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

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Abstract

Human induced selection, including mortality caused by recreational angling, can cause phenotypic changes in wild populations. Brown trout (*Salmo trutta*) is an intensively fished salmonid that is also impacted by unintended hatchery-induced domestication, and thus provides a relevant model to study both angling- and hatchery-induced selection. We produced crosses of fish with high or low vulnerability to angling using 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. Our results show that angling selection had population-specific effects on risk taking -related latency and exploration tendency, and that 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 juvenile personality and suggests that metabolic rate and stress sensitivity may also be affected. The context-dependent effects of angling selection indicate that easy solutions for fisheries management and conservation purposes to mitigate these changes may not exist.

Introduction

Selective harvesting of natural populations by humans induces strong selection (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 (Kern et al. 2016; Wong et al. 2012). In empirical and simulation studies of heavily exploited fish stocks, fisheries-induced evolution has decreased the size at maturity and growth rate of fish (Audzijonyte et al. 2013; Biro and Post 2008; Devine et al. 2012; Nussle et al. 2009).

In addition to 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). Regarding behavioral traits, selection from recreational fishing is expected to particularly affect boldness and exploration tendency (Arlinghaus et al. 2017). 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, slow-growth 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).

Mechanisms mediating angling-induced selection are poorly understood, but several studies have found a correlation between behaviors affecting energy balance and minimum metabolic rate (meta-analysis by Mathot et al. 2018), implying that selection acting on personality could thereby affect metabolism in fish. 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) and more empirical studies in common garden conditions are needed to address this question.

Angling selection can be understood also 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.

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 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). Brown trout (*Salmo trutta*), are one of the most targeted game fish species globally and endangered in parts of its native range in Europe. To understand the human impact on brown trout populations, empirical research on the consequences of angling is urgently needed. 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 to assess the potential interplay between fishing- and hatchery-induced selection. We hypothesized that offspring from angling-vulnerable parents would have 1) higher scores in boldness-related 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 licence obtained from the national Animal Experiment Board in Finland (licence 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 broodstock). 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 established from two source populations and reared in nearby hatcheries for 3–4 generations. These stocks originated from predominantly migratory (adfluvial) populations in the region (further details in Lemopoulos et al. (2019)). Despite originating from the same River Varisjoki watershed, the used populations showed moderate genetic divergence based on fixation index (F_{ST}-value) of 0.11 (Lemopoulos et al. 2019). The wild population had been exposed to angling more recently than the hatchery population, although fishing pressure had been weaker than the fishing pressure on the migratory strain prior to hatchery rearing (P. Hyvärinen, unpublished observation).

During the whole study, fish were fed with commercial fish pellets (Raisio Oyj). In 2015, hatchery-origin and wild-origin adult fish were exposed to experimental fly fishing and divided into captured (high vulnerability, HV) and uncaptured (low vulnerability, LV) groups. Fish were fished in two size-assortative pools for each population during June and July with fly fishing gear adjusted by the size of the fish in the pools. The wild fish were fished in semi-natural 50-m^2 ponds with a gravel-bottom outer riffle sections and *ca.* 1 m deep, concrete inner pool sections (53 and 91 visually size-sorted fish in two ponds). The hatchery fish were fished in 75-m^2 concrete ponds with no structures (64 larger and 167 smaller fish from two different cohorts in two ponds). Angling was performed by experienced fly fishers (mainly A.V.) using unnaturally coloured woolly bugger -type fly patterns tied to barbless hooks. During angling sessions, an angler fished a pond until a fish took the fly or five minutes passed, after which angling was continued at earliest one hour later. If a fish was captured, angling was continued immediately after processing, which included anaesthesia with benzocaine (40 mg L^{-1}), identification of passive integrated transponder (Oregon RFID) code or tagging when a pre-existing tag was missing, and measuring total length (to 1 mm) and weight (to 2 g). Fish that were missing PIT-tags were tagged under the skin next to the dorsal fin using 12 mm tags at this point. After processing, the fish were transferred to similar ponds (hatchery fish to a 50 m^2 otherwise similar concrete pond) as used for each population during angling. After angling trials were finished, on 25 June 2015, all remaining wild fish that were not captured were collected by dip-netting after draining the experimental angling ponds, anaesthetized, measured and weighed (mean body lengths of fish uncaptured and captured by angling: in large fish 457 and 475 mm, respectively, and in small fish 344 and 354, respectively). Uncaptured wild fish were then combined in the same ponds as the fish captured by angling. The captured hatchery strain fish were subjected to a second round of angling ~2 weeks later, where in total eight fish were captured and prioritized for breeding the HV-line, but this was not done on wild fish due to their limited availability. Angling trials finished on 8 July 2015, and also hatchery fish were transferred back to their original ponds. Because of the warm water at the time of finishing the second round of angling, the uncaptured hatchery fish were not measured to avoid handling-induced stress and mortality. One deep-hooked small hatchery fish was found dead 5 days and one large hatchery fish 41 days after capture, but otherwise no mortality occurred between angling trials and the breeding.

The offspring used in this study were obtained from fish bred in four groups (i.e. high- and low-vulnerability [HV and LV, respectively] within each population) in the autumn of 2015. A replicated, fully factorial 3 × 3 breeding design was used to create the F_1-generation; males were crossed with females in all combinations in one matrix, and the matrices replicated three times for each group, details in Electronic Supplemental Material (ESM1, available online). In the autumn of 2016, the one-summer-old fish were tagged with individual 12-mm PIT-tags in the abdominal cavity under anaesthesia (benzocaine). After tagging, the selection lines were mixed together in two 3.2 m^2 fibreglass 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. 4 h 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, available online.

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.

The fish were allowed to recover from respirometry for at least four days before behavioral trials to minimize potential effects of handling stress on behavior. They were not fed for 24-h prior to behavioral trials. The trials were conducted in custom-made mazes (Fig. 1) (size 400 mm wide x 1500 mm long, water depth 100 mm in the open area). During the trials, temperature in the maintenance tanks and test arenas was on average 4.5 ± SD 1.3°C. Water flow rate during the trials was adjusted to ~8 L min^{-1} (~7.6–8.8 L min^{-1}). This allowed for at minimum 1.26 times the arena volume of water to flow between consecutive trials, which was considered sufficient to minimize potential carry-over effects of chemical cues between trials. The arena was lit by LED lights (CRI90 LED chain in waterproof silicon tube, 3000-3300K, 4.8W m^{2}) situated along one long edge of the arena (>70 lux across the arena depending on distance from light source). Half-way across the arena was a brick gate situated next to one side, allowing entry from the other side. Behind the brick, natural pebbles (~3–5 cm in diameter) were scattered unevenly on the floor, and one large stone was provided for shelter. A second large stone was placed in the center of the arena in front of the start box. Four similar arenas were used in the experiment, but they differed in the visual appearance of the natural stones and two of the arenas were mirror images of the other two with respect to the location of the gate.

Upstream from the flow-through test arena was a section divided by a metal grid (5 mm mesh size) where a hatchery-reared burbot (*Lota lota*) (length ~30-40 cm) was placed to introduce olfactory cues of a natural predator of juvenile brown trout. Burbot are nocturnal bottom-dwelling predators that are likely difficult for prey to detect visually, but their odor induces antipredator responses in prey species (Ylönen et al. 2007). Burbot were regularly fed with pieces of various cyprinids and vendace (*Coregonus albula*) during rearing, and only with fresh pieces of brown trout for two days prior to and during the trials. Burbot were moved to the test arenas at least one day before the trials. The burbot were fed with trout pieces in separate tanks and changed in each arena every 10–15 trials (2–3 days).

Before each trial, individual brown trout were haphazardly removed from their rearing tanks using a dip-net under red light and placed into black 10-L buckets filled with ~8L of water from the flow-through system. Fish were identified by PIT tags and left undisturbed for 10 min before being transferred into the start box located downstream from the test arena by pouring. During each trial, the trout was acclimatized in the start box for 3 min, after which the door of the box was opened by pulling a string from behind a curtain, and fish movements recorded from above using two CCTV infrared cameras (two arenas simultaneously filmed using the same camera) for 10 min (of which first 9 min 45 s was included in the behavior analysis). The behavioral trial was repeated three times between 8:00 and 11:00 for each focal fish, with an average time of 4.3 days (range 1–8 days) between consecutive trials. One trial from four fish was omitted from analysis due to error in data collection. The order in which batches of four fish were captured on the same day from the same tank for the four arenas was recorded (batch from hereon, levels 1–5, four individuals from batch 6/7 combined to batch 5).

Testing behavioral responses to burbot

To confirm that burbot odor was perceived risky in the personality assays, we tested for the response of brown trout to burbot in separate controlled tests using individuals from wild HV and wild LV groups (N=10 in each). These fish were acclimated to similar tanks as the personality-tested fish at 12h:12h L:D photoperiod for one week before trials started. One individual died after the first trial. The behavior of each individual was tested on six different days in the presence and absence of predator (3 trials in each condition in haphazard order). 3–4 different arenas were used for each fish on different days to reduce fish habituation to the arenas. These trials were conducted between 14:40 and 17:00. Control arenas were emptied and thoroughly rinsed with pressurized tap water and water flow maintained for >2h before the trials to avoid carry-over effects from burbot odor in earlier experiments. The water used in the flow-through system originates from lake Kivesjärvi, where burbot is a common species; thus, traces of burbot odor may have been present in all trials.

Analysis of video recordings

Behavioral data were collected from videos using manual tracking with AV Bio-Statistics 5.2 timing software. The observer was blind to the identity of fish in all recordings. Analyses were conducted in haphazard order, and each trial was analyzed once. In total four people analyzed the videos. Four behaviors were recorded from the arena trials: 1) *latency* as the time from the start of the experiment until the whole body of fish emerged from the start box, (after Boulton et al. 2014; Moran et al. 2016; Vainikka et al. 2016); 2) time until fish passed the gate to the upstream section of the arena (arrow in Fig. 1), but this was not analyzed because of many fish not entering this section; instead we recorded 3) *exploration tendency* as a binary variable indicating whether the whole body of the fish passed the gate within the arena; and 4) *activity* of fish as the proportion of time spent actively swimming after emerging from the start box. We used the proportion of time rather than absolute time active to reduce the dependence of activity from latency. Activity was thus calculated by dividing the total time when fish did not move when outside the start box by the total time spent outside the start box and subtracting the value from 1. Stillness was characterized as the fish not moving forward, backward or sideways for longer than ~2 s. Notably, activity by our definition refers 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, available online.

Sex determination from DNA samples

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

Statistical analyses

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

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

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

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

Results

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

The LMM indicated significantly higher $\dot{M}O_{2,min}$ in the offspring of wild fish than of hatchery fish, and a moderate interaction effect between photoperiod and population, wild population having higher values than hatchery population in the 12:12 L:D photoperiod. Interaction was also found for population and angling selection line, hatchery LV fish tending to have higher oxygen uptake than HV fish, while selection lines did not differ in the wild population (Fig. 2A; Table 3). $\dot{M}O_{2,ave}$ was higher in wild than in hatchery population, with a modest interaction effect of angling selection in the two populations (non-significant, $P = 0.085$), observed as higher $\dot{M}O_{2,ave}$ in hatchery LV compared to hatchery HV, but no effect of angling selection in the wild population (Fig. 2B). Sex did not have a significant effect on either $\dot{M}O_{2,min}$ (Type III test, $F_{1,58} = 0.901$, $P = 0.346$) or $\dot{M}O_{2,ave}$ ($F_{1,58.577} = 0.1823$, $P = 0.671$).

Plasma cortisol increased ~seven-fold in individuals subjected to confinement stress (mean = 140.62 ng mL^{-1}, SD = 41.00) compared to non-stressed fish (mean = 19.22 ng mL^{-1}, SD = 20.65), (t-test, $t = 11.125$, $df = 29.523$, $P < 0.001$). Angling selection or population did not significantly affect the level of post-stress plasma cortisol, although it showed a similar tendency as observed in $\dot{M}O_{2,ave}$ (Table 3, Fig. S3, available online).

Behavior in angling selection lines

Fish emerged from the start box during the recorded time in ~84% of the trials. There was a slightly non-significant interaction effect ($P = 0.054$) of population background and angling selection on latency (Table 4). This was observed as an elevated probability to emerge in fish from LV background compared to HV background in the hatchery population, but not in the wild population (Fig. 3A).

Fish were less active after acclimation in constant light compared to the 12:12 L:D photoperiod, but activity did not differ between populations or angling selection lines (Fig. 3B; Table 4). Angling selection had contrasting effects on exploration tendency in each population: in the hatchery population, a higher proportion of fish from LV selection line were explorative than from HV selection line, while there was an opposite tendency in the wild population (Fig. 3C; Table 4). In addition, exploration tendency increased with repeats of the behavioral trial. Sex did not have a significant effect on any behavior trait (female vs 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

Population-specific effects of angling selection on boldness

Captured and non-captured parent brown trout produced offspring that differed in boldness-related traits during their second summer. Surprisingly, boldness, measured as latency to explore a novel arena, was lower in the HV fish than in the LV fish in the hatchery population. This was a contradictory finding as angling is expected to select against boldness

(Arlinghaus et al. 2017). The wild population showed a weaker difference between selection lines, but its direction was more in line with the theory, with HV fish being bolder than LV fish. Activity during the behavioral trials was not affected by angling selection. In contrast, activity was affected by photoperiod, which suggests a connection between energy demand/expenditure and photoperiod through activity.

The unexpected result of angling-vulnerability related low boldness in hatchery population may be explained by the hatchery rearing environment through several indirect effects. First, the parent fish that were vulnerable to angling may have had the least strict preference for the standard feeds, as they accepted the colourful, unnatural fly patterns. Second, the vulnerable fish may have had the lowest status in the dominance hierarchy within the ponds, and therefore been the hungriest and likeliest to attack lures. Third, the hatchery-history itself, i.e. inadvertent domestication, might have selected for the most proactive individuals. This, in turn, could have shifted the vulnerability in this population towards more reactive fish that may have more flexibility in their behavior.

In contrast to the hatchery population, angling trials on wild parent fish were more representative of real angling situations in the field. The wild fish had natural invertebrate food available in their ponds, and the structured ponds offered more hiding places. The wild fish had clearly lower catchability than the hatchery fish, and the wild fish could only be captured when approaching the undisturbed pond from a distance. Very few wild fish were captured in one angling session (maximum 4) compared to the hatchery fish (maximum 11). The captured and non-captured parent fish did not show evident size-differences, indicating that the effects of angling were most likely mediated by size-independent traits.

Stress coping styles and angling selection

A possible consequence of angling selection is a change in the frequencies of not only bold and timid individuals, but also stress-tolerant and stress-sensitive individuals, due to the association of angling vulnerability, behavior and stress coping styles (Vindas et al. 2017a). Furthermore, the POLS theory suggests that stress coping styles, along with metabolic rate and other traits, are connected to life-history traits, which vary along a slow-fast continuum (e.g., (Dammhahn et al. 2018; Reale et al. 2010). Stress coping styles and POLS concepts therefore provide frameworks for testing the consequences of angling induced selection.

In largemouth bass, cortisol response to a standard stressor was negatively associated with capture probability, as expected (Louison et al. 2017). Koeck et al. (2018) found a weak negative effect (~0.5% change in risk) of high cortisol response on vulnerability to angling in a domestic strain of rainbow trout (*Oncorhynchus mykiss*), but there was no similar relationship in a wild strain of brown trout. In our study, stress sensitivity, expressed as a cortisol response to confinement, was not affected by angling-selection, although these tests suffered from low statistical power. However, a higher response in hatchery LV fish compared to HV was more visible through $\dot{M}O_{2,ave}$, which is notable given that confinement in the respirometer can induce a stress response in fish (Murray et al. 2017) and increase their oxygen uptake. Thus, the trends between HV and LV in the hatchery population observed in the cortisol response and $\dot{M}O_{2,ave}$ suggest potential for angling selection for increased stress sensitivity, which might become more visible after multiple generations of selection. These results suggest that the individuals of LV selection line within the hatchery population showed a more reactive stress coping style than HV line. Differences in coping styles could also partly explain why the behavior interpreted as boldness showed a pattern contradicting our expectations; if the LV fish were more reactive compared to the HV fish, their behavior in the personality trial may have indicated a higher stress response to the experiment and heightened escape behavior (Laskowski et al. 2016). The stress test and $\dot{M}O_{2,ave}$ results are in agreement with the hypothesis that the wild population displayed a more reactive coping style compared to the hatchery population.

Whether $\dot{M}O_{2,min}$ is connected to the coping styles/POLS's or angling selection remains inconclusive based on our results. A lack of association between metabolic rate and personality has been reported previously in other species, such as the Trinidadian guppy (*Poecilia reticulata*) (White et al. 2016). We found a trend of higher $\dot{M}O_{2,min}$ in the hatchery LV line compared to HV line, and an opposite trend in wild fish, under 12:12 L:D photoperiod. The contrast between the populations in the response to angling selection is therefore in line with the behavioral results in latency and exploration tendency, but also with activity in the response to photoperiod, although there was no correlation between activity and $\dot{M}O_{2,min}$ after accounting for group differences. Again, as in boldness, the result in hatchery population was opposite to the prevailing theory, and it is possible that stronger effects from angling could be observed over several generations of selection in natural conditions. Growth rate is unlikely to explain the differences in $\dot{M}O_{2,min}$ between groups, as the body mass of fish at the end of the experiment did not differ between groups (Table 1). Despite not directly addressing questions on trait covariances, as physiological traits were measured only once (Mathot and Frankenhuis 2018; Niemelä and Dingemanse 2018), our results add to the literature to promote the understanding of evolution in traits due to angling induced selection. From an angling selection perspective, some of the most interesting traits to include in further experiments would be neurochemical stress responses and their links to the bold-shy behavioral axis, and a role for metabolic rate remains to be understood better in connection to these traits.

Genetic and parental effects in population divergence and angling selection

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

Potential effects of photoperiod on energy balance

We incorporated environmental variation in our study as two different photoperiods. The results demonstrate, on one hand, that metabolic rate and swimming activity are sensitive to photoperiod, and on the other hand, that the other behavioral traits lack this sensitivity. Constant light is not encountered by brown trout during the winter months; hence the 24-hour light regime could be considered unnatural and potentially stressful for the fish. Constant light can disrupt entrainment of endogenous rhythms by inhibiting the synthesis of melatonin and by directly affecting photosensitive proteins (Falcón et al. 2010; Peirson et al. 2009). Based on our results, constant light had an inhibiting effect on fish swimming activity, and also decreased $\dot{M}O_{2,min}$ in the wild population, indicating that energy metabolism in brown trout can be affected by (an unnatural) photoperiod. In general, non-tropical species are expected to be particularly sensitive to photoperiod disturbances due to the role of day length in anticipating seasonal changes in environmental conditions (Borniger et al. 2017).

Innate vs learned antipredatory responses

Our goal was to study risk-taking behavior/boldness of offspring by subjecting fish to the olfactory cues of a natural predator that had fed on conspecifics. Wild brown trout typically increase the use of refuges under predation threat, while hatchery brown trout do not (Álvarez and Nicieza 2003). None of the individuals in the behavior trials in this study had been exposed to predators before the trials apart from potential traces of piscivore odors in the rearing water. The scarcity of responses to the presence of predator odor, measured in the offspring of wild fish, suggests only weak innate responses. Nevertheless, the tendency for lower activity in the presence of burbot than in control conditions resembles previously shown antipredator responses in fish (Álvarez and Nicieza 2003; Kopack et al. 2015).

Conclusions

Our results demonstrate the potential for rapid fishing-induced evolution in a very popular target species. The effects of angling selection are somewhat contradictory between wild and hatchery populations of fish, which leads to new questions on the mechanisms behind the observed differences. For practical fisheries management, the results with the wild fish are more representative and generalizable and showed little evidence of selection in physiological or behavioral traits. Overall, our study supports earlier findings according to which angling may be a potentially significant driver of evolution in behavioral and physiological traits in harvested 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.

Conflict of Interest

The authors declare that they have no conflict of interest.

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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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Tables

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.

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

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

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.

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

	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
	Log₁₀ 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 × log₁₀ body mass	**-0.51 ± 0.21**	**1**	**70**	**5.65**	**0.020**
	Random effects	**Variance (SD^2)**				
	Chamber	0				
	Residual	0.07^2				

	Fixed effects	**Estimate ± SE**	**Num df**	**Res / Den df**	**F**	**P**
$Log_{10}(\dot{M}O_{2,ave})$ (LMM)	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
	Log₁₀ 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 × log₁₀ 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				

	Fixed effects	**Estimate ± SE**	**Sum sq.**	**Df**	**Test statistic**	**P**
Post-stress cortisol (LM)	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 behaviour 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.

	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			

Table 5. Results of models for activity, latency and exploration tendency in the presence of predatory olfactory cues and control conditions in brown trout. For activity, the t-test was used with Satterthwaite approximations to degrees of freedom. The model was fit with restricted maximum likelihood. For latency, proportional hazard estimates (± standard error) are shown with hazard ratios (e^{coef}). For latency and exploration tendency, Wald Chisquare test was used to determine significance of fixed effects. The zero levels for contrasts in all models were: treatment control and selection line HV. Significant effects ($P < 0.05$) shown in 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	$(1.847 \times 10^{-5})^2$

Figure Legends

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

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

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

4). In B and C, predictions were made for the first trial repeat. For N in each group, see Table 1.

872 Fig. 1.

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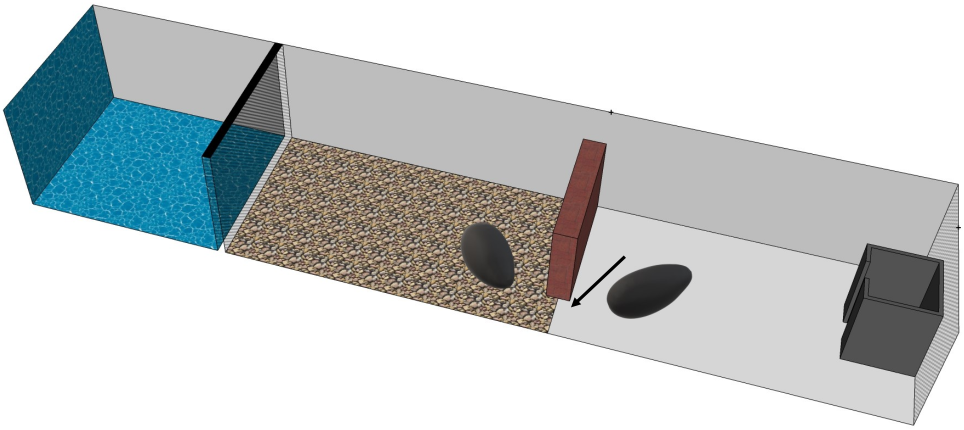

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875 Fig. 2.

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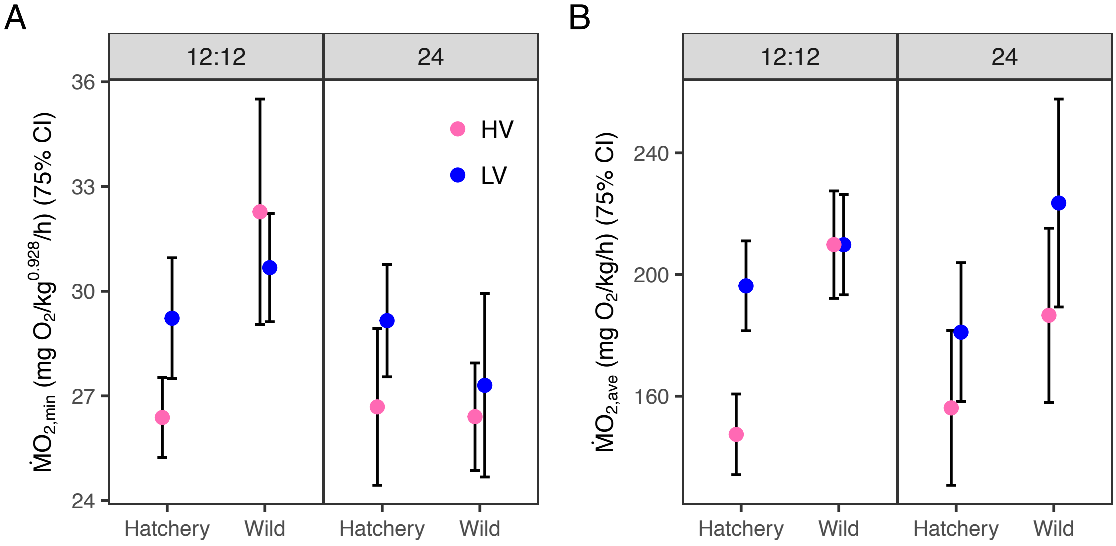

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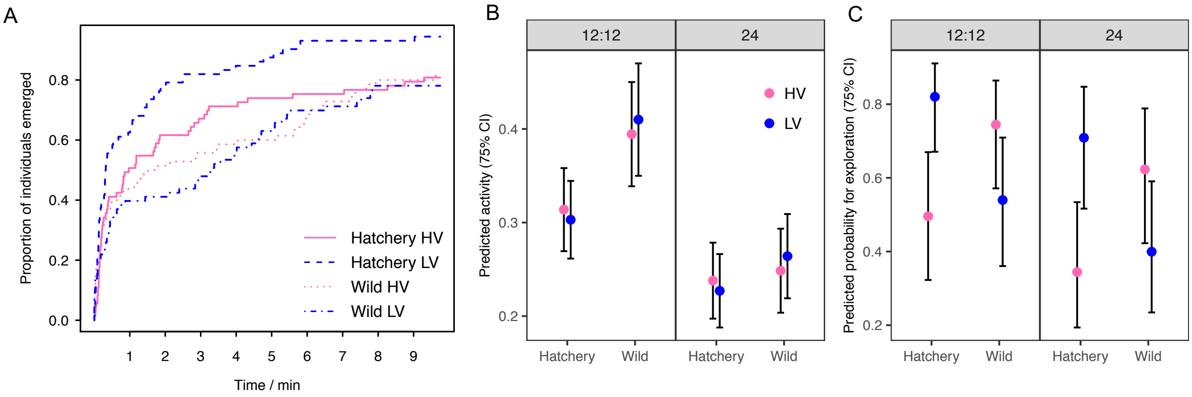
Fig. 3.


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
Predicted activity (75% CI)
12:12
24
0.2
0.3
0.4
HV
LV
Hatchery
Wild
Hatchery
Wild
C
Predicted probability for exploration (75% CI)
12:12
24
0.2
0.4
0.6
0.8
Hatchery
Wild
Hatchery
Wild