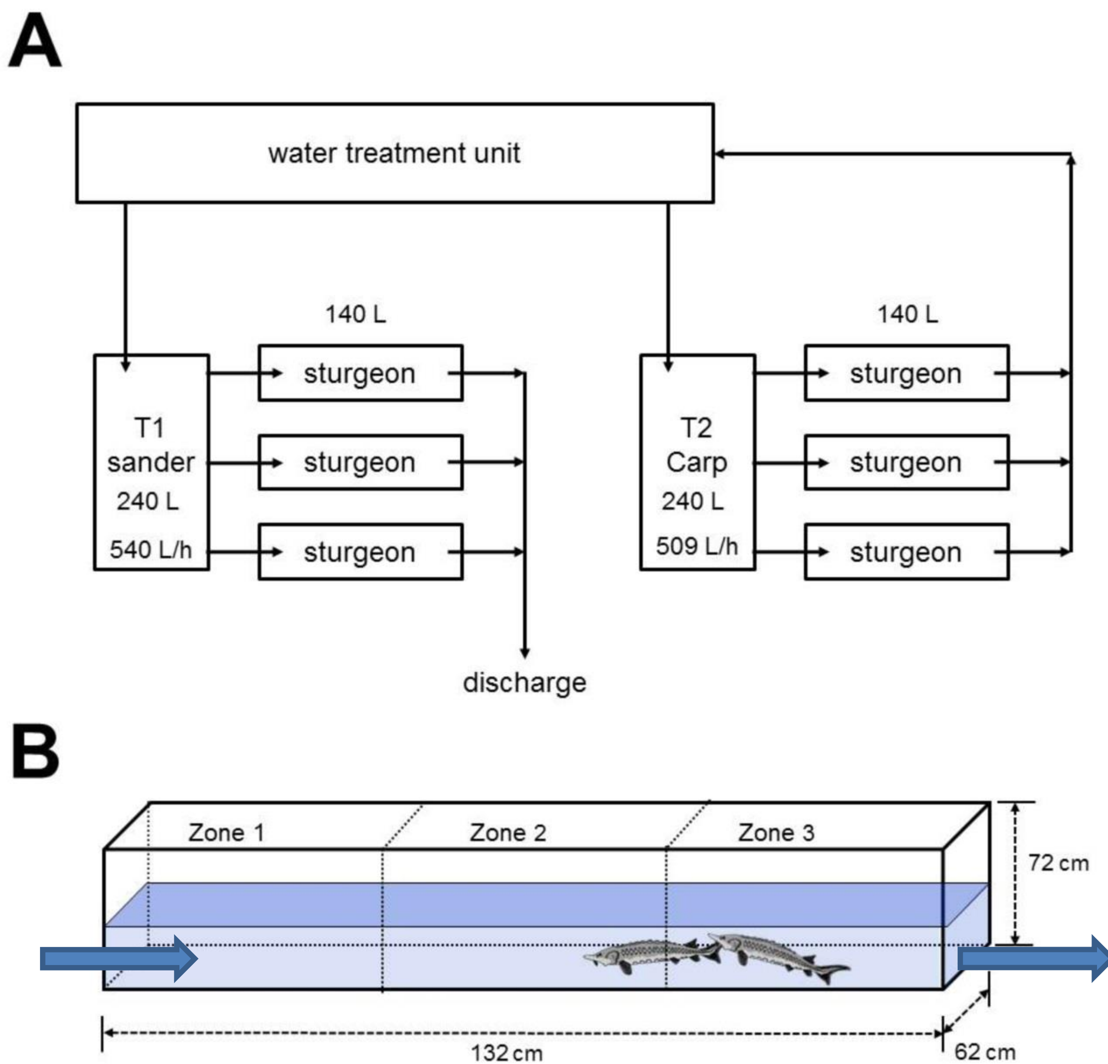
Figures



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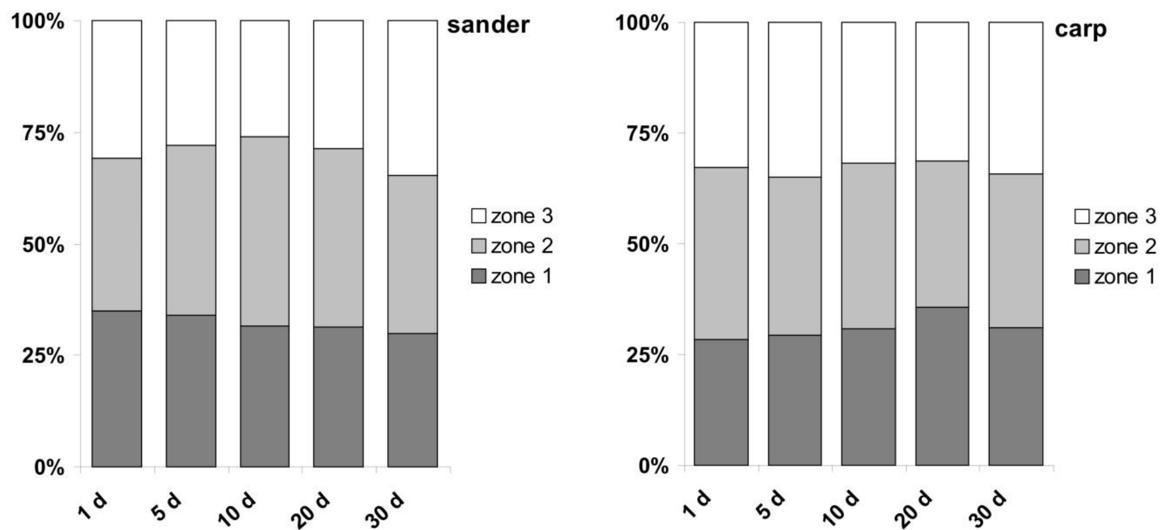

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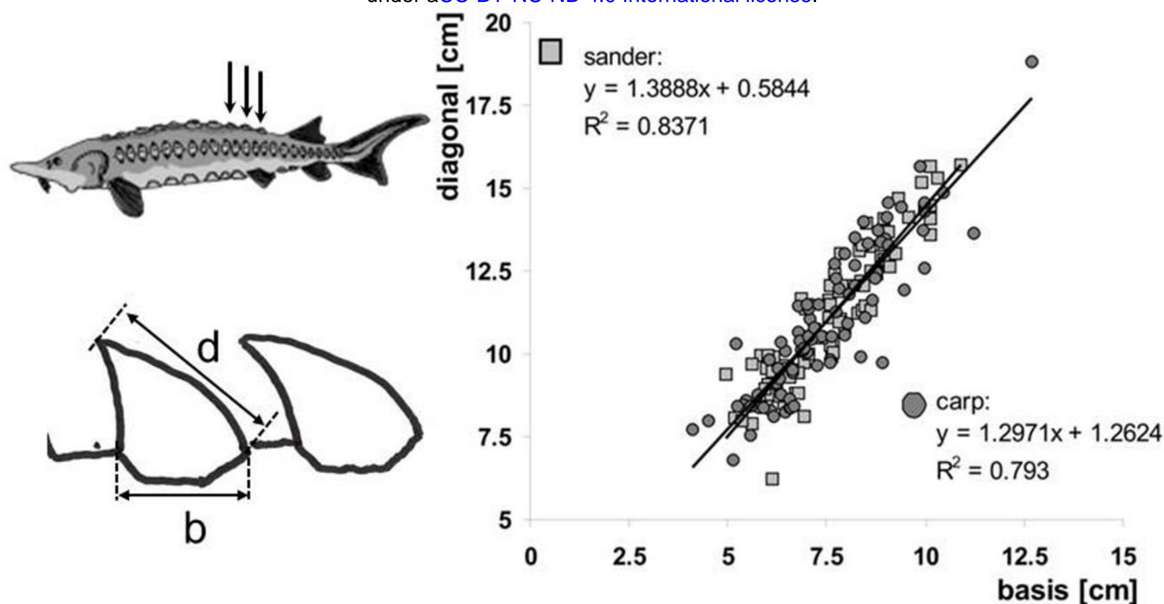

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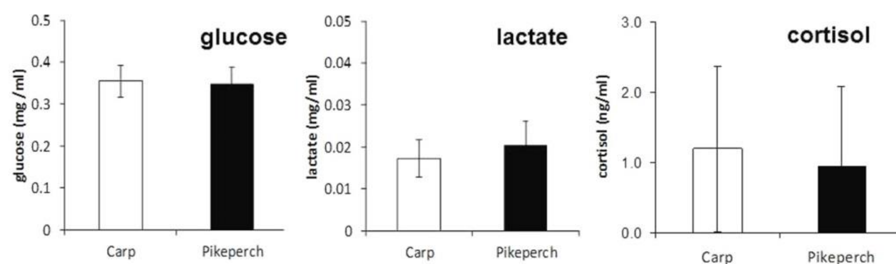

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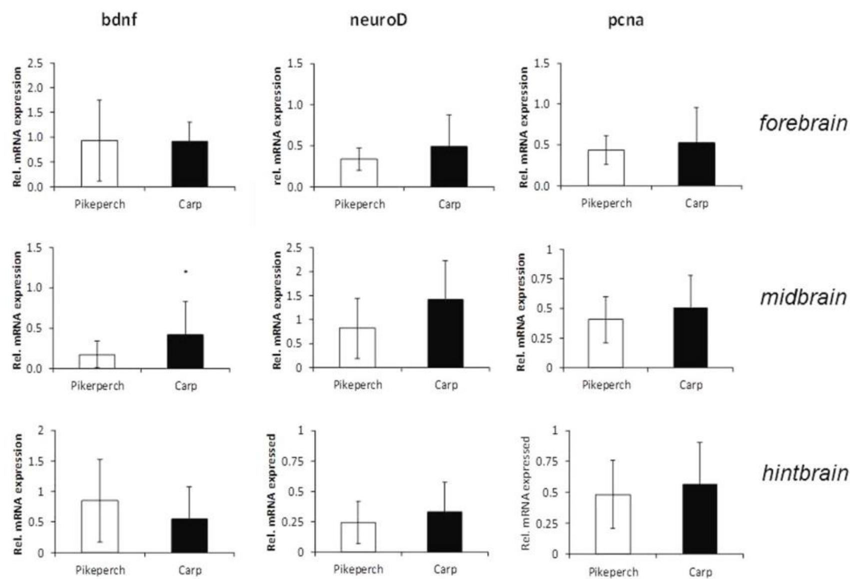

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