Behavioral and metabolic consequences after a single generation of angling selection in brown trout

Running title: Angling selection in risk-taking behavior

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Abstract

The behavior of organisms can affect their vulnerability to human induced selection, including recreational angling. Angling is expected to select fish with bold behavior, which may be linked to low stress responses through stress coping styles. Brown trout (*Salmo trutta*) is an intensively fished salmonid, and thus provides a relevant model to study artificial human-induced selection by angling. We used a selection experiment with fish possessing high or low vulnerability to angling to understand the consequences on traits related to stress coping styles and metabolic rate. We produced selection lines in two populations of brown trout –one wild and one reared in captivity for several generations– and reared the offspring in common garden conditions. We then assessed minimum and average metabolic rates, boldness and sensitivity to stress in juveniles at the age of 1 year. Angling selection had population-specific effects on risk taking -related latency and exploration tendency, and populations differed on average in several measured traits, which could be due to a combination of genetic and non-genetic effects. Our study provides evidence for angling induced selection in fish personality and suggests that metabolic rate and stress sensitivity might also be affected. The results can be explained by contrasting frequencies of proactive and reactive stress coping style in the two populations.

Significance statement:

Hunting and fishing by humans, as any predation, can select individuals with bold behaviors, which potentially leads to an increase in shyness in prey populations. Because this is expected to occur in many fish stocks, we experimentally tested the consequences of angling vulnerability in brown trout in their offspring. Angling selection had different effects on a wild and a hatchery population of brown trout, making hatchery juveniles from low vulnerability parents more willing to take risks and have a faster metabolism than offspring

52 from highly vulnerable parents, but having only a weak effect on in juveniles from the wild
53 population. Our study implies that angling selection can lead to accumulating behavioral,
54 stress sensitivity and metabolic change over time. Whether this will manifest in increased
55 shyness may depend on the background of the population.

Introduction

The behavior of organisms in their environment affects their vulnerability to be captured by predators or by humans. Artificial selection by hunting and fishing can have strong effects on various phenotypes of a species over time (Fugère and Hendry 2018) and can increase the relative frequencies of maladaptive phenotypes (Allendorf and Hard 2009; Coltman et al. 2003). Empirical studies have shown that responses to human-induced selection can be rapid at both genetic (Cooke et al. 2007; Sutter et al. 2012; Uusi-Heikkilä et al. 2015) and phenotypic levels, including behavior (Kern et al. 2016; Wong et al. 2012).

Besides large-scale fisheries using gillnets, trawls and other commercial gear, recreational and small-scale fisheries can also induce selection on vulnerability to fishing and traits that explain vulnerability (Cooke et al. 2007; Hollins et al. 2018; Redpath et al. 2010; Sutter et al. 2012; Uusi-Heikkilä et al. 2008). Selection from recreational fishing is expected to particularly affect boldness and exploration tendency (Arlinghaus et al. 2017), as bold and explorative fish are often the most vulnerable to angling likely due to the required behavioral decisions from the fish (Cooke et al. 2007; Härkönen et al. 2014; Wilson et al. 2015), reviewed in (Lennox et al. 2017), though not in all studies (Louison et al. 2017; Vainikka et al. 2016). Over time, angling selection could increase the frequency of shy phenotypes in the population, which could lead to less efficient resource use and decreased population growth and thus diminished productivity (Andersen et al. 2018; Arlinghaus et al. 2017).

The consequences of angling selection can be understood in the light of stress coping styles (Louison et al. 2017). Coping styles can be defined as consistent behavioral differences driven by varying neurochemical stress responses (Schjolden et al. 2005; Vindas et al. 2017a; Vindas et al. 2017b). Proactive coping style is generally bold, routine-based and relies on a

sympathetic stress response (involving catecholamines), while reactive type is shyer, more flexible in behavior, and relies on a parasympathetic stress response (involving glucocorticoids) (Koolhaas et al. 2010; Schjolden et al. 2005), although distinct types have not been identified in all studies, e.g., (Thomson et al. 2011). Selection by angling may therefore affect the neurochemical stress response of fish due to underlying correlations with behavior. In this scenario, a fish that responds to the presence of an angler with a high cortisol response is less likely caught than a non-stressed fish.

Selection acting on personality could also affect metabolism in fish due to a correlation between behaviors affecting energy balance and minimum metabolic rate (meta-analysis by Mathot et al. 2018). This relationship may be bidirectional, as metabolic rate is affected by behavior, but may also be the underlying cause for risk-taking behavior, depending on food availability (Killen et al. 2011). According to the pace-of-life syndrome (POLS) theory, boldness should correlate positively with metabolic rate because a fast metabolic machinery requires high food intake, which again requires bold behavior (Réale et al. 2010). In one of the first empirical angling selection studies, standard metabolic rate was found to be 10% lower in a low vulnerability selection line compared to a high vulnerability selection line in largemouth bass (*Micropterus salmoides*) (Redpath et al. 2010). This supports the expectation of a positive correlation between vulnerability to angling and metabolic rate, however, several studies have found no association between these traits (Louison et al. 2017; Louison et al. 2018; Väätäinen et al. 2018). Thus, more empirical studies in common garden conditions are needed to address this question.

As many other taxa, salmonids can display distinctive behavioral strategies/syndromes and coping styles (Adriaenssens and Johnsson 2011; Brelin et al. 2008; Huntingford and Adams

2005; Näslund and Johnsson 2016; Vindas et al. 2017b), which may provide resource- and life stage -dependent survival benefits. Salmonids, such as the brown trout (*Salmo trutta*), are also affected by domestication in hatchery rearing, which impacts their life-history strategies, growth and behavior (Araki et al. 2008; Horreo et al. 2018; Huntingford 2004) and can increase their vulnerability to angling (Klefoth et al. 2013). In this study, we asked whether already one generation of angling selection could induce observable changes in the behavior, metabolic rate, or cortisol response of brown trout. We studied fish from both wild and hatchery origin that may exhibit different coping styles. We hypothesized that offspring from angling-vulnerable parents would have 1) higher scores in risk-taking behavior, 2) higher minimum metabolic rate, and 3) lower stress sensitivity compared to fish from non-vulnerable parents, and 4) that fish from hatchery stock parents would display more proactive stress coping styles compared to fish from wild parents.

Material and methods

Angling experiment and fish husbandry

Experiments on brown trout were carried out between 2015 and 2017 at the Natural Resources Institute Finland (Luke) Kainuu Fisheries Research Station (www.kfrs.fi) under license obtained from the national Animal Experiment Board in Finland (license number ESAVI/3443/04.10.07/2015). Two strains of brown trout were used. Wild, predominantly non-migratory, parental fish from River Vaarainjoki were captured by electrofishing (generally non-selective fishing gear) during spawning time in 2010–2012 and brought to the research station. The second parental strain used was a hatchery strain (so-called Lake Oulujärvi hatchery brood stock). The parental fish were taken from two year-classes of the 2nd generation of the brood stock maintained in the same research station. The founders of the brood stock came from three hatchery stocks reared in nearby hatcheries for 3–4 generations

131 and established from two source populations. These stocks originated from predominantly
132 migratory (adfluvial) populations in the region (further details in Lemopoulos et al. (2019)).
133 Despite originating from the same River Varisjoki watershed, the populations used in this
134 study showed moderate genetic divergence based on fixation index (F_{ST}-value) of 0.11
135 (Lemopoulos et al. 2019). The wild population had been exposed to angling more recently
136 than the hatchery population, although fishing pressure had been weaker than the fishing
137 pressure on the migratory strain prior to hatchery rearing (P. Hyvärinen, unpublished
138 observation).
139
140 During the whole study, fish were fed with commercial fish pellets (Raisio Oyj). In 2015,
141 hatchery-origin and wild-origin adult fish were exposed to experimental fly fishing and
142 divided into captured (high vulnerability, HV) and uncaptured (low vulnerability, LV) groups.
143 Fish were fished in two size-assortative pools for each population during June and July with
144 fly fishing gear adjusted by the size of the fish in the pools. The wild fish were fished in semi-
145 natural 50-m^2 ponds with a gravel-bottom outer riffle sections and *ca.* 1 m deep, concrete
146 inner pool sections (53 and 91 visually size-sorted fish in two ponds). The hatchery fish were
147 fished in 75-m^2 concrete ponds with no structures (64 larger and 167 smaller fish from two
148 different cohorts in two ponds). Angling was performed by experienced fly fishers (mainly
149 A.V.) using unnaturally colored woolly bugger -type fly patterns tied to barbless hooks.
150 During angling sessions, an angler fished a pond until a fish took the fly or five minutes
151 passed, after which angling was continued at earliest one hour later. If a fish was captured,
152 angling was continued immediately after processing, which included anesthesia with
153 benzocaine (40 mg L^{-1}), identification of passive integrated transponder (Oregon RFID) code
154 or tagging when a pre-existing tag was missing, and measuring total length (to 1 mm) and
155 weight (to 2 g). Fish that were missing PIT-tags were tagged under the skin next to the dorsal

156 fin using 12 mm tags at this point. After processing, the fish were transferred to similar ponds
157 (hatchery fish to a 50-m^2 otherwise similar concrete pond) as used for each population during
158 angling. After angling trials were finished, on 25 June 2015, all remaining wild fish that were
159 not captured were collected by dip-netting after draining the experimental angling ponds,
160 anaesthetized, measured and weighed (mean body lengths of fish uncaptured and captured by
161 angling: in large fish 457 and 475 mm, respectively, and in small fish 344 and 354,
162 respectively). Uncaptured wild fish were then combined in the same ponds as the fish
163 captured by angling. The captured hatchery strain fish were subjected to a second round of
164 angling ~2 weeks later, where in total eight fish were captured and prioritized for breeding the
165 highly vulnerable line, but this was not done on wild fish due to their limited availability.
166 Angling trials finished on 8 July 2015, and also hatchery fish were transferred back to their
167 original ponds. Because of the warm water at the time of finishing the second round of
168 angling, the uncaptured hatchery fish were not measured to avoid handling-induced stress and
169 mortality. One deep-hooked small hatchery fish was found dead 5 days and one large
170 hatchery fish 41 days after capture, but otherwise no mortality occurred between angling trials
171 and the breeding.
172
173 The offspring used in this study were obtained from fish bred in four groups (i.e. high- and
174 low-vulnerability [HV and LV, respectively] within each population) in the autumn of 2015.
175 A replicated, fully factorial 3 × 3 breeding design was used to create the F_1-generation; males
176 were crossed with females in all combinations in one matrix, and the matrices replicated three
177 times for each group, details in Electronic Supplemental Material (ESM1, available online).
178 In the autumn of 2016, the one-summer-old fish were tagged with individual 12-mm PIT-tags
179 in the abdominal cavity under anesthesia (benzocaine). After tagging, the selection lines were
180 mixed together in two 3.2 m^2 fiberglass rearing tanks.

Photoperiod acclimations

In mid-March 2017, after being reared under constant light, 100 fish were divided into two different photoperiod groups in 0.4-m^2 green, plastic, flow-through tanks. The tanks were covered with green nets. The first group continued to be reared under constant light (at water surface approximately 9 lux, N= 10/group, 40 fish in tank), and the second group received a 12h:12h light-dark (L:D) acclimation (at water surface approximately 12 lux during light period, N = 15/group divided equally in two tanks, details in ESM1, available online). Fish were fed using automatic belt feeders (~0.3% fish mass per day) on 5–6 days per week during approx. 4h between 8:00 and 20:00 to avoid the entrainment of endogenous rhythms by feeding. After a minimum two-week acclimation, the metabolic rate measurements were started.

Measurement of O_2 consumption

The O_2 consumption ($\dot{M}O_2$) was measured as a proxy of metabolic rate (Nelson 2016) using intermittent flow-through respirometry (Svendsen et al. 2016) with 15–17-min cycles. The fish were caught by dip-netting under a dim red light into 10-L buckets, identified with a PIT-reader and transferred to the flow-through measurement chambers immersed in a water bath, which was also immersed in a flow-through buffer tank. Measurements were started immediately and continued for approximately 23h, corresponding to 90–96 measurement cycles for all individuals. After measurements, fish were anesthetized with benzocaine, measured for total length (to 1 mm) and weighed (to 0.1 g), after which they were transferred to new 0.4 m^2 tanks similar to those used prior to measurements, with the same photoperiods as before the measurements. Respirometer chamber oxygen levels were then measured empty for one cycle to quantify bacterial respiration rates. No measurable respiration was detected

without fish. The slope of the decrease in oxygen level during each 3.5-minute measurement period was calculated using linear regression in AV Bio-Statistics v. 5.2 (by A.V., available at http://www.kotikone.fi/ansvain/). Because the $\dot{M}O_2$ of fish was extremely low due to cold water temperature, we accepted all measurement periods with regression coefficients $R^2>0.2$ in the calculation (in total 28 slopes were excluded across all measurements). This was justified as visual inspection of the data revealed clear negative trends and excluding slopes with low R^2 would have biased $\dot{M}O_2$ estimates strongly upwards. Further details of the method are given in ESM1.

The minimum oxygen consumption ($\dot{M}O_{2,min}$) was calculated from the average of the four least negative slopes after discarding the first, the last and the least negative slope. Values from three individuals were discarded as outliers (>3x SD difference to the mean). In addition, we calculated the average consumption across all measurements excluding the first and last slope for each fish ($\dot{M}O_{2,ave}$) because the stress of being confined in the measurement chamber is reflected in oxygen consumption (Morgan and Iwama 1996; Murray et al. 2017). The coefficient of the relationship of $\log_{10}(\dot{M}O_{2,min})$ and $\log_{10}$(body mass in kg) was used to calculate mass-specific $\dot{M}O_{2,min}$ for visualization, after (Killen et al. 2011).

Behavioral trial setup

Quantification of boldness in animals should involve an element of risk-taking. In experimental settings different measures, such as latency to explore a novel environment, are often used as proxies for boldness (Conrad et al. 2011; Johnsson and Näslund 2018). Here, we quantified the boldness of fish using different behaviors expressed in the presence of predator cues in a novel environment.

231 The fish were allowed to recover from respirometry for at least four days before behavioral
232 trials to minimize potential effects of handling stress on behavior. They were not fed for 24-h
233 prior to behavioral trials. The trials were conducted in custom-made mazes (Fig. 1) (size 400
234 mm wide x 1500 mm long, water depth 100 mm in the open area). During the trials,
235 temperature in the maintenance tanks and test arenas was on average 4.5 ± SD 1.3°C. Water
236 flow rate during the trials was adjusted to ~8 L min^{-1} (~7.6–8.8 L min^{-1}). This allowed for at
237 minimum 1.26 times the arena volume of water to flow between consecutive trials, which was
238 considered sufficient to minimize potential carry-over effects of chemical cues between trials.
239 The arena was lit by LED lights (CRI90 LED chain in waterproof silicon tube, 3000-3300K,
240 4.8W m^2) situated along one long edge of the arena (>70 lux across the arena depending on
241 distance from light source). Half-way across the arena was a brick gate situated next to one
242 side, allowing entry from the other side. Behind the brick, natural pebbles (~3–5 cm in
243 diameter) were scattered unevenly on the floor, and one large stone was provided for shelter.
244 A second large stone was placed in the center of the arena in front of the start box. Four
245 similar arenas were used in the experiment, but they differed in the visual appearance of the
246 natural stones and two of the arenas were mirror images of the other two with respect to the
247 location of the gate.
248
249 Upstream from the flow-through test arena was a section divided by a metal grid (5 mm mesh
250 size) where a hatchery-reared burbot (*Lota lota*) (length ~30-40 cm) was placed to introduce
251 olfactory cues of a natural predator of juvenile brown trout. Burbot are nocturnal bottom-
252 dwelling predators that are likely difficult for prey to detect visually, but their odor induces
253 antipredator responses in prey species (Ylönen et al. 2007). Burbot were regularly fed with
254 pieces of various cyprinids and vendace (*Coregonus albula*) during rearing, and only with
255 fresh pieces of brown trout for two days prior to and during the trials. Burbot were moved to

256 the test arenas at least one day before the trials. The burbot were fed with trout pieces in
257 separate tanks and changed in each arena every 10–15 trials (2–3 days).
258
259 Before each trial, individual brown trout were haphazardly removed from their rearing tanks
260 using a dip-net under red light and placed into black 10-L buckets filled with ~8L of water
261 from the flow-through system. Fish were identified by PIT tags and left undisturbed for 10
262 min before being transferred into the start box located downstream from the test arena by
263 pouring. During each trial, the trout was acclimatized in the start box for 3 min, after which
264 the door of the box was opened by pulling a string from behind a curtain, and fish movements
265 recorded from above using two CCTV infrared cameras (two arenas simultaneously filmed
266 using the same camera) for 10 min (of which first 9 min 45 s was included in the behavior
267 analysis). The behavioral trial was repeated three times between 8:00 and 11:00 for each focal
268 fish, with an average time of 4.3 days (range 1–8 days) between consecutive trials. One trial
269 from four fish was omitted from analysis due to error in data collection. The order in which
270 batches of four fish were captured on the same day from the same tank for the four arenas was
271 recorded (batch from hereon, levels 1–5, four individuals from batch 6/7 combined to batch
272 5).
273
274 *Testing behavioral responses to burbot*
275 To confirm that burbot odor was perceived risky in the personality assays, we tested for the
276 response of brown trout to burbot in separate controlled tests using individuals from wild HV
277 and wild LV groups (N=10 in each). These fish were acclimated to similar tanks as the
278 personality-tested fish at 12h:12h L:D photoperiod for one week before trials started. One
279 individual died after the first trial. The behavior of each individual was tested on six different
280 days in the presence and absence of predator (3 trials in each condition in haphazard order).

281 3–4 different arenas were used for each fish on different days to reduce fish habituation to the
282 arenas. These trials were conducted between 14:40 and 17:00. Control arenas were emptied
283 and thoroughly rinsed with pressurized tap water and water flow maintained for >2h before
284 the trials to avoid carry-over effects from burbot odor in earlier experiments. The water used
285 in the flow-through system originates from lake Kivesjärvi, where burbot is a common
286 species; thus, traces of burbot odor may have been present in all trials.
287
288 *Analysis of video recordings*
289 Behavioral data were collected from videos using manual tracking with AV Bio-Statistics 5.2
290 timing software. The observer was blind to the identity of fish in all recordings. Analyses
291 were conducted in haphazard order, and each trial was analyzed once. In total four people
292 analyzed the videos. Four behaviors were recorded from the arena trials: 1) *latency* as the
293 time from the start of the experiment until the whole body of fish emerged from the start box,
294 (after Boulton et al. 2014; Moran et al. 2016; Vainikka et al. 2016); 2) time until fish passed
295 the gate to the upstream section of the arena (arrow in Fig. 1), but this was not analyzed
296 because of many fish not entering this section; instead we recorded 3) *exploration tendency* as
297 a binary variable indicating whether the whole body of the fish passed the gate within the
298 arena; and 4) *activity* of fish as the proportion of time spent actively swimming after emerging
299 from the start box. We used the proportion of time rather than absolute time active to reduce
300 the dependence of activity from latency. Activity was thus calculated by dividing the total
301 time when fish did not move when outside the start box by the total time spent outside the
302 start box and subtracting the value from 1. Stillness was characterized as the fish not moving
303 forward, backward or sideways for longer than ~2 s. Notably, activity by our definition refers
304 to short-term activity in a risky, novel environment, not in a familiar environment as it is

classically defined (Conrad et al. 2011), and it was recorded only from the trials in which the fish emerged from the start box.

Cortisol response to confinement stress

We measured the plasma cortisol levels from of a subset of the fish after exposure to a standardized confinement stress. During the tests, the fish were transferred to individual dark brown 10-L plastic buckets with 1.5 L water for 30 min (except for one fish in each Wild HV and LV and Hatchery HV when the time was 36 min by mistake). The water was aerated using air stones and pump (Sera Air 550R and Sera AS30 air stone) during the test. The buckets were placed in a flow-through buffer tank at a temperature matching the acclimation tanks (temperatures increased during the days of the measurement, 26–29 June 2017, from 13.4 to 16.1°C), and left undisturbed in the dark for the duration of the confinement. Fish were then removed from buckets by dip-netting, anaesthetized using benzocaine solution, measured (to 1 mm) and weighed (to 0.1 g). Blood samples were collected within 2–5 min from the start of anesthesia. The sampling order of fish from the same tank during the same day was recorded. Blood was collected using 23 G heparinized needles and syringes and kept on ice temporarily until centrifuged at 4000 x g for 10 min. Plasma was collected in Eppendorf tubes and frozen at –20°C until analysis. Control samples for establishing baseline plasma cortisol concentrations were collected after terminal anesthesia as described above, omitting the confinement stress treatment. Plasma cortisol concentration was determined using enzyme-linked immunosorbent assay (ELISA) (Enzo cortisol assay) as described in ESM1.

328 **Sex determination from DNA samples**

329 To consider potential sex differences in the studied traits, we identified the sex of fish using
330 PCR amplification of the sexually dimorphic *sdY* locus, which identifies the correct sex in
331 brown trout with nearly 100% accuracy (Quéméré et al. 2014); details in ESM1.

332

333 **Statistical analyses**

334 The number of individuals included in each analysis is shown in Table 1. We built univariate
335 models for each response variable (metabolic and behavioral variables and cortisol level) to
336 assess the differences between breeding group and acclimation conditions (Table 2). All
337 analyses were conducted in R v.3.3.2 (R Core Team, 2016). Linear (LMM) and generalized
338 mixed-effects models (GLMM) were fitted using package *lme4* (Bates et al. 2015) with
339 *lmerTest* (Kuznetsova et al. 2017) and the frailty models using package *coxme* (Therneau,
340 2018). The data were visualized using ggplot2 (Wickham 2009) and patchwork
341 (https://github.com/thomasp85/patchwork). Statistical significance was determined as $\alpha =$
342 0.05 in all models. Predicted means within groups were estimated for behavior traits with
343 package *ggeffects* (Lüdecke 2018). The effect of sex was analyzed in separate models,
344 including the fixed effect of sex as well as the effects from original models, except
345 photoperiod or its interactions due to limited sample size with known sex. All linear models
346 were checked for homoscedasticity and normality of residuals.

347

348 Log_{10}-transformed $\dot{M}O_{2,min}$ or $\dot{M}O_{2,ave}$ were analyzed using an LMM with function *lmer*. The
349 main effects of population, selection line, photoperiod and $\log_{10}$-body mass (in kg) were
350 separately tested using linear hypothesis testing (function *lht* in package *car*) using restricted
351 models, where each respective main effect and its interactions were defined zero and
352 compared to the full model using F-tests.

353

354 The difference in cortisol level of control fish and fish exposed to confinement stress was first
355 tested using a one-tailed t-test. The post-confinement stress cortisol level was then analyzed
356 using a linear model using function *lm*.

357

358 Behavioral traits were analyzed using an LMM (*activity*), a frailty model (i.e. mixed effect
359 Cox proportional hazards models for time-to event data (Collett 2015)) (*latency*) and a
360 GLMM (Bernoulli-distributed *exploration tendency*). Trial repeats were encoded as -1, 0, and
361 1 in data from angling selection experiment as 1–6 from burbot vs. control experiment. In 8
362 trials, the fish jumped out of the start box prior to the trial and their behavior was analyzed for
363 9 min 45 s min after the jump. Correlations between metabolic traits and activity were
364 calculated from model residuals and best linear unbiased predictions (BLUPs), respectively,
365 to assess potential underlying associations between the traits across all individuals.
366 Correlations were not calculated for time-to-event data (*latency*) or binary data (*exploration*
367 *tendency*). For further details see ESM1, available online, and Data accessibility.

368

369 **Results**

370 $\dot{M}O_{2,min}$, $\dot{M}O_{2,ave}$ *and stress-sensitivity*

371 The LMM indicated significantly higher $\dot{M}O_{2,min}$ in the offspring of wild fish than of hatchery
372 fish, and a moderate interaction effect between photoperiod and population, wild population
373 having higher values than hatchery population in the 12:12 L:D photoperiod. Interaction was
374 also found for population and angling selection line, hatchery LV fish tending to have higher
375 oxygen uptake than HV fish, while selection lines did not differ in the wild population (Fig.
376 2A; Table 3). $\dot{M}O_{2,ave}$ was higher in wild than in hatchery population, with a modest
377 interaction effect of angling selection in the two populations (non-significant, $P = 0.085$),

378 observed as higher $\dot{M}O_{2,ave}$ in hatchery LV compared to hatchery HV, but no effect of angling
379 selection in the wild population (Fig. 2B). Sex did not have a significant effect on either
380 $\dot{M}O_{2,min}$ (Type III test, $F_{1,58} = 0.901$, $P = 0.346$) or $\dot{M}O_{2,ave}$ ($F_{1,58.577} = 0.1823$, $P = 0.671$).
381
382 Plasma cortisol increased ~seven-fold in individuals subjected to confinement stress (mean =
383 140.62 ng mL^{-1}, SD = 41.00) compared to non-stressed fish (mean = 19.22 ng mL^{-1}, SD =
384 20.65), (t-test, $t = 11.125$, $df = 29.523$, $P < 0.001$). Angling selection or population did not
385 significantly affect the level of post-stress plasma cortisol, although it showed a similar
386 tendency as observed in $\dot{M}O_{2,ave}$ (Table 3, Fig. S3, available online).
387
388 *Behavior in angling selection lines*
389 Fish emerged from the start box during the recorded time in ~84% of the trials. There was a
390 slightly non-significant interaction effect ($P = 0.054$) of population background and angling
391 selection on latency (Table 4). This was observed as an elevated probability to emerge in fish
392 from LV background compared to HV background in the hatchery population, but not in the
393 wild population (Fig. 3A).
394
395 Fish were less active after acclimation in constant light compared to the 12:12 L:D
396 photoperiod, but activity did not differ between populations or angling selection lines (Fig.
397 3B; Table 4). Angling selection had contrasting effects on exploration tendency in each
398 population: in the hatchery population, a higher proportion of fish from LV selection line
399 were explorative than from HV selection line, while there was an opposite tendency in the
400 wild population (Fig. 3C; Table 4). In addition, exploration tendency increased with repeats
401 of the behavioral trial. Sex did not have a significant effect on any behavior trait (female vs
402 male, Activity: $F_{1,39.612} = 1.217$, $P = 0.277$; Latency: $e^{coef} = 1.03$, $z = 0.29$, $P = 0.770$;

Exploration tendency: $z = -0.514$, $P = 0.607$). There was no correlation between the BLUPs of activity and residual $\dot{M}O_{2,min}$ (*Pearson r* = 0.02) or $\dot{M}O_{2,ave}$ (*Pearson r* = –0.04).

Behavioral responses to predator presence

The fish tended to be less active ($P = 0.072$) in the presence of burbot than under control conditions (Table 5). The variance of activity between individuals appeared higher in the presence of burbot, but this was not significant in Levene's test of homogeneity of variance ($F_{1,93} = 0.214$, $P = 0.645$). Activity decreased slightly with increasing behavior trial repeats. Latency was not affected by predator cues (non-significant increase in probability to emerge by 9%), but it increased with increasing behavior trial repeats and between-individual variation in latency was high (~10% higher variance in burbot vs control data compared to data from angling selection lines). The exploration tendency of fish was not affected by predator cues.

Discussion

Stress coping styles and angling selection

We found that captured and non-captured parent brown trout produced offspring that differed in boldness-related behaviors. Against the expectations, boldness, measured as latency to explore a novel arena, was lower in the HV selection line than in the LV line in the hatchery population, while a weaker but more expected effect was found in the wild population. Stress sensitivity was not affected by angling-selection, although these tests suffered from low statistical power. However, a higher response in hatchery LV line compared to HV line was more visible through $\dot{M}O_{2,ave}$. This is notable given that confinement in the respirometer can induce a stress response in fish (Murray et al. 2017) which would likely increase their oxygen uptake. Thus, the trends between HV and LV in the hatchery population observed in the

428 cortisol response and $\dot{M}O_{2,ave}$ suggest potential for angling selection for increased stress
429 sensitivity, which might become more visible after multiple generations of selection. Overall,
430 the results suggest that the individuals of LV selection line within the hatchery population
431 showed a more reactive stress coping style than HV line.
432
433 Differences in coping styles could also partly explain why the boldness-related behaviors
434 showed a pattern contradicting our expectations; if the LV fish were more reactive compared
435 to the HV fish, their behavior in the personality trial may have indicated a higher stress
436 response to the experiment and heightened escape behavior (Laskowski et al. 2016).
437 Population- and species differences in coping styles may also explain some of the
438 inconclusiveness of earlier studies. In largemouth bass, cortisol response to a standard stressor
439 was negatively associated with capture probability (Louison et al. 2017). However, Koeck et
440 al. (2018) only found a weak negative effect (~0.5% change in risk) of high cortisol response
441 on vulnerability to angling in a domestic strain of rainbow trout (*Oncorhynchus mykiss*), but
442 there was no similar relationship in a wild strain of brown trout.
443
444 Whether $\dot{M}O_{2,min}$ is connected to the coping styles/POLS's or angling selection remains
445 unresolved based on our results. A lack of association between metabolic rate and personality
446 has been reported previously in other species, such as the Trinidadian guppy (*Poecilia*
447 *reticulata*) (White et al. 2016). We found a trend of higher $\dot{M}O_{2,min}$ in the hatchery LV line
448 compared to the HV line, and an opposite trend in wild fish, under the 12:12 L:D photoperiod.
449 The result did not entirely conform to the prevailing theory given that photoperiod had a
450 population-specific effect on metabolic rate, but not on behavior. Growth rate is unlikely to
451 explain the differences in $\dot{M}O_{2,min}$ between groups, as the body mass of fish at the end of the
452 experiment did not differ between groups (Table 1). Overall, despite not directly addressing

453 questions on trait covariances, as physiological traits were measured only once (Mathot and
454 Frankenhuis 2018; Niemelä and Dingemanse 2018), our results add to the literature to
455 promote the understanding of evolution in traits due to angling induced selection. From an
456 angling selection perspective, some of the most interesting traits to include in further
457 experiments would be neurochemical stress responses and their links to bold and explorative
458 behavior.
459
460 *Population-specific effects of angling selection on boldness*
461 Although stress coping styles can explain our findings on behavioral responses, the hatchery
462 rearing environment can also have contributed to the pattern through indirect effects. For one,
463 the vulnerable fish may have had the lowest status in the dominance hierarchy within the
464 ponds, and therefore been the hungriest and likeliest to attack lures. In contrast to the hatchery
465 population, angling trials on wild parent fish were more representative of real angling
466 situations in the field. The wild population showed a weaker difference between selection
467 lines, but its direction was more in line with theory, with HV fish being bolder than LV fish.
468 The wild fish had natural invertebrate food available in their ponds, and the structured ponds
469 offered more hiding places. The wild fish had clearly lower catchability than the hatchery
470 fish, and the wild fish could only be captured when approaching the undisturbed pond from a
471 distance. Very few wild fish were captured in one angling session (maximum 4) compared to
472 the hatchery fish (maximum 11). The captured and non-captured parent fish did not show
473 evident size-differences, indicating that the effects of angling were most likely mediated by
474 size-independent traits.
475
476 *Genetic and parental effects between populations and selection lines*

477 Populations frequently differ in e.g., metabolic rate and behavioral syndromes (Dingemanse et
478 al. 2007; Lahti et al. 2002; Polverino et al. 2018), driven by environmental differences,
479 natural selection, founder effects, and genetic drift. The differences we found between
480 populations can therefore be explained by several factors, including the level of
481 domestication, as the hatchery stock had been reared in captivity for several generations. They
482 also differed in their life-histories, with the wild population being clearly less migratory than
483 the hatchery population (A. Lemopoulos, unpublished data). In addition, although we reared
484 offspring under common garden conditions and maximized genetic diversity within each
485 group, it is possible that differences in the early rearing environments of wild and hatchery
486 parents could have had contrasting effects on offspring through parental or epigenetic effects
487 (Crews et al. 2012; Reddon 2012). We studied individuals in their second summer, and
488 parental effects usually affect early life-stages the most; for instance, maternal effects on
489 metabolic traits have been shown to be negligible from 90 days post hatching in coral reef
490 fish (Munday et al. 2017), although maternal stress affects many life-stages in three-spined
491 stickleback (*Gasterosteus aculeatus*) (Bell et al. 2016; Metzger and Schulte 2016).
492 Additionally, parental effects may have also contributed to differences between the selection
493 lines *via* stress resulting from angling (briefly increased cortisol level after angling shown,
494 e.g., in Wilson et al. (2011)). It is nevertheless likely that for both population and angling
495 selection line differences, genetic inheritance may explain our results at least partly, as both
496 angling vulnerability and personality traits can be heritable in the studied populations (Ågren
497 et al. 2019) and in other species (Dingemanse et al. 2009; Philipp et al. 2009).
498
499 *Potential effects of photoperiod on energy balance*
500 We incorporated environmental variation in our study as two different photoperiods. The
501 results demonstrate, on one hand, that metabolic rate and swimming activity are sensitive to

502 photoperiod, and on the other hand, that the other behavioral traits lack this sensitivity.
503 Constant light is not encountered by brown trout during the winter months; hence the 24-hour
504 light regime could be considered unnatural and potentially stressful for the fish. Constant light
505 can disrupt entrainment of endogenous rhythms by inhibiting the synthesis of melatonin and
506 by directly affecting photosensitive proteins (Falcón et al. 2010; Peirson et al. 2009). Based
507 on our results, constant light had an inhibiting effect on fish swimming activity, and also
508 decreased $\dot{M}O_{2,min}$ in the wild population, indicating that energy metabolism in brown trout
509 can be affected by (an unnatural) photoperiod. In general, non-tropical species are expected to
510 be particularly sensitive to photoperiod disturbances due to the role of day length in
511 anticipating seasonal changes in environmental conditions (Borniger et al. 2017).
512
513 *Innate vs learned antipredatory responses*
514 Our goal was to study risk-taking behavior/boldness of offspring by subjecting fish to the
515 olfactory cues of a natural predator that had fed on conspecifics. Wild brown trout typically
516 increase the use of refuges under predation threat, while hatchery brown trout do not (Álvarez
517 and Nicieza 2003). None of the individuals in the behavior trials in this study had been
518 exposed to predators before the trials apart from potential traces of piscivore odors in the
519 rearing water. The scarcity of responses to the presence of predator odor, measured in the
520 offspring of wild fish, suggests only weak innate responses. Nevertheless, the tendency for
521 lower activity in the presence of burbot than in control conditions resembles previously
522 shown antipredator responses in fish (Álvarez and Nicieza 2003; Kopack et al. 2015).
523
524 **Conclusions**
525 Our results demonstrate the potential for rapid human-induced evolution in the behavior of a
526 popular fishing target species. The effects of angling selection were contradictory between

wild and hatchery populations of fish, which leads to new questions on the mechanisms behind the observed differences. Stress coping styles may explain the result, as indicators of stress sensitivity tended differ between the wild and hatchery populations of fish. Overall, our study supports earlier findings according to which angling may be a potentially significant driver of evolution in behavioral and physiological traits in natural populations.

Author contributions

A.V. and P.H. produced the selection lines, J.M.P, N.A. and A.V. designed the experiment, J.M.P., N.A., S.M. and A.L. collected the data, J.M.P. and L.M. analysed the data, J.M.P. wrote the initial draft of the manuscript. All authors contributed to preparing the manuscript.

Compliance with Ethical Standards

All applicable institutional and/or national guidelines for the care and use of animals were followed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data accessibility

All data and R codes for the models in this manuscript are available in Github (https://github.com/jprokkola/Strutta_repo). Videos of behavior trials will be made publicly available in Figshare (accession) upon acceptance for publication.

Electronic Supplementary Material

ESM1. Pdf-file including supplemental figures and methods.

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779
780 Tables

781 Table 1. The number of individuals in each group in each analysis, and fish total body length and mass (mean ± SD) at the end of the experiment.

782

Photoperiod	Group	N (metabolic rate)	N (latency)	N (activity)	N (stress response)	N (body size)	N males / females (unknown)	Body length / mm	Body mass / g
12:12	Hatchery HV	15	15	15	7	14	9 / 5 (1)	117 ± 10	17.1 ± 4.3
	Hatchery LV	7	14	14	6	14	6 / 2 (7)	115 ± 13	16.5 ± 6.7
	Wild HV	11	14	14	4	14	6 / 6 (3)	117 ± 7	17.4 ± 3.2
	Wild LV	14	15	15	9	14	7 / 7 (1)	115 ± 9	16.6 ± 4.3
24	Hatchery HV	10	10	10	0	9	6 / 3 (1)	119 ± 9	17.7 ± 4.7
	Hatchery LV	7	10	10	0	6	0 / 4 (6)	124 ± 9	19.6 ± 3.9
	Wild HV	8	10	10	0	6	2 / 3 (5)	116 ± 9	17.8 ± 7.1
	Wild LV	6	10	10	0	5	2 / 1 (7)	119 ± 9	17.8 ± 3.0

783 Table 2. The main statistical models used in this study. Abbreviations explained below the

784 table.

785

Study section	Response variable	Model
I. Angling selection experiment	$Log_{10}(\dot{M}O_{2,min})$ or $Log_{10}(\dot{M}O_{2,ave})$	$y_{ij} = \beta_0 + \beta_1 PHO_{ij} + \beta_2 POP_{ij} + \beta_3 SEL_{ij} + \beta_4 POP_{ij} \times SEL_{ij} + \beta_5 POP_{ij} \times PHO_{ij} + \beta_6 POP_{ij} \times logBM_{ij} + \beta_7 logBM_{ij} + \beta_8 WT_{ij} + p_l + e_{ij}$
	Stress sensitivity (post-stress plasma cortisol)	$y_i = \beta_0 + \beta_1 POP_i + \beta_2 SEL_i + \beta_3 POP_i \times SEL_i + \beta_4 ORD_i + \beta_5 BL_i + \beta_6 WT_i + e_i$
	Activity (proportion of time spent swimming during the trial)	$y_{ijk} = \beta_0 + \beta_1 PHO_{ijk} + \beta_2 POP_{ijk} + \beta_3 SEL_{ijk} + \beta_4 POP_{ijk} \times SEL_{ijk} + \beta_5 POP_{ijk} \times PHO_{ijk} + \beta_6 REP_{ijk} + b_i + c_j + d_k + e_{ijk}$
	Latency to emerge from the box	$\lambda(t) = \lambda_0(t) e^{\beta_1 PHO_{ijk} + \beta_2 POP_{ijk} + \beta_3 SEL_{ijk} + \beta_4 POP_{ijk} \times SEL_{ijk} + \beta_5 POP_{ijk} \times PHO_{ijk} + \beta_6 REP_{ijk} + b_i + c_j + d_k}$
	Exploration tendency (1 = explorative, 0 = unexplorative)	$y_{ij} \sim Bernoulli(p_{ijk})$ $logit(p_{ijk}) = \beta_0 + \beta_1 PHO_{ijk} + \beta_2 POP_{ijk} + \beta_3 SEL_{ijk} + \beta_4 POP_{ijk} \times SEL_{ijk} + \beta_5 POP_{ijk} \times PHO_{ijk} + \beta_6 REP_{ijk} + b_i + c_j + d_k$
II. Behaviour responses to burbot olfactory cues	Activity	$y_{ijk} = \beta_0 + \beta_1 SEL_{ijk} + \beta_2 TRE_{ijk} + \beta_3 REP_{ijk} + \beta_4 BL_{ijk} + b_i^{(1)} CON + b_i^{(2)} BUR + c_j + d_k + e_{ijk}$
	Latency to emerge from the box	$\lambda(t) = \lambda_0(t)\, e^{\beta_1 SEL_{ijk} + \beta_2 GR_{ijk} + \beta_3 REP_{ijk} + \beta_4 BL_{ijk} + b_i + c_j + d_k}$
	Exploration tendency	$y_{ijk} \sim Bernoulli(p_{ijk})$ $logit(p_{ijk}) = \beta_0 + \beta_1 SEL_{ijk} + \beta_2 GR_{ijk} + \beta_3 REP_{ijk} + b_i + c_j + d_k$

β_0 Intercept, *PHO* Photoperiod, *POP* Population, *SEL* Selection, *logBM* Log_{10} body mass in kg, *WT* Water temperature in °C, *ORD* Capture order from the same tank, *BL* Body length in mm – mean (118.8182 mm for I, 122.4464 mm for II), *REP* Trial repeat, *TRE* Treatment, p_l the random effect for chamber l, b_i random effect for fish i, c_j the random effect for arena j, d_k the random effect for batch k, *e* Residual, λ_0 baseline hazard, *t* time, *CON / BUR* binary explanatory variables for burbot and control treatments.

786 Table 3. Results from models for $\dot{M}O_{2,min}$ and $\dot{M}O_{2,ave}$ and post-stress plasma cortisol. The
787 zero levels for contrasts were: photoperiod 12:12, population hatchery, and selection line HV.
788 For the LMMs, F and P- values for the interactions and temperature effect were obtained from
789 Type III sums of squares and Satterthwaite approximation for degrees of freedom. For the
790 other fixed effects, linear hypothesis tests using F-test on restricted models with each main
791 effect and its interactions set to zero were used – residual degrees of freedom are given for
792 these tests. For cortisol, Type III F-test shown with population and selection line fixed effects
793 estimated using linear hypothesis tests. Significant ($P < 0.05$) effects shown in bold. For
794 intercepts, t-test values are shown.
795

	Fixed effects	Estimate ± SE	Num df	Res / Den df	F	P
$Log_{10}(\dot{M}O_{2,min})$ (LMM)	Intercept	1.82 ± 0.29	1	70	6.32 (t)	<0.001
	Photoperiod	**0.0024 ± 0.02**	**2**	**67.48**	**4.03**	**0.022**
	Population	**-0.84 ± 0.38**	**4**	**52.71**	**3.01**	**0.026**
	Selection	0.054 ± 0.02	2	68.63	2.50	0.090
	Temperature	-0.0009 ± 0.02	1	70	0.002	0.968
	Log_{10} body mass	**1.15 ± 0.15**	**2**	**68.86**	**35.22**	**<0.001**
	Pop × selection	-0.068 ± 0.03	1	70	3.86	0.053
	Pop × photoperiod	**-0.07 ± 0.03**	**1**	**70**	**4.51**	**0.037**
	Pop × log_{10} body mass	**-0.51 ± 0.21**	**1**	**70**	**5.65**	**0.020**
	Random effects	**Variance (SD^2)**				
	Chamber	0				
	Residual	0.07^2				
$Log_{10}(\dot{M}O_{2,ave})$ (LMM)	**Fixed effects**	**Estimate ± SE**	**Num df**	**Res / Den df**	**F**	**P**
	Intercept	1.18 ±0.44	1	72.50	2.66 (t)	0.010
	Photoperiod	-0.027 ± 0.04	2	70.178	1.52	0.226
	Population	**-0.72 ± 0.54**	**4**	**70.271**	**5.38**	**<0.001**
	Selection	**0.09 ± 0.04**	**2**	**70.763**	**3.17**	**0.048**
	Temperature	-0.03 ± 0.04	1	72.742	0.68	0.412
	Log_{10} body mass	0.38 ± 0.22	2	71.883	1.60	0.209
	Pop × selection	-0.09 ± 0.05	1	70.660	3.05	0.085
	Pop × photoperiod	-0.03 ± 0.05	1	70.373	0.36	0.550
	Pop × log_{10} body mass	-0.49 ± 0.30	1	71.343	2.67	0.107
	Random effects	**Variance (SD^2)**				
	Chamber	0.029^2				
	Residual	0.109^2				
Post-stress cortisol (LM)	**Fixed effects**	**Estimate ± SE**	**Sum sq.**	**Df**	**Test statistic**	**P**
	Intercept	331.91 ± 225.90	2963.0	1	2.16 (t)	0.162
	Population	47.84 ± 30.43	3392.6	2	2.47	0.142
	Selection	19.35 ± 25.88	767.1	2	0.56	0.466
	Temperature	-15.22 ± 15.38	1343.9	1	0.98	0.338
	Body length	0.07 ± 0.84	8.3	1	0.006	0.939
	Sampling order	-0.75 ± 3.93	50.4	1	0.04	0.851
	Pop × selection	-19.81 ± 37.59	381.1	1	0.28	0.606
	Residual		20587.6	15		

Table 4. Results of models for behavior traits in brown trout from hatchery and wild populations and two angling selection lines (HV and LV). The zero levels for contrasts in all models were: photoperiod 12:12, population hatchery, and selection line HV. For model equations, see Table 2. For Activity, F and P-values for the interactions and trial repeat were obtained from Type III test, and for the other main effects from linear hypothesis tests using restricted models with each main effect and its interactions set to zero. Fixed effects with $P < 0.05$ shown in bold. For intercepts, t- or z-test values shown.

806

	Fixed effects	Estimate ± SE	Num Df	Res/Den Df	F	P
Activity (LMM)	**Intercept**	**0.30 ± 0.04**	**1**	**33.18**	**7.78 (t)**	**<0.001**
	Photoperiod	**-0.75 ± 0.04**	**3**	**84.03**	**6.53**	**0.001**
	Population	0.08 ± 0.06	3	42.50	1.40	0.242
	Selection	-0.01 ± 0.04	2	74.67	0.10	0.903
	Pop × selection	0.025 ± 0.06	1	197.41	1.28	0.259
	Photoperiod × pop	-0.070 ± 0.06	1	81.43	1.37	0.245
	Trial repeat	-0.017 ± 0.02	1	44.24	0.20	0.655
	Random effects	**Variance (SD^2)**				
	ID	0.054^2				
	Batch	0.036^2				
	Arena	0.016^2				

	Fixed effects	Coef	e^{coef}	SE	z	P
Latency (frailty model)	Photoperiod	0.13	1.14	0.26	0.49	0.630
	Population	-0.083	0.92	0.30	-0.27	0.780
	Selection	**0.57**	**1.76**	**0.26**	**2.21**	**0.027**
	Pop × selection	-0.71	0.49	0.37	-1.92	0.054
	Photoperiod × pop	-0.060	0.94	0.38	-0.16	0.870
	Trial repeat	**0.23**	**1.26**	**0.082**	**2.83**	**0.005**
	Random effects	**Variance (SD^2)**				
	ID	0.625^2				
	Batch	0.079^2				
	Arena	0.097^2				

	Fixed effects	Estimate ± SE	Wald χ^2	Df	P
Exploration tendency (GLMM)	Intercept	0.55 ± 0.63	0.88 (z)	1	0.380
	Photoperiod	-0.63 ± 0.63	1.80	1	0.179
	Population	1.07 ± 0.72	0.058	1	0.810
	Selection	1.54 ± 0.64	0.43	1	0.511
	Pop × selection	**-2.46 ± 0.92**	**7.18**	**1**	**0.007**
	Photoperiod × pop	0.07 ± 0.88	0.006	1	0.936
	Trial repeat	**0.58 ± 0.21**	**7.79**	**1**	**0.005**
	Random effects	**Variance (SD^2)**			
	ID	1.398^2			
	Batch	0.270^2			
	Arena	0.762^2			

807
808

809 Table 5. Results of models for activity, latency and exploration tendency in the presence of
810 predatory olfactory cues and control conditions in brown trout. For activity, the t-test was
811 used with Satterthwaite approximations to degrees of freedom. The model was fit with
812 restricted maximum likelihood. For latency, proportional hazard estimates (± standard error)
813 are shown with hazard ratios (e^{coef}). For latency and exploration tendency, Wald Chisquare
814 test was used to determine significance of fixed effects. The zero levels for contrasts in all
815 models were: treatment control and selection line HV. Significant effects ($P < 0.05$) shown in
816 bold.

	Fixed effects	**Estimate ± SE**	**Den Df**	**t**	**P**
Activity (LMM)	**Intercept**	**0.435 ± 0.060**	**23.25**	**7.198**	**<0.001**
	Selection line	0.012 ± 0.065	9.59	0.18	0.861
	Treatment	-0.081 ± 0.042	15.85	-1.928	0.072
	Trial repeat	**-0.029 ± 0.010**	**71.62**	**-2.97**	**0.004**
	Body length	-0.00009 ± 0.004	8.63	-0.02	0.984
	Random effects	**Variance (SD^2)**			
	ID (burbot)	0.122^2			
	ID (control)	0.077^2			
	Batch	0.000			
	Arena	0.000			
	Residual	1.400^2			
Latency (frailty model)	**Fixed effects**	**Coef ± SE**	**e^{coef}**	**z**	**P**
	Selection line	0.336 ± 0.372	1.399	0.900	0.370
	Treatment	0.089 ± 0.220	1.093	0.400	0.690
	Trial repeat	0.113 ± 0.065	1.120	1.759	0.080
	Body length	-0.006 ± 0.026	0.994	-0.230	0.820
	Random effects	**Variance (SD^2)**			
	ID	0.661^2			
	Batch	0.210^2			
	Arena	0.080^2			
Exploration tendency (GLMM)	**Fixed effects**	**Estimate ± SE**	**z**	**P**	
	Intercept	1.449 ± 0.801	1.808	0.0706	
	Selection line	0.659 ± 0.724	0.910	0.363	
	Treatment	-0.63 ± 0.487	-1.294	0.196	
	Trial repeat	-0.066 ± 0.142	-0.465	0.642	
	Random effects	**Variance (SD^2)**			

ID	1.140^2
Batch	0.000
Arena	$(1.847 \times 10^{-5})^2$

819 Figure Legends
820

821 Fig.1. 3D-illustration of the arena used in personality trials without the left side wall. Water
822 flow direction is left-right. Burbot was placed in the area indicated by blue color, upstream
823 from the net (inaccessible to the brown trout). The grey box indicates the start box, where the
824 fish was placed before the start of a trial. Latency was measured as time to emerge from the
825 box. Activity was measured as swimming activity outside the start box arena after emergence.
826 Exploration tendency was measured as the whole body of fish passing the gate indicated by
827 an arrow.
828

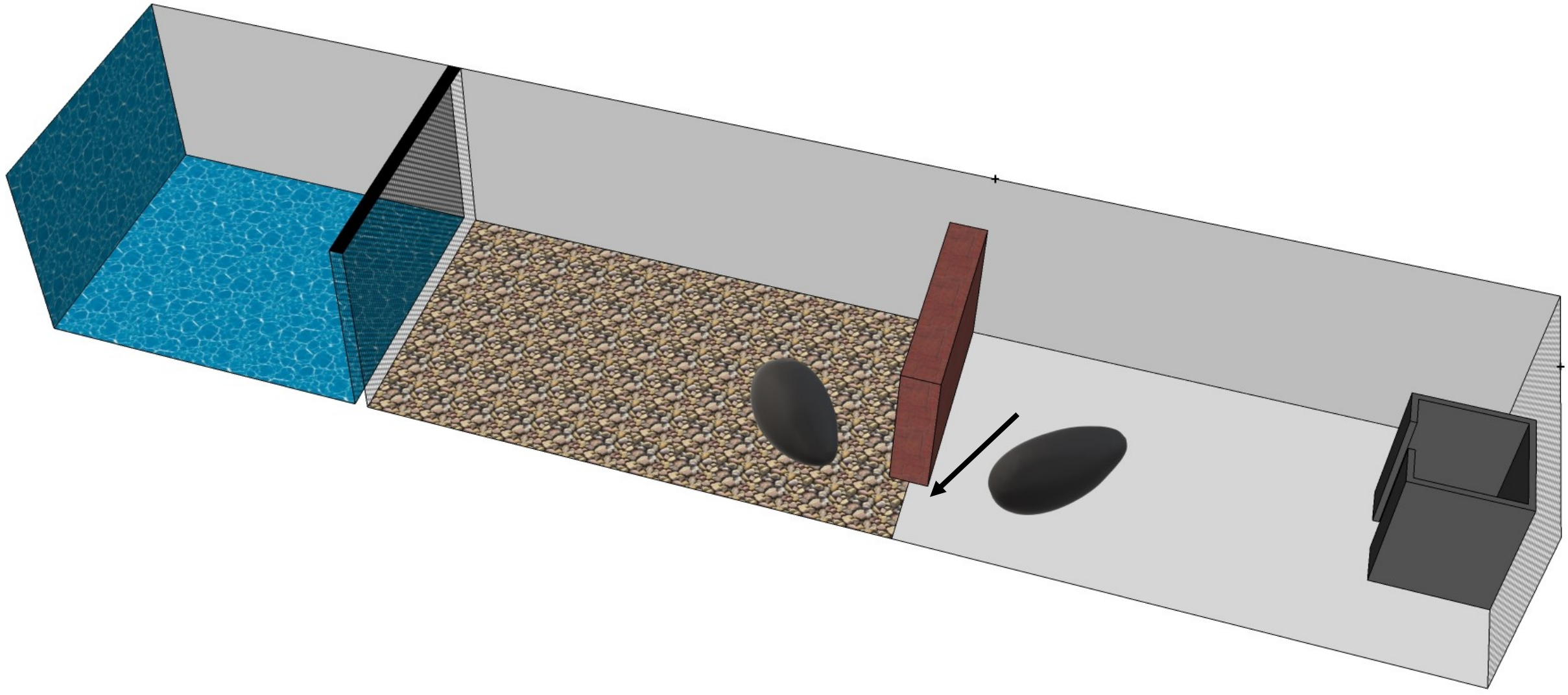
829 Fig. 2. Means and 75% confidence intervals for A) mass-specific $\dot{M}O_{2,min}$, using mass-scaling
830 exponent 0.928; and B) mass-specific $\dot{M}O_{2,ave}$ using raw body mass (scaling exponent was
831 0.19 and was not used). For statistical significance, see Table 3. Wild and hatchery
832 populations shown with two angling selection lines produced from individuals with high
833 (HV) or low (LV) vulnerability to angling. 12:12 = L:D rhythm, 24 = continuous light. N in
834 each group shown in Table 1. Legend shown in A.
835

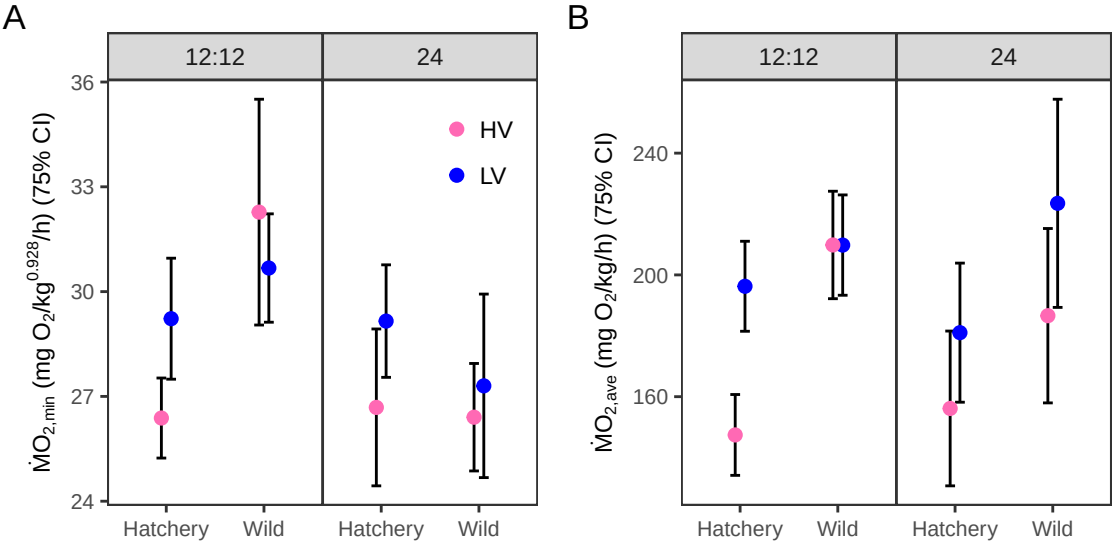
836 Fig. 3. Behavioral differences between two angling vulnerability selection lines (HV – high
837 vulnerability, LV – low vulnerability) within the hatchery and wild populations. A) Curves
838 showing the proportion of individuals emerged from the start box, drawn with Kaplan-Meier
839 estimator. Photoperiods are combined within each breeding group, confidence intervals
840 omitted for clarity. Higher proportion indicates higher boldness. B) Predicted activity from
841 LMM with 75% confidence intervals for predicted values. Significantly lower activity was
842 observed in 24 (constant light) compared to 12:12 (light-dark rhythm) (Table 4). C) Predicted
843 exploration tendency from GLMM with 75% confidence intervals for predicted values.
844 Angling selection had opposing effects on exploration tendency in the two populations (Table

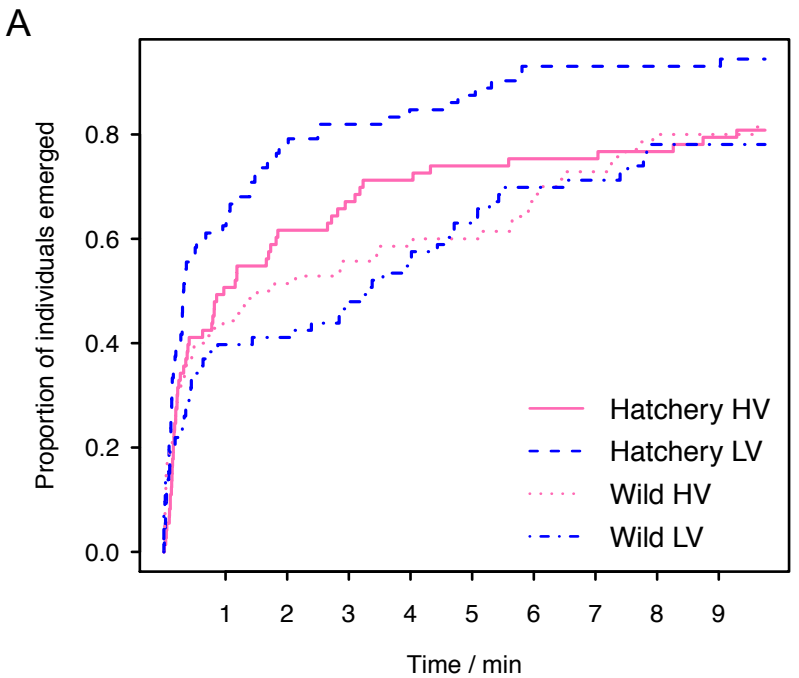
845 4). In B and C, predictions were made for the first trial repeat. For N in each group, see Table

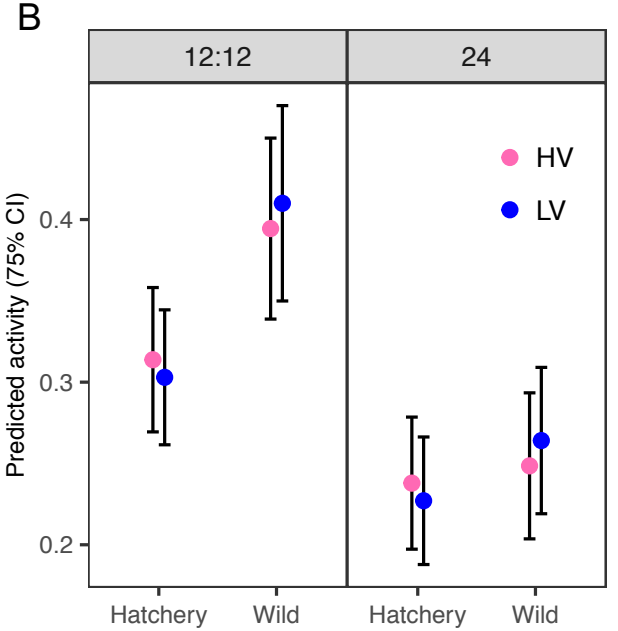
846 1.

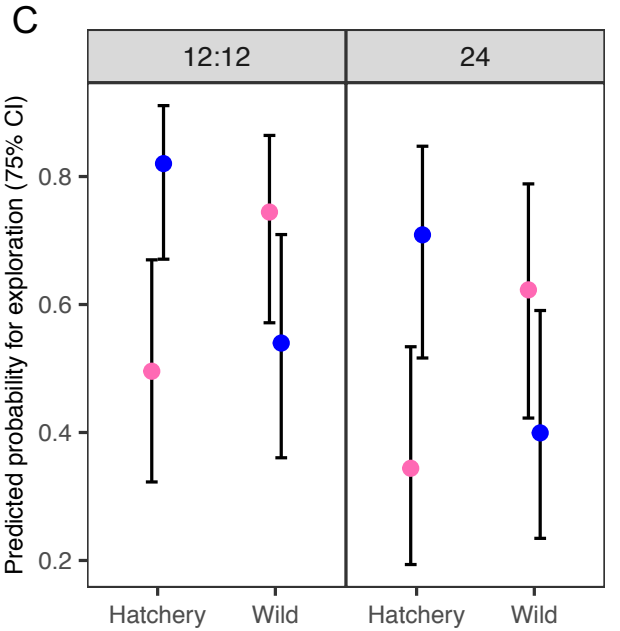
847




A
12:12
24
36
33
30
27
24
HV
LV
$\dot{M}O_{2,min}$ (mg O_2/kg$^{0.928}$/h) (75% CI)
Hatchery
Wild
Hatchery
Wild
B
12:12
24
240
200
160
$\dot{M}O_{2,ave}$ (mg O_2/kg/h) (75% CI)
Hatchery
Wild
Hatchery
Wild


A
Proportion of individuals emerged
0.0
0.2
0.4
0.6
0.8
1
2
3
4
5
6
7
8
9
Time / min
Hatchery HV
Hatchery LV
Wild HV
Wild LV


B
12:12
24
Predicted activity (75% CI)
0.2
0.3
0.4
HV
LV
Hatchery
Wild
Hatchery
Wild


C
12:12
24
Predicted probability for exploration (75% CI)
0.2
0.4
0.6
0.8
Hatchery
Wild
Hatchery
Wild