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1 **Laboratory captivity can affect scores of metabolic rates**
2 **and activity in wild brown trout**

3
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17
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19
20 *Short title: Laboratory captivity can affect plastic traits*

27

28

29 ***Abstract***

30 Phenotypic scoring of wild animals under standardized laboratory conditions is
31 important as it allows field ecologists and evolutionary biologists to understand the
32 development and maintenance of inter-individual differences in plastic traits (*e.g.* behavior
33 and physiology). However, captivity is associated with a shift from a natural familiar
34 environment to an unfamiliar and artificial environment, which may affect estimates of plastic
35 phenotypic traits. In the present study, we tested how previous experience with laboratory
36 environments and time spent in captivity affects behavioral (*i.e.* activity) and metabolic (*i.e.*
37 standard and maximum metabolic rates) scoring of our model species, wild brown trout *Salmo*
38 *trutta*. We found that individuals with previous experience of laboratory captivity (10.5 month
39 earlier) showed higher activity in an open field test than individuals with no prior experience
40 of laboratory captivity. Previous experience with captivity had no significant effect on
41 metabolic rates. However, metabolic rates seemed to increase with increasing time spent in
42 captivity prior to the collection of measurements. Although there are benefits of keeping wild
43 animals in captivity prior to scoring, our results suggest that whilst allowing for sufficient
44 acclimatisation researchers should aim at minimizing time in captivity of wild animals to
45 increase accuracy and ecological relevance of the scoring of plastic phenotypic traits.

46

47 ***Key words:*** phenotypic plasticity, sampling bias, phenotypic scoring, animal personality,
48 oxygen consumption, salmonids

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Introduction

A growing body of studies on plastic phenotypic traits, such as behavior and physiology, aim to understand the development and maintenance of consistent phenotypic differences between individuals (Sih et al., 2014; Auer et al., 2015) and their ecological implications (Des Roches et al. 2018; Raffard et al. 2018). Studies of wild free-ranging animals exposed to the selection pressures of their natural environment play an irreplaceable role in this type of research (see Archard & Braithwaite, 2010; Adriaenssens & Johnsson, 2013). In order to test the repeatability of phenotypic traits, it is necessary to measure these parameters on the same set of individuals under identical environmental conditions at different time points (Dingemanse & Wolf, 2013). These measurements are usually impossible to carry out in the field due to the spatio-temporal heterogeneity of environmental conditions (physical and social environment) in the wild. Neglecting the basic assumption that all individuals need to be scored under the same ambient conditions can lead to biased estimates of repeatability of differences between individuals (*e.g.* pseudo-repeatability; Dingemanse & Dochterman, 2013). Therefore, mark-recapture studies combined with repeated phenotypic scoring of wild animals under standardized laboratory conditions are necessary to bridge this methodological gap (Johnsson & Näslund 2018). The main advantage of such studies is that focal individuals are residing in their natural environment between scorings and yet are scored under the same ambient conditions. However, by nature, captivity is inherently associated with novel environmental conditions for wild animals and related to sources of stress (*e.g.* removal from the natural environment, handling, transport, novel food and social conditions, confinement in an artificial environment of holding tanks or cages), which can affect the measurements of

77 plastic phenotypic traits (Niemelä & Dingemanse, 2014).

78 To be able to generalize findings from mark-recapture studies on wild animals when
79 utilizing repeated standardized laboratory scorings of phenotypic traits, we need to understand
80 how estimates of phenotypic traits in laboratory settings are affected by captivity. Previous
81 methodological studies have highlighted the effects of acclimation period (Biro, 2012;
82 Edwards et al., 2013) and the design of laboratory assays (Näslund, Bererhi & Johnsson,
83 2015; Polverino et al., 2016; Chabot, Steffensen, & Farrell, 2016) on the determination of
84 plastic phenotypic traits. These studies were conducted over short time intervals (*i.e.* several
85 days or weeks) and focal individuals were obtained from hatcheries or kept in the laboratory
86 during the entire study period. The effect of captivity on the phenotypic traits of wild animals
87 over a longer time period still remains unknown.

88 In the present study, we used wild brown trout *Salmo trutta* as a model species to
89 repeatedly measure individual open field test activity, standard metabolic rate (SMR), and
90 maximum metabolic rate (MMR). Activity measured in an open field test is a common
91 behavioural test in animal personality research (David & Dall, 2016). Standard metabolic rate
92 (*i.e.* basic post-digestive energetic costs required to sustain life) and maximum metabolic rate
93 (*i.e.* maximum aerobic performance capacity of an organism) are widely used physiological
94 traits that are linked to the fitness of animals (Metcalf et al., 2015). Specifically, we tested i)
95 how activity, SMR, and MMR differ between individuals with previous experience of
96 laboratory captivity (*i.e.* 10.5 months before the scoring) and naïve control individuals (no
97 previous experience of captivity), and ii) how the time spent in the laboratory (*i.e.* in holding
98 tanks) prior to respirometry affects SMR and MMR.

99

100 ***Materials and Methods***

101 *Study site and fish sampling*

102 The sampling was conducted from April 2015 to April 2016 within the upstream stretch of
103 Ringsbäcken, a small stream running through a sub-boreal forest in southern Sweden
104 (57°40.318'N, 12°59.300'E). The initial sampling of individuals was conducted by
105 electrofishing between April 7 to April 10, 2015 at four sampling sites. Environmental factors
106 in the stream (*i.e.* water temperature and pH, depth and width of stream channel, bottom and
107 canopy characteristics) were similar across the four sampling sites, but non-native brook trout,
108 *Salvelinus fontinalis*, reside in the three upstream sampling sites (Závorka et al. 2017). A
109 previous study has revealed that co-existence with non-native brook trout can affect the
110 phenotypic syndrome of native brown trout (Závorka et al. 2017). Therefore, the site where
111 the experimental brown trout were collected was included in the statistical analyses (see
112 details below).

113 Captured brown trout (219 individuals: body mass mean \pm SD = 10.9 \pm 7.1 g, fork
114 length mean \pm SD = 95.7 \pm 22.6 mm) were anaesthetized (benzocaine; 0.5 ml L⁻¹), measured
115 for fork length (from the tip of the upper jaw, to the end of the central-most caudal fin ray)
116 and body mass, and fin clipped (0.5 cm² of the left pelvic fin). Fin clips were taken for stable
117 isotope analyses published elsewhere (Závorka et al. 2017). Individuals were implanted with
118 12-mm PIT-tags (HDX ISO 11784/11785, Oregon RFID, Portland, OR, USA) in the body
119 cavity, and following recovery the fish were released back into the stream. During the first
120 recapture session using electrofishing between June 3 and June 10, 2015, 72 tagged
121 individuals (body mass mean \pm SD = 11.7 \pm 6.7 g, fork length mean \pm SD = 99.9 \pm 19.2 mm)
122 were recaptured. During the second recapture session between April 18 and April 21, 2016,
123 63 tagged individuals (body mass mean \pm SD = 20.4 \pm 8.8 g, fork length mean \pm SD = 122.9
124 \pm 17.6 mm) were recaptured. Among the 63 individuals caught during the second recapture
125 session in 2016, 31 individuals had been previously recaptured and kept in the lab in 2015.
126 The other 32 recaptured individuals had not experienced laboratory conditions and only

127 underwent the initial sampling and tagging in April 2015. After each recapture, individuals
128 were transported to the laboratory facility, measured for fork length and body mass, fin
129 clipped (left pelvic fin), and placed in holding tanks. Holding tanks (71 L, 0.65×0.32×0.34 m)
130 contained shelter (rocks, plastic tubes, and plastic plants) and aerated freshwater from a semi-
131 recirculating flow-through filtration system (flow rate 2 L min⁻¹) and housed 10-11
132 individuals per tank. Photoperiod followed natural light cycles and water temperature in the
133 holding tanks were kept at 11 – 13°C throughout the laboratory captivity. Individuals were
134 fed daily till apparent satiation during the whole period with a mix of chironomid larvae,
135 maggots, and earthworms. After completing the lab scoring which took three weeks in both
136 years (June-July 2015 and April-May 2016), individuals were released back into the
137 Ringsbäcken stream. Focal individuals were therefore exposed to natural conditions for the
138 majority of the experimental period.

139

140 *Scoring of the phenotypic traits*

141 The scoring protocol was identical in both years of the study and followed the protocol
142 used in Závorka et al. (2017). In order to allow evacuation of food contents and to standardize
143 hunger levels, individuals were not fed during acclimation to behavioural scoring (one day
144 before the assay) and respirometry (four days before the assay). Previous studies have shown
145 that these fasting periods are sufficient and appropriate to provide behavioural and metabolic
146 scores of long-term ecological significance in brown trout (Závorka et al. 2015, 2016, 2017).
147 Activity of individuals was scored four days after capture of individuals in 2015 and two days
148 after capture of individuals in 2016. Activity was scored by open field test using a still water
149 in barren tank with a rectangular base (0.61 × 0.45 m, water level 0.10 m) as arena and a
150 video camera (Toshiba Camileo S20, Tokyo, Japan) positioned above the trial tanks to record
151 fish tracks. Total distance moved over 10 minutes after a 15 minutes acclimation period was

152 extracted from the recordings using an automated tracking software (LoliTrack 4.0 Loligo
153 Systems ApS, Viborg, Denmark) and used as proxy for individual activity. When subjected to
154 the trial, fish were gently netted from the holding tank and placed into trial tanks. Trial tanks
155 were cleaned and refilled with fresh water for each trial. Trials were performed from 08.00
156 until 17.00 under the same environmental conditions (homogenously distributed dim
157 fluorescent light ~100 lux, water temperature ~12 ° C, pH ~7.5, oxygen concentration ~10.7
158 mg/l, and conductivity ~170 μ S/cm). SMR and MMR were determined using intermittent
159 flow-through respirometry (Clark, Sandblom & Jutfelt 2013). Depending on the size of the
160 individual, fish were introduced into either a small (volume: 0.584 L, diameter: 6.4 cm,
161 length: 15.5 cm) or large (volume: 1.112 L, diameter: 6.4 cm, length: 31.0 cm) custom-made
162 ‘static’ intermittent flow-through cylindrical perspex respirometers. These respirometers were
163 submerged in a larger experimental tank with recirculating aerated freshwater (temperature
164 ~10 °C. salinity ~0.1 ppt, pH ~7.9, conductivity ~275 μ S/cm, Na⁺ ~5 mmol/L, K⁺ ~0.3
165 mmol/L, Ca²⁺ ~0.4 mmol/L). Water was continuously circulated through each respirometer
166 using an in-line submersible pump within a recirculation loop, and the partial pressure of
167 oxygen in the water within the respirometer was measured continuously at 0.5 Hz using a
168 FireSting O2 system (PyroScience, Aachen, Germany), which was calibrated in accordance
169 with the supplier’s manual. Water within the respirometer was refreshed with automated flush
170 pumps for 5 min in every 20 min period, ensuring that oxygen levels in the respirometers
171 always remained above 90% air saturation. The slope of the decline in the partial pressure of
172 oxygen in the water within the respirometers during each 15 min period between flush cycles
173 was then used to calculate oxygen uptake using the following formula:

$$174 \quad \text{oxygen uptake} = [(V_r - V_f) \times \Delta C_{wO_2}] / \Delta t$$

175 where V_r is the volume of the respirometer, V_f is the volume of the fish (assuming that the
176 overall density of the fish is 1g/ml of tissue), ΔC_{wO_2} is the change in the oxygen concentration

177 of the water within the respirometer (C_{wO_2} is the product of the partial pressure and
178 capacitance of oxygen in the water, the latter being dependent on salinity and temperature),
179 and Δt is the time during which ΔC_{wO_2} is measured (Clark, Sandblom & Jutfelt 2013). SMR
180 was measured as the average of the lowest 20% of oxygen uptake measurements that were
181 recorded over the time the fish were in the respirometers (~18 h over night, Chabot et al.
182 2016). MMR was determined by recording oxygen uptake immediately after the individual
183 had been subjected to an exhaustive exercise protocol where fish were chased for 3 min
184 around a circular tank (diameter 0.3 m, water depth 0.2 m) containing 10°C, aerated
185 freshwater (Clark, Sandblom & Jutfelt 2013).

186

187 *Statistical analyses*

188 The effect of experience with laboratory captivity on plastic phenotypic traits (*i.e.*
189 activity, SMR, and MMR) was tested with a linear model using experience (categorical
190 variable with two levels: experience or naïve), body mass, interaction between experience and
191 body mass, and sampling site of individuals origin (categorical variable with four levels) as
192 independent variables.

193 The effect of the time spent in the laboratory captivity prior to metabolic
194 measurements on SMR and MMR of individuals was analysed using a linear model that
195 contained time spent in captivity in days, year of the experiment (categorical variable with
196 two levels: 2015 and 2016), interaction between time spent in captivity in days and year of the
197 experiment, sampling site of individual origin, and body mass as independent variables.

198 In order to test hypothetic explanations of our findings that could be resolved with our
199 data (see discussion), we tested the following two post hoc hypotheses: *i*) specific growth rate
200 (SGR) differed between experience and naïve trout (hypothesis was tested by a linear model
201 using experience, sampling site of individual origin, and their body mass as independent

202 variables), *ii*) time spent in captivity before metabolic measurements was related to initial
203 body mass (*i.e.* body mass at capture) and activity of individuals (hypothesis was tested by a
204 linear model using activity, sampling site of individual origin, and their body mass as
205 independent variables).

206 The significance of the response variables of the fitted models was evaluated using an
207 ANOVA (Type III sums of squares) using the car package for R (Fox & Weisberg 2011). Fit
208 of the models was evaluated by a Shapiro–Wilk test and by visual inspection of the normality
209 of the models' residual distribution. SGR, SMR, MMR, and body mass were \log_{10}
210 transformed in all models. Non-significant interactions among the independent variables were
211 removed from tested models. Statistical analyses were made in R 3.2.3 (R Core Team,
212 Vienna, Austria).

213

214 **Results**

215 We found that individuals with previous experience of laboratory captivity had a significantly
216 higher activity at the second scoring occasion in 2016 than naïve individuals ($F_{1;57} = 10.03$, p
217 $= 0.0025$, Fig. 1a). Activity of individuals was not significantly related to the interaction of
218 laboratory experience and body mass ($F_{1;56} = 0.72$, $p = 0.3999$), body mass ($F_{1;57} = 0.31$, $p =$
219 0.5822) or sampling site of origin ($F_{3;57} = 0.57$, $p = 0.6379$). There was no significant effect of
220 previous experience with laboratory captivity on mass specific SMR ($F_{1;57} = 2.32$, $p = 0.1333$,
221 Fig. 1b) or mass specific MMR ($F_{1;57} = 1.15$, $p = 0.2875$, Fig. 1c). SMR and MMR of
222 individuals were increasing with body mass of individuals (SMR: $F_{1;57} = 331.05$, $p < 0.0001$;
223 MMR: $F_{1;57} = 426.99$, $p < 0.0001$), but were not significantly related to the interaction term
224 between laboratory experience and body mass (SMR: $F_{1;56} = 0.04$, $p = 0.8414$; MMR: $F_{1;56} =$
225 0.40 , $p = 0.5317$) or sampling site of origin (SMR: $F_{3;57} = 0.05$, $p = 0.9843$; MMR: $F_{3;57} =$
226 0.26 , $p = 0.8560$).

227 We found that both mass specific SMR ($F_{1;128} = 4.61, p = 0.0336$, Fig. 2a) and mass
228 specific MMR ($F_{1;128} = 11.27, p = 0.0010$, Fig. 2b) were higher in individuals that were kept
229 in the holding tanks for longer periods prior to exhaustive exercise and respirometry. There
230 was no significant effect of interaction between time spent in captivity and year of the
231 experiment on mass specific SMR and MMR (SMR: $F_{1;127} = 2.61, p = 0.1090$; MMR: $F_{1;127} =$
232 $2.64, p = 0.1064$). Mass specific MMR was higher in 2016 than in 2015 ($F_{1;128} = 15.82, p =$
233 0.0001), but there was no difference in SMR between the two years of the experiment ($F_{1;128}$
234 $= 0.01, p = 0.9042$). Similar to the model described in the previous paragraph, SMR and
235 MMR of individuals increased with their body mass (SMR: $F_{1;128} = 240.98, p < 0.0001$;
236 MMR: $F_{1;128} = 1085.86, p < 0.0001$), but was not related to their sampling site of origin
237 (SMR: $F_{3;128} = 0.6268, p = 0.5990$; MMR: $F_{3;128} = 0.15, p = 0.9320$).

238 In the test of the first post hoc hypothesis, we found no significant difference in
239 specific growth rate of naïve and experienced individuals ($F_{1;58} = 0.36$; p-value = 0.5482). For
240 the second post hoc hypothesis, we found that activity measured at the beginning of
241 laboratory captivity was not significantly related to the time spent in the lab before the
242 respirometry ($F_{1;128} = 0.71$; p-value = 0.4022). However, there was a significant negative
243 relationship between the initial body mass and the time that individuals spent in the lab before
244 the respirometry ($F_{1;128} = 13.33$; p-value = 0.0003).

245

246 ***Discussion***

247 We found that individuals with previous experience of laboratory captivity (10.5 month
248 earlier) displayed higher activity in an open field test than individuals with no prior
249 experience. Previous experience with captivity had no significant effect on metabolic rates
250 (*i.e.* SMR and MMR). However, we found that SMR and MMR were apparently increasing
251 with increasing time spent in laboratory captivity. While these findings are limited only to our

252 model species (*i.e.* brown trout), we suggest that captivity in laboratory environment may
253 similarly affect plastic phenotypic traits in other animal model species (McPhee and Carlstead
254 2010).

255 There are at least three potential mechanisms that could explain why individuals with
256 previous experience to laboratory captivity displayed a significantly higher activity when
257 compared to naïve individuals. First, laboratory-experienced individuals may recognize the
258 test conditions from their previous time in captivity, which could have subsequently changed
259 their response in the open field test. For example, experienced individuals may have
260 perceived the scoring environment more familiar than naïve individuals. Along these lines, it
261 has been suggested that measurement of activity in the open field test with an unfamiliar
262 environment corresponds to boldness, and exploratory behaviour, while the same test in a
263 familiar environment corresponds predominantly to activity (Réale et al., 2007). Since
264 experienced individuals have previously been scored in the lab only once, the change in their
265 behaviour was more likely a response to a change in the context of the behavioural test (*i.e.*
266 the context of the behavioural test has changed for experience individuals from unfamiliar to
267 familiar) rather than habituation to the repeated treatment (Edwards et al., 2013). This
268 explanation would require that the individuals retain the information about laboratory
269 captivity for over 10 months. Substantial variability exists amongst fishes in their capacity to
270 retain information, which differs across species and contexts. For example, brook sticklebacks
271 *Culaea inconstans* forget foraging skills after 8 days (Croy & Hughes, 1991), whereas the
272 same skills can be retained by rainbow trout *Oncorhynchus mykiss* for over 3 months (Ware
273 1971). In an angling experiment, Beukema (1969) showed that carp *Cyprinus carpio*
274 previously hooked, remain harder to catch a year later when compared to unhooked carp,
275 which suggests that stressful stimuli may be retained for a long time by fish. Here, it may be
276 possible that the fish perceived the first test as a negative and stressful experience, leading to

277 a faster initiation of the exploratory escape response, which then could explain the increased
278 activity when compared to the first trial the preceding year. The second alternative is that
279 captivity may alter post-release performance of experienced individuals in the wild, which
280 subsequently changes their behaviour. For example, brown trout fry in captivity can grow
281 slower than conspecifics from the same population that remained in the native stream
282 (Näslund, Sandquist & Johnsson, 2017). In addition, brown trout released in a stream after lab
283 captivity may lose their territory (Závorka et al., 2015), which may lead to further reductions
284 in growth, followed by compensatory growth (Johnsson & Bohlin, 2006) with associated
285 long-term increases in activity (Orpwood, Griffiths & Armstrong, 2006). However, in our
286 study we found no difference in specific growth rate of naïve and experienced individuals.
287 The third possible explanation of higher activity of experienced individuals is a sampling bias
288 during recapture with respect to an individual's activity, as active individuals may be more
289 susceptible to capture (Howard 1982; Biro & Dingemanse, 2009; Koeck et al. 2018).
290 Therefore, it is more likely to have a high proportion of active individuals amongst those
291 captured twice than those only captured once. However, earlier results suggest either that no
292 activity related sampling bias occurs in our model species (*i.e.* brown trout) when recapturing
293 using electric fishing (Adriaenssens and Johnsson 2013), or that recapture probability is
294 driven by an interaction between fish activity and body size (Näslund et al. 2018). All these
295 explanations can bias conclusions of mark-recapture studies using repeated laboratory scoring
296 of wild animals. Changes in individual behaviour would lead to an overestimation of open
297 field test activity of repeatedly scored individuals, while the sampling bias could lead to an
298 underestimation of survival in the less active individuals that may have a lower probability to
299 be caught.

300 The trends between metabolic rates (*i.e.* mass specific SMR and MMR) and time in
301 captivity observed in this study indicate either that metabolic rates of individuals are

302 increasing with time spent in captivity, or that individuals with high metabolic rates tend to be
303 inadvertently scored later than individuals with low metabolic rates. The tendency for an
304 increasing SMR with time spent in captivity could be due to the differences in the quality and
305 quantity of food supplied in captivity compared to that available in the wild. Auer *et al.*
306 (2016) have demonstrated that individuals fed *ad libitum* display a higher post-digestive SMR
307 than individuals fed on a lower ration. Individuals in our study were fed daily till apparent
308 satiation during the entire period of captivity. Therefore SMR may have increased over time
309 in captivity as a consequence of plastic changes in their metabolic machinery or changes in
310 specific dynamic action (Secor, 2009) in response to the abundant food availability under
311 laboratory conditions. The MMR of vertebrates is thought to be predominantly affected by
312 oxygen consumption of skeletal muscle (Weibel *et al.*, 2004), and thus should not be affected
313 by a short-term change in food availability (Auer *et al.*, 2016). A second explanation of the
314 increase of SMR and MMR with time in captivity could be an inadvertent sampling bias
315 during collection of individuals from the holding tanks. Such a bias could occur if SMR and
316 MMR were associated with a behavioural trait that affects probability of individuals being
317 collected by a dip net (Biro & Dingemans, 2009). We found that activity measured at the
318 beginning of laboratory captivity was not related to the time spent in the lab prior to
319 respirometry (*i.e.* active individuals were not collected from holding tanks for metabolic
320 scoring prior to less active individuals). However, there was a significant negative
321 relationship between the body mass at the beginning of laboratory captivity and the time that
322 individuals spent in the lab prior to respirometry. This suggests that we may have
323 inadvertently scored the larger individuals earlier than the small ones. Nonetheless, the latter
324 finding does not directly explain the relationship between time spent in the lab and mass
325 specific metabolic rates as those are mass independent.

326 In summary, we found that laboratory captivity can have an effect on the standardized
327 scores of plastic behavioural and metabolic traits. We emphasize that there can be benefits of
328 keeping wild animals in captivity prior to scoring (*i.e.* using acclimation period) when
329 maintained under adequate holding conditions (Niemelä & Dingemanse 2014; Näslund &
330 Johnsson 2016; Johnsson & Näslund 2018). Benefits may include reductions in stress,
331 acclimation to surroundings, or standardization of environmental conditions prior to testing.
332 However, our results also indicate potential drawbacks of laboratory captivity. Therefore, we
333 suggest that researchers should aim to minimize the time that wild animals need to spend in
334 laboratory captivity whilst allowing for a sufficient acclimatisation period to the novel
335 conditions in order to increase accuracy of phenotypic scoring. Field ecologists and
336 evolutionary biologists frequently use laboratory scores for evaluation of phenotypes in the
337 wild animals. Therefore, we emphasize, in agreement with Niemelä & Dingemanse (2014),
338 that laboratory scores of plastic phenotypic traits need to be interpreted with caution and
339 preferably in association with phenotypic scoring in the wild.

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347

348 ***Data accessibility statement***

349 Should the manuscript be accepted, data will be archived at figshare.com (doi:
350 10.6084/m9.figshare.4685032).

351

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460 **Figure legend**

461 **Figure 1** – Laboratory scores of naïve and experienced individuals of juvenile brown trout.
462 Boxplots demonstrate the distribution of a) activity, b) mass specific SMR, and c) mass
463 specific MMR (n = 32 and 31 for naïve and experienced individuals for all measured traits,
464 respectively). The experienced individuals were scored for the same traits under the same
465 conditions 10.5 months earlier, while naïve individuals had no previous experience with
466 laboratory conditions. Box edge represents the mean and 25th and 75th percentiles and
467 whiskers cover the 95th percentiles. Filled circles represent individual data points.

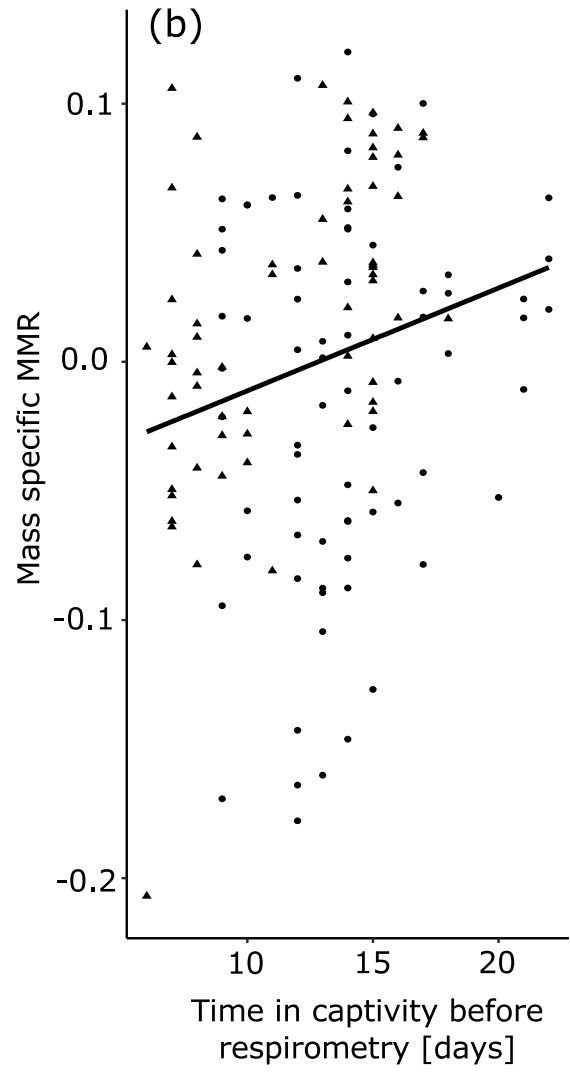
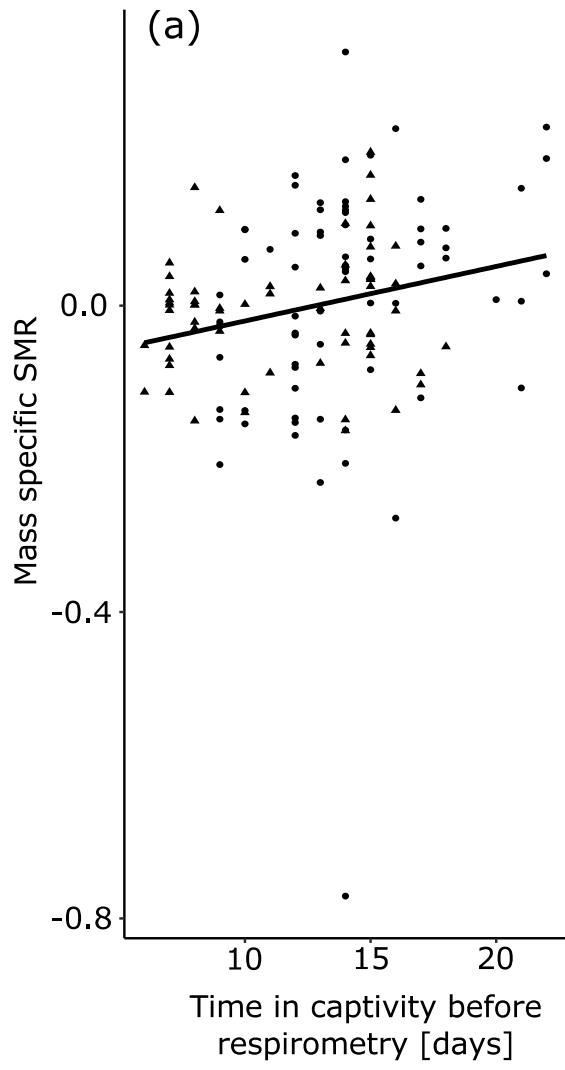
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469 **Figure 2** – Relationship between time spent in captivity before exhaustive exercise and
470 respirometry and a) mass specific SMR, b) mass specific MMR (n = 72 and 64 in the year
471 2015 and 2016 respectively for both measured metabolic rates) of juvenile brown trout. Filled
472 circles and triangles represent measurements collected in 2015 and 2016 respectively. Naïve
473 and experienced individuals were analysed together, as laboratory experience had no effect on
474 the scores of metabolic rates.

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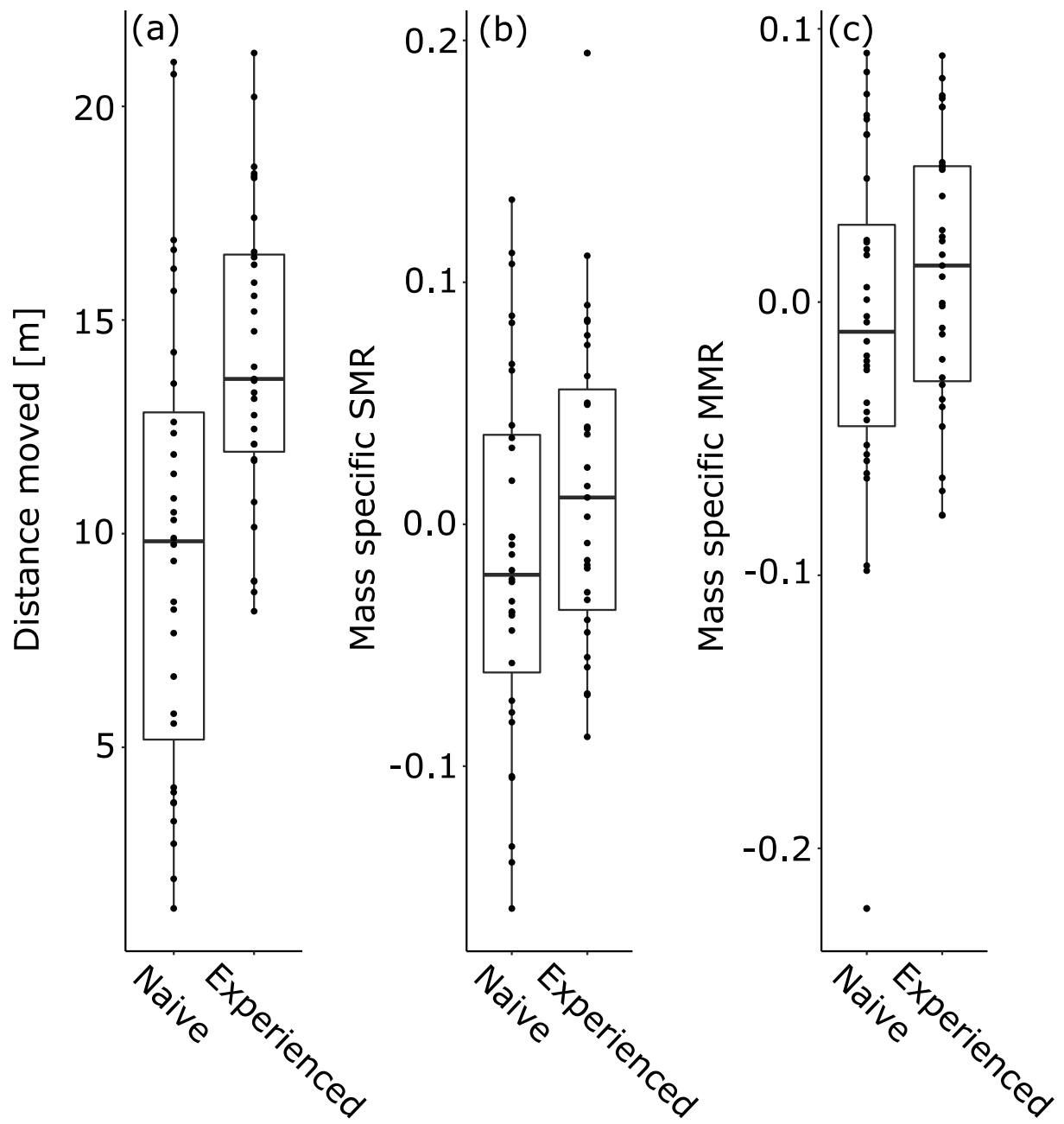
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479 Figure 1



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481 Figure 2