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# Do Metabolic Traits, Vulnerability to Angling, or Capture Method Explain Boldness Variation in Eurasian Perch?

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## ABSTRACT

The pace-of-life syndrome (POLS) concept predicts that individuals with high baseline metabolic rates demonstrate high boldness, aggressiveness, and activity, especially in food acquisition, with associated relatively greater energy requirements. In fishes, these behaviors may increase individual vulnerability to angling. To test the predictions of the POLS concept, we quantified individual standard metabolic rate (SMR) and boldness in both wild-caught and hatchery-reared Eurasian perch (*Perca fluviatilis*). We found both SMR and boldness to be repeatable traits but detected no correlation between them. Individual vulnerability to angling was assessed in the hatchery-reared perch, but we found no difference in boldness or SMR between vulnerable and nonvulnerable perch. Wild-caught perch were ice fished using either natural or artificial bait, and we observed no differences in boldness or SMR with respect to bait type or capture order. Our findings do not support the predictions of the POLS concept and, consistent with earlier studies in perch, suggest that angling may not drive selection against boldness in this species.

**Keywords:** personality, standard metabolic rate, thyroxine, growth rate, recreational fishing.

## Introduction

Individuals demonstrate consistent differences in behavior (personalities) that govern how they respond to external stimuli across time and context (Conrad et al. 2011; Mather and Logue 2013; Van Oers and Naguib 2013). Certain aspects of animal personalities (i.e., personality traits) are at least partly heritable (Van Oers and Sinn 2013; Kortet et al. 2014). Personality traits may be associated with both physiological and life-history traits (Nakayama et al. 2017) due to intrinsic and extrinsic factors, as predicted by the pace-of-life syndrome (POLS) concept (Réale et al. 2010).

The POLS concept can be used to derive hypotheses on how key traits of physiology (e.g., metabolic rate), personality (e.g., boldness or activity), and life history (e.g., age at maturation or growth rate) are expected to correlate among individuals within populations (Ricklefs and Wikelski 2002; Careau et al. 2008; Réale et al. 2010). Because individuals with higher metabolic rates have increased energy demands, higher food-intake rates are a prerequisite to achieving equal fitness and somatic growth rates with individuals of low metabolic rate (Cooke et al. 2007; but see Van Leeuwen et al. 2011). Higher food-intake rates in turn often involve increased behavioral activity and boldness or risk-taking in food acquisition (Biro and Stamps 2010), which are expected to increase predation risk and create growth/mortality trade-offs (Mangel and Stamps 2001; Stamps 2007; Biro and Sampson 2015).

If certain fish personality traits, such as greater boldness in foraging, increase vulnerability to capture (Lewin et al. 2006; Cooke et al. 2007, 2017; Härkönen et al. 2014; but see Vainikka et al. 2016), fishing may induce evolution of those and any other covarying traits, regardless of whether these syndromes are physiologically or genetically driven (Biro and Post 2008; Uusi-Heikkilä et al. 2008). For example, high vulnerability to angling may be coupled with high metabolic rates in largemouth bass (*Micropterus salmoides*; Cooke et al. 2007; Redpath et al. 2010; see also Hessenauer et al. 2015). The possible linkage between individual metabolic rate, personality, and life-history productivity (Yamamoto et al. 1998; Careau et al. 2008; Biro and Stamps 2010) may have severe microevolutionary implications for the productivity of harvested fish stocks, highlighting the need for further examination of their relationships (Hutchings and Fraser 2008).

Standard metabolic rate (SMR) represents the minimum energetic demand of an ectotherm to maintain vital processes (e.g., Gupta and Thapliyal 1991) and is usually quantified via oxygen consumption (e.g., Metcalfe et al. 1995; McCarthy 2000; Seppänen et al. 2009). In fishes, thyroid hormones (THs; mainly thyroxine T4 and triiodothyronine T3) primarily affect oxygen consump-

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tion and lipid, carbohydrate, and protein metabolism in tissues (McCormick 2011). Moreover, they affect somatic growth (Higgs et al. 1982; Sheridan 2011), early development, and metamorphosis (Dickhoff et al. 1978; Power et al. 2001), as well as sexual maturation and reproduction (Hulbert 2000; Squires 2003; Peter 2011). Prior studies in ectotherms have suggested a modulatory effect of T4 on SMR (e.g., John-Alder 1990; reviewed in Gupta and Thapliyal 1991) and even on behavior, with T4 administration being associated with higher running endurance (John-Alder 1991) and excitability (Steinberg et al. 1993) in lizards.

For this work, Eurasian perch (*Perca fluviatilis* L., hereafter perch) was chosen as the model species, as it is fished both recreationally and commercially in Europe. Perch have also been used as a model species in several comparative studies of life-history variation and behavior (e.g., Westerberg et al. 2004; Heibo and Magnhagen 2005; Vainikka et al. 2005) and demonstrate consistent individual differences in several traits (Magnhagen and Bunnefeld 2009; Kekäläinen et al. 2014; Härkönen et al. 2016).

Both wild-caught and hatchery-reared perch were used to address three questions regarding the relationship between physiology and behavior: (1) Do individual differences in SMR explain variation in boldness? (2) Does vulnerability to angling explain variation in boldness or SMR in hatchery-reared perch? (3) Do wild perch captured with natural versus artificial baits differ in individual boldness, SMR, or plasma concentrations of T4? Plasma T4 concentration was selected to complement SMR measurements and obtain a broader picture of individual metabolism (Hulbert 2000) in relation to behavioral characteristics.

## Methods

### Wild Perch

Wild perch were ice fished from Lake Pohjalampi (62°40'N, 29°33'E) in Liperi, eastern Finland. The lake (61 ha; mean depth: 3.2 m; maximum depth: 5.3 m) has an abundant perch population, with northern pike (*Esox lucius*) as the main predator (Leppä et al. 2003). To avoid possible sampling bias (e.g., induced by weather conditions; Bégout Anras and Lagardère 1998), perch were collected on three occasions between 0900 and 1500 hours in 2013: on February 6 ( $n = 40$ ), February 14 ( $n = 12$ ), and March 21 ( $n = 30$ ). Ice fishing locations were chosen by drilling holes along the littoral slopes of the lake until a perch shoal was encountered (the method is further described in Kekäläinen et al. 2014).

With the aim of capturing perch with high variation in behavioral traits related to angling vulnerability (Härkönen et al. 2016), fish were captured using two distinct fishing gears: (1) an unbaited copper/silver-colored artificial spoon lure (ViM 34 mm; ViM Pirken, Växjö, Sweden) with a brightly colored treble hook (size 14 Mustad) and (2) a small, inconspicuous, copper-covered mormyshka hook (i.e., a tear-shaped, 4-mm-diam. tungsten lure attached to a size 14 single hook) with natural bait consisting of two to four live bloodworms (chironomid larvae; see also Härkönen et al. 2016). The artificial lure, used with active vertical stimulation (jigging), was attached to a rigid rod with a 0.16-mm fishing line (Stroft GTM, Reinfeld, Germany)—whereas the

natural bait was attached to a rod with a sensitive indicator tip with a 0.10-mm fishing line (Trabucco Super Elite T1 Tournament, Parma, Italy) and held stationary or stimulated only moderately. Both capture methods were used on all capture dates and locations (but at independent ice holes) to minimize day bias and spatial variation between capture methods.

Following each capture, the perch were anesthetized in a 10-L plastic pail using a 60-mg L<sup>-1</sup> concentration of clove oil (Oy Anders Meder Ab, Helsinki, Finland)/ethanol solution (with 1:9 clove oil-ethanol; Hoskonen and Pirhonen 2004). The perch were tagged under anesthesia with passive integrated transponders (PIT tags: 7 mm × 1.35 mm FDX-B PIT tag; Lolligo Systems, Tjele, Denmark) inserted under the skin next to the dorsal fin using a syringe-style implanter and needle. The perch were transferred in a water-filled 50-L cooler to 500-L laboratory tanks at the University of Eastern Finland (UEF), Joensuu campus. The perch were allowed to acclimate for at least 4 d before respirometry.

### Hatchery Perch

The parental population (total  $\bar{L} = 170 \pm 19$  [SD] mm and wet  $\bar{M} = 42.8 \pm 19.3$  [SD] g;  $n = 48$ ) of the hatchery-reared perch was captured by ice fishing from Lake Kivesjärvi in April 2010 and brought to Kainuu Fisheries Research Station of the Natural Resources Institute Finland (Luke; formerly Finnish Game and Fisheries Research Institute). The perch were allowed to spawn in 3.2-m<sup>2</sup> indoor fiberglass tanks provided with rice-root spawning beds. After spawning, the beds were transferred to indoor flow-through flumes to incubate until hatching. The hatched fry were moved outdoors into two seminatural 400-m<sup>2</sup> ponds to feed on natural food consisting of benthos and plankton provided by water flow from nearby Lake Kivesjärvi.

At age 3, the perch from one of the ponds were assigned to two groups based on their vulnerability (or lack thereof) to angling under ice cover and open water conditions. The perch were ice fished between April 8 and 11, 2013, using the same two capture methods (artificial lure or natural bait) described above. Captured perch (i.e., vulnerable to angling) were anesthetized, individually PIT tagged, and returned to the pond.

After the ice cover had melted, on May 19 and 20, 2013, the perch were angled with a standard telescopic fishing rod (5 m), line, sink, and hook (size 14) with a blowfly (Calliphoridae) larva (replaced every 5 min) as bait. The pond was angled for 15 min from each of the four sides, followed by a minimum 1-h break before repeating the angling process. Again, the captured perch (vulnerable to angling) were PIT tagged if they had not already been and returned to the pond for possible recapture. After the angling experiments, the pond was drained and all the perch were collected with dip nets. The fish were subsequently transferred to the laboratory at UEF Joensuu campus in water-filled 50-L coolers. After an acclimation period of 3 d, all the untagged (i.e., uncaptured) perch considered nonvulnerable to angling were anesthetized and PIT tagged. After tagging, the hatchery perch were left undisturbed for 48 h.

Further experiments were carried out separately for the wild-caught and hatchery-reared perch groups due to the presence of ice cover and to keep laboratory confinement time minimal. All fish within groups were held in one 500-L tank filled with unchlorinated tap water before respirometry. The fish were maintained at  $8.0^\circ \pm 0.3^\circ\text{C}$  (mean  $\pm$  SD) using a cooling unit and fed with freeze-dried bloodworms or food flakes (NovoFil/NovoBel; JBL, Neuhofen, Germany) once a week. Light rhythm was set to 12L:12D from 0700 to 1900 hours.

### Respirometry

SMR of the perch was measured before behavioral trials, as suggested by Biro and Stamps (2010). We used an automated intermittent-flow respirometry system (DAQ-PAC-F4; Loligo Systems) with fiber-optic sensors to record oxygen consumption in three parallel transparent acrylic chambers with volumes of 295–297 or 825–830 mL (for fish with a body mass greater than 23 g). For respirometry, individual perch were haphazardly dip netted from the tank, and after removing excess water with a paper towel, fish body mass was measured in a water-filled container as wet mass. Handling time and air exposure were kept minimal, as the fish were transferred to the respirometry chambers under water.

The perch were fasted for 48 h and temperature acclimated to  $8^\circ\text{C}$  for at least 4 d after transport before the SMR measurements, which were started on work days at 0800 hours. Oxygen consumption was measured at  $8.0^\circ \pm 0.1^\circ\text{C}$ . Water temperature was kept constant by a water bath with a cooling unit and an immersion thermostat (Lauda T). Oxygen consumption measurement was started as each fish was placed in a chamber (Seppänen et al. 2009). Measurements were taken over a 24-h period (Voutilainen et al. 2011), using a 25-min loop consisting of 5- and 10-min flush and wait periods, respectively, and a 10-min measuring period. Flow rate was approximately one chamber volume  $\text{min}^{-1}$ . The respirometry data were saved using AutoResp software (Loligo Systems). The oxygen consumption rate of 13 perch was measured twice within 6–32 d to estimate the repeatability of SMR.

SMR ( $\text{mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) was calculated from the slope of linear regression on oxygen depletion (percent air saturation  $\text{h}^{-1}$ ) during the 10-min measuring period, with regression line selection being based on the highest  $R^2$  value when fitting on an 80% span of each  $\text{O}_2$ -measuring period. Measuring periods with a fitted regression curve  $R^2$  value greater than 0.8 were accepted, and for the 24-h period, data from chambers only with at least 25 accepted loops were included. From the five measurement periods with the lowest  $\text{O}_2$ -depletion levels, the average of the three highest (to mitigate the effect of possible measurement error) was used to calculate SMR for each individual (Voutilainen et al. 2011). Microbial respiration was measured from empty chambers before and after SMR measurement of each fish, and the average of these two was subtracted from the total oxygen consumption of each chamber. From the total oxygen consumption, the mass-specific SMR was calculated using the equation

$$\text{SMR} = \frac{\text{DO} \times V_{\text{CH}}}{\text{SO}_2} \cdot \frac{(m - m_{\text{MICR}})}{M_{\text{F}}},$$

where DO = amount of dissolved oxygen in ambient water ( $\text{mg L}^{-1}$ ),  $V_{\text{CH}}$  = chamber volume (L),  $\text{SO}_2$  = air saturation (100%),  $m$  = decline in  $\text{O}_2$  concentration (percent air saturation  $\text{h}^{-1}$ ),  $m_{\text{MICR}}$  = microbial respiration (percent air saturation  $\text{h}^{-1}$ ), and  $M_{\text{F}}$  = fish mass (g). Following metabolic measurements, test perch were moved into a second 500-L holding tank. The correlation between the days spent in the laboratory before respirometry and mass-corrected SMR (rSMR) was estimated for both groups using Spearman's  $\rho$ .

### Boldness Trials

To assess boldness, novel arena tests were carried out after respirometry (minimum one night) in a rectangular plastic arena (fig. 1) filled with 22 L of water (cf. Budaev and Brown 2011; Kortet et al. 2014). At one end of the arena there was a “start box,” where each perch was placed for a 5-min acclimation before the behavioral trial, which was started by lifting a hatch opening to the arena. At the opposite end there was a mirror, and the midway was indicated by a marker line. Glued to the bottom was a standardized shelter composed of a plastic plant with a small rock on each side (fig. 1).

The boldness trials were conducted in  $8^\circ\text{C}$  water under dim lighting with an infrared LED light and a web camera placed over the arena: captive perch demonstrate greater degrees of activity in low-light conditions, despite being a visual predator (Vainikka et al. 2016). During each trial, we recorded (1) time to swim out of the start box, (2) time to reach shelter, (3) time to reach the midway line, and (4) time to reach the mirror. The timing of the four behavioral events was measured using AV Bio-Statistics software (v. 4.9 by A. Vainikka).

The trials, taking place between 0900 and 1500 hours, lasted for a maximum of 20 min and ended when the focal fish reached the mirror. In the absence of activity, the maximum time (20 min) was assigned for a behavioral event. Two trials were carried out for each perch, with a minimum of one and a maximum of 18 nights between the trials in the wild perch and two or three nights in the hatchery perch, as Vainikka et al. (2016) found that the repeatability of behavior in perch was independent of the time between the behavioral trials. The boldness trials for the wild-caught perch were conducted between February 28 and April 17, 2013, and for the hatchery-reared perch were conducted between May 27 and July 17, 2013.

### Plasma T4 and Fish Age Determination in the Wild-Caught Perch

T4 concentration was measured in wild-caught perch. On the day of the last boldness trial for a given fish (between 1600 and 1800 hours; Loter et al. 2007), a blood sample was drawn with a heparinized syringe from the caudal vein under anesthesia (via clove oil, as above). Plasma was separated via 10-min centrifugation at 14,000 rpm (20,000 g, Sigma 201M; Bioblock Sci-

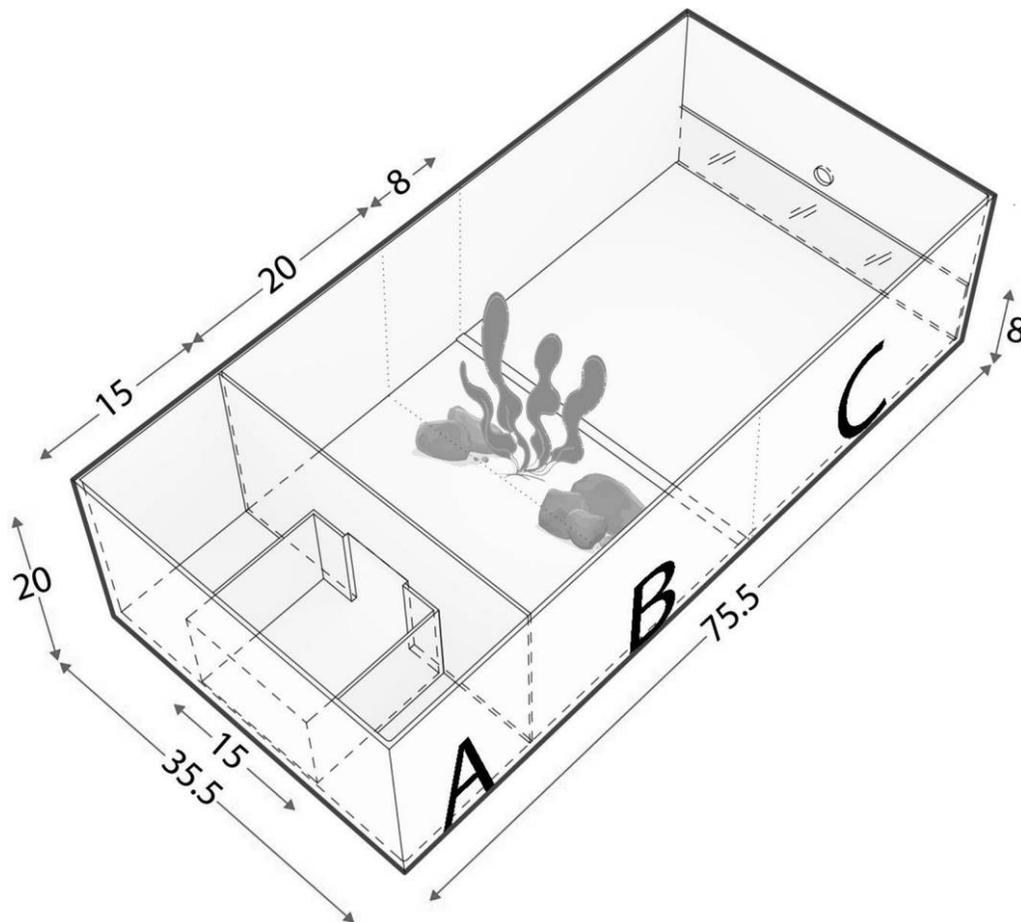


Figure 1. Behavioral trial arena for quantifying individual Eurasian perch boldness. The four measuring points for behavioral time variables are shown, starting from the left: (1) exiting the start box, (2) reaching shelter (vegetation and rocks), (3) reaching the midarena line, and (4) reaching the mirror at the far end. The inner length of section C is 28 cm. A color version of this figure is available online.

entific, Illkirch-Graffenstaden, France), and samples were stored at  $-80^{\circ}\text{C}$  until analysis. Finally, the perch were measured for wet mass and length and euthanized by cervical dislocation.

The total plasma concentration of T4 was determined from 10- $\mu\text{L}$  plasma samples in duplicates using a commercial solid-phase enzyme-linked immunosorbent assay (DRG Total T4 ELISA, EIA-4568; DRG Instruments, Marburg, Germany), following the manufacturer's instructions. We consulted with an expert about the suitability of the assay for fish plasma. The detection limit of the assay was 8  $\text{nmol L}^{-1}$ , and the intra-assay coefficient of variation was 9.3% ( $n = 27$ ). Absorbance was measured at 450 nm with a microplate reader (FLUOstar Omega; BMG LABTECH, Ortenberg, Germany). T4 concentrations were determined based on four-parameter regression ( $R^2 > 0.99$ ) with the BMG LABTECH MARS data analysis software. The average concentration of the duplicates was used in statistical analyses.

For age determination, the opercula of the euthanized wild perch were removed and cleaned, and scale samples were taken below the lateral line between the two dorsal fins. Perch age was determined from the operculum under a binocular microscope—the more unambiguous method of the two—consulting

the pressed scale samples under a microfilm reader for support when necessary. Individual relative growth rates were subsequently calculated by subtracting individual fish total length from the average length of each year class and dividing the difference by the average length. The same procedure was used for the hatchery perch, both groups thus being directly comparable in this measure. This study was conducted in accordance with the Finnish legislation on animal experimentation and licensed by the National Animal Experiment Board of Finland (license ESAVI/1906/04.10.03/2012).

#### Statistical Methods

Histogram-based graphical assessment, the Kolmogorov-Smirnov Z-test, and Q-Q plots were used to evaluate the distributions of variables. Normality transformations were deemed unnecessary. Unless stated otherwise, all statistical tests were performed in SPSS for Windows (v. 25; IBM, Armonk, NY).

Accounting for mass dependence in metabolic rate studies is generally advisable (McCarthy 2000), especially when focusing on individual differences, due to the power-law dependence of

metabolic rate on body size (Kleiber's law). In our study, mass dependence in SMR was removed by calculating the residuals from the linear regression of  $\log_{10}$ -transformed SMR ( $\text{mg O}_2 \text{ h}^{-1}$ ) on  $\log_{10}$ -transformed fresh mass (g; McCarthy 2000; Seppänen et al. 2009). The residual values (i.e., rSMR) were used to represent metabolic rate. For subsequent analyses, the rSMR values were calculated both across perch groups and separately for the wild-caught and hatchery-reared perch groups, and the appropriate rSMR was used depending on the statistical model. Repeatabilities of SMR and body mass (Maciak and Konarzewski 2010) were evaluated using the intraclass correlation coefficient (ICC)  $r$  (Lessells and Boag 1987; McCarthy 2000) using AV Bio-Statistics.

Principal component (PC) analysis (PCA) was used to reduce the four behavioral time variables into a single component. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were used to estimate the suitability of the data for PCA. Since the results of the PCA were very similar for both wild-caught and hatchery-reared perch, if tested separately (in both analyses, PC loadings for all of the four behavioral variables were above 0.9 and one component was extracted with an eigenvalue of 3.7), the perch groups were combined.

Based on a histogram and a Q-Q plot, the distribution of the regression factor scores derived from the PCA came nearest to the gamma distribution. To achieve a better fit, the scores were first ranked starting from the highest value (to represent boldness instead of shyness) using fractional ranking (Templeton 2011). The ranked values were then transformed to follow gamma distribution (positive, nonzero) by using the inverse gamma function in SPSS ( $\alpha = 2, \beta = 1$ ; applying Templeton's [2011] method to gamma distribution).

The resulting values (boldness score) thus described individual boldness, so that larger values were associated with higher boldness. The boldness scores were calculated (starting from PCA) both across perch groups and separately for the wild-caught and hatchery-reared perch groups. The boldness scores were also calculated separately for the active perch only (excluding perch that did not leave the start box in either of the two boldness trials) for all three groups (active wild, hatchery, both). Since the PCA factor scores for active perch remained non-normally distributed, the same gamma transformation was used, facilitating comparison between different models. Depending on the statistical model in question, the appropriate boldness score was used as the response variable.

The repeatability of boldness was assessed following the procedure of Nakagawa (2017) for estimating the ICC for gamma-distributed data ( $\text{ICC}_{\text{GLMM}}$ ) in a generalized linear mixed model (GLMM) with a log link function. The boldness score was set as the repeated response variable, perch group (wild vs. hatchery) was added as a fixed effect, and perch individual was added as a source of random effect. This full model was compared to a null model (with a random intercept but no fixed effect) using the Akaike information criterion (AIC) values.

Following the supplemental material in Nakagawa (2017), we used the `glmer` function for exploration models provided in the R package `lme4` (Bates et al. 2015) in the R environment

(RStudio 2016; R Development Core Team 2017) to estimate the adjusted  $\text{ICC}_{\text{GLMM}}$  with the recommended trigamma function. The corresponding  $R^2_{\text{GLMM}}$  values for marginal  $R^2$ , as variance explained by fixed effects ( $R^2_{\text{GLMM}(m)}$ ), and conditional  $R^2$ , as variance explained by both fixed and random effects ( $R^2_{\text{GLMM}(c)}$ ), were estimated following Nakagawa (2017).

To verify the repeatability of boldness to be independent of the time between the two boldness trials (Vainikka et al. 2016), correlation was estimated for the boldness score difference between the first and the second boldness trials and the number of days between the trials. This correlation was estimated as Spearman's  $\rho$  in the wild-caught perch group only, since in the hatchery-reared group all trial pairs were only 2 or 3 d apart.

### Statistical Models

Since the boldness score followed gamma distribution, GLMMs with gamma distribution and a log link function were used to study the effects of the measured predictors on the response variable boldness. Boldness was set as a repeated variable. Scaled identity was chosen as the covariance structure for the repeated variable, since variance was considered in effect constant between the two trials—as also suggested by the smallest AIC value between candidate structures (267, compared to 272 of the nearest covariance structure, compound symmetry) in the main model (GLMM I).

All models included an intercept—as well as a random intercept in the GLMMs, with perch individual (PIT number) specified as subject and source of random effect, to allow for individual variation across groups. Scaled identity was set as the covariance structure for the random effect, since there was only one random effect.

The fixed predictors selected for the main model, GLMM I, combining both perch groups (wild-caught and hatchery-reared perch) were growth rate and rSMR, as well as group and group interactions with the other predictors. To evaluate the effect of having inactive perch in the data, an alternative main model with the active perch (GLMM I<sub>A</sub>) was fitted with the exclusion of the perch that did not leave the shelter in either of the boldness trials. Group and rSMR were set as fixed predictors in the alternative model.

GLMM II was used to estimate the effect of vulnerability to angling (vulnerable or nonvulnerable) on boldness among the hatchery-reared perch. Fish length and the number of days spent in the laboratory before the first boldness trial (lab days) were included as fixed predictors. An alternative model (GLMM II<sub>A</sub>) excluding the inactive perch was fitted for comparison.

GLMM III was used to explain boldness variation in the wild-caught perch with the fixed predictors capture order and capture hole. In this model, the factorial effect of capture order was nested within the term capture hole—since capture order was dependent on capture hole. These were further nested within capture method—since only one method was used per capture hole—to determine whether there were differences in behavior between the two capture methods (Vainikka et al. 2012; Kekäläinen et al. 2014). The resulting nested terms were

thus capture order(capture hole[capture method]) and capture hole(capture method). Lab days (until the first boldness trial) was included as a fixed predictor. Again, an alternative model was fitted with the exclusion of the behaviorally inactive perch (GLMM III<sub>A</sub>).

The association between T4 concentration and boldness among the wild-caught perch group was tested in a separate GLMM IV, since the T4 sample size was limited ( $n = 27$ ), due to selection of only high-quality plasma samples for the T4 assay. Lab days as the total number of days spent in the laboratory was included in the model. Correlations between plasma T4 concentration, rSMR, and relative growth rate were estimated using Pearson's product-moment correlation coefficient (Pearson's  $r$ ).

A general linear model (GLM I) was used to explain rSMR variation among the hatchery-reared perch with vulnerability to angling, growth rate, and the number of days spent in the laboratory before respirometry (fixed predictors). For the wild-caught perch group, a second GLM II was used to explain rSMR variation with capture method, capture order, and capture hole. Again, the capture terms were nested in the GLM II, as in GLMM III.

GLM requirements were confirmed using Levene's test of equality of error variances and model fits using adjusted  $R^2$  values. GLMM fits were assessed graphically from observed versus model predicted plots, as well as residual plots (Pearson residual vs. predicted) and Q-Q plots. GLMM AIC values were used to find as parsimonious yet biologically justifiable a model as possible, and fitted model (with fixed effects) AIC values were compared to those of null models (random terms only). A significance level of 0.05 was used for all statistical tests, and all means are reported as mean  $\pm$  SD.

## Results

### Respirometry

The mass-specific SMR values varied between 17.2 and 43.4 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> in the hatchery perch ( $\overline{\text{SMR}} = 33.4 \pm 5.3[\text{SD}]$  mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>,  $n = 43$ ) and between 11.8 and 54.0 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> in the wild perch ( $\overline{\text{SMR}} = 29.3 \pm 8.9[\text{SD}]$  mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>,  $n = 44$ ). SMR was repeatable (ICC  $r = 0.52$ ,  $n = 13$ ,  $P = 0.025$ ), as was the corresponding body mass (ICC  $r = 0.98$ ,  $P < 0.001$ ). The linear regression equation describing the association between body mass and SMR was  $\log \text{SMR}(\text{mg O}_2 \text{ h}^{-1}) = 0.88 \cdot \log M_F - 1.35$ , with  $R^2 = 0.80$  for the wild-caught and hatchery-reared perch combined (wild:  $\log \text{SMR} = 0.933 \cdot \log M_F - 1.43$ , with  $R^2 = 0.78$ ; hatchery:  $\log \text{SMR} = 0.68 \cdot \log M_F - 1.08$ , with  $R^2 = 0.36$ ). Group-specific rSMR was used in further analyses when applicable.

SMR levels did not significantly differ between wild and hatchery perch in a GLM ( $F_{1,84} = 2.983$ ,  $n = 87$ ,  $P = 0.088$ ), in which mass was included as a covariate ( $F_{1,84} = 0.304$ ,  $P = 0.583$ ). There was no significant correlation between the days spent in the laboratory before respirometry and rSMR among the wild perch group (Spearman's  $\rho = 0.180$ ,  $n = 44$ ,  $P = 0.242$ ), but there was a negative correlation in the hatchery perch group ( $\rho = -0.354$ ,  $n = 43$ ,  $P < 0.05$ ).

### Boldness

The mean time delays for movement to different areas of the test arena for the wild-caught and hatchery-reared perch groups were relatively long (table 1). Forty-five out of 87 perch (52%) did not leave the start box in either of the two boldness trials. A

Table 1: Mean times (minutes  $\pm$  SD) for tracked behaviors in the boldness trials (total span: 20 min) of wild-caught perch caught with artificial lure or natural bait and hatchery-reared perch vulnerable or nonvulnerable to angling

Behavioral variable	Wild perch		Hatchery perch		PC loading
	Artificial lure	Natural bait	Vulnerable	Nonvulnerable	
N:					
All perch	21	23	8	35	
Active perch only	12	17	2	11	
Time out of start box:					
All perch	14.8 $\pm$ 5.9	13.5 $\pm$ 5.8	19.0 $\pm$ 1.9	17.2 $\pm$ 4.9	.94
Active perch only	10.8 $\pm$ 5.0	11.2 $\pm$ 5.1	15.9 $\pm$ .3	11.2 $\pm$ 4.8	.91
Time to shelter:					
All perch	15.9 $\pm$ 5.7	14.5 $\pm$ 6.0	20.0 $\pm$ 0	17.7 $\pm$ 4.4	.98
Active perch only	12.8 $\pm$ 5.9	12.5 $\pm$ 5.8	20.0 $\pm$ 0	12.6 $\pm$ 4.9	.97
Time to midarena:					
All perch	16.3 $\pm$ 5.4	15.5 $\pm$ 6.0	20.0 $\pm$ 0	17.7 $\pm$ 4.1	.98
Active perch only	13.6 $\pm$ 5.8	13.9 $\pm$ 6.3	20.0 $\pm$ 0	13.2 $\pm$ 4.8	.97
Time to mirror:					
All perch	16.5 $\pm$ 5.3	15.6 $\pm$ 6.0	20.0 $\pm$ 0	18.1 $\pm$ 3.9	.97
Active perch only	13.8 $\pm$ 5.7	14.1 $\pm$ 6.2	20.0 $\pm$ 0	14.2 $\pm$ 5.0	.95

Note. The corresponding factor loadings in principal component (PC) analysis are shown. Mean times of two trials are shown both for all perch (total trial span was assigned for lack of action for a behavior) and for active perch only (excluding the perch that did not show any activity in the behavioral arena during the trial in either of the two trials).

Table 2: Generalized linear mixed model results on gamma-transformed boldness variation in pooled data of wild-caught and hatchery-reared perch

Explanatory variable	<i>F</i>	df numerator	df denominator	$\beta$	SE	<i>P</i>
Intercept				.412	.349	<.005
Group	9.265	1	162	.324	.106	<.005
rSMR	1.135	1	162	.736	1.091	.288
Growth rate	.258	1	162	2.135	1.786	.612
Group $\times$ growth rate	2.903	1	162	-3.288	1.930	.090
Group $\times$ rSMR	.014	1	162	-.148	1.243	.905

Note. The fixed effects and coefficients for boldness (repeated response variable) are shown ( $n = 84$ ). Group estimates are for wild perch, with hatchery perch as the reference. rSMR = mass-corrected standard metabolic rate.

high KMO measure (0.804) and a statistically significant Bartlett's test ( $P < 0.001$ ) allowed us to perform the PCA. The PC extracted from the PCA explained 93.3% of the variation in the behavioral time variables, with an eigenvalue of 3.74 (table 1).

According to the GLMM for estimating the repeatability of the boldness scores between the two trials, the behavior of the perch individuals was repeatable ( $ICC_{GLMM} = 0.539$ ). The full model with group as a fixed effect had a lower AIC value than the null model (343.7 vs. 369.8, respectively), making it preferable. The corresponding  $R^2_{GLMM}$  values were  $R^2_{GLMM(m)} = 0.35$  and  $R^2_{GLMM(c)} = 0.70$  (see "Methods"). The estimate for the effect of group was significant in the model ( $F_{1,170} = 61.73$ ,  $\beta_w$  for wild group = 0.810, SE = 0.132,  $n = 87$ ,  $P < 0.005$ ).

The repeatability of boldness was indicated to be independent of the time between the trials by the lack of correlation between the difference between the two boldness scores and the

number of days between the two trials (Spearman's  $\rho = 0.045$ ,  $n = 44$ ,  $P = 0.771$ ) in the wild-caught group.

#### Explaining Boldness Variation across Perch Groups

According to the main model (GLMM I), rSMR did not explain boldness variation across the two perch groups (table 2; fig. 2). Growth rate did not explain boldness either, but there was a significant difference in boldness between the two perch groups (table 2; fig. 2). The mean boldness across both trials was higher in the wild-caught perch ( $2.415 \pm 1.961$ ,  $n = 88$ ) than in the hatchery-reared perch ( $1.664 \pm 0.927$ ,  $n = 86$ ).

A possible seasonal effect could not be separated from group differences, since boldness was measured in different months for the two groups. However, the difference in mean boldness between hatchery-reared and wild-caught perch was most likely

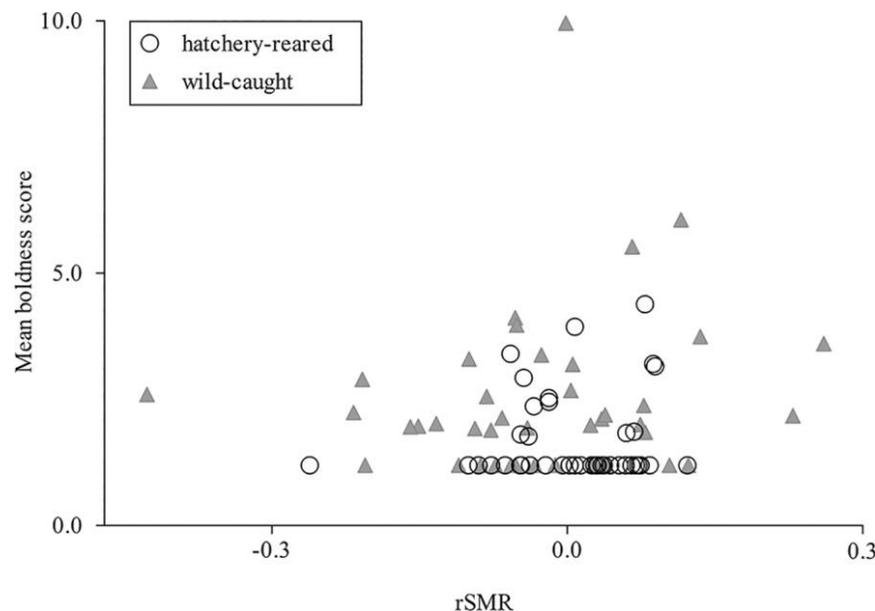


Figure 2. Mass-corrected standard metabolic rate (rSMR) did not explain mean boldness variation (gamma transformed) of two boldness trials in two groups of Eurasian perch (Spearman's  $\rho = 0.037$ ,  $n = 87$ ,  $P = 0.733$ ). On average, the wild-caught perch were bolder than the hatchery-reared ones, however. All measurements were performed in 8°C unchlorinated tap water. A color version of this figure is available online.

due to the greater number of inactive perch that did not leave the start box to enter the arena (in either of the boldness trials) among the hatchery-reared perch (30 [70%] vs. 15 [34%]; table 1; fig. 2). This interpretation was supported by the alternative model (GLMM I<sub>A</sub>)—from which all behaviorally inactive perch were excluded—showing no differences between groups in boldness ( $F_{1,80} = 1.041, \beta_W = 0.254, SE = 0.248, n = 42, P = 0.311$ ). The rSMR did not explain boldness without the inactive perch either ( $F_{1,80} = 1.927, \beta_W = 1.343, SE = 0.968, P = 0.169$ ).

#### *Differences in Hatchery-Reared Perch Vulnerable or Nonvulnerable to Angling*

Out of the 64 hatchery-reared perch measured ( $\bar{M} = 16.9 \pm 2.4$  g,  $\bar{L} = 125 \pm 6$  mm), 11 (17%) had previously been captured by ice fishing or rod angling and thus classified as vulnerable to angling. Length did not statistically significantly differ between vulnerable and nonvulnerable perch groups (vulnerable [v]:  $\bar{L} = 125.7 \pm 5$  mm; nonvulnerable [nv]:  $\bar{L} = 124.6 \pm 6$  mm; ANOVA:  $F_{1,62} = 0.309, n = 64, P = 0.580$ ), nor did relative growth rate (v mean =  $0.008 \pm 0.043$ ; nv mean =  $-0.002 \pm 0.047$ ; ANOVA:  $F_{1,62} = 0.415, P = 0.522$ ).

Variation in boldness among the hatchery-reared perch, according to the GLMM II, was not explained by vulnerability to angling ( $F_{1,83} = 3.878, n = 8v + 35nv = 43, \beta$  for v =  $-0.235, SE = 0.120, P = 0.052$ ) or fish length ( $F_{1,83} = 1.348, \beta = 0.014, SE = 0.012, P = 0.249$ ). The number of days spent in the laboratory (before the first boldness trial; range = 7–27 d) was nonsignificant in the original model ( $F_{1,82} = 1.204, \beta = -0.017, SE = 0.016, P = 0.276$ ) and was excluded from the final model based on AIC. The alternative model (GLMM II<sub>A</sub>), including the active hatchery perch only, with vulnerability to angling as the only predictor, showed very similar results ( $F_{1,24} = 3.266, n = 2v + 11nv = 13, \beta = -0.680, SE = 0.376, P = 0.098$ ).

Neither vulnerability to angling ( $F_{1,39} = 0.071, n = 8v + 35nv = 43, P = 0.424$ ) nor growth rate ( $F_{1,39} = 0.003, P = 0.955$ ) explained rSMR variation in the hatchery-reared perch, according to the GLM I ( $R^2_{\text{adj}} = 0.068$ , lack of fit test,  $P = 0.229$ ). However, time spent in the laboratory before respirometry was negatively associated with the level of metabolism in the model ( $F_{1,43} = 5.682, \beta = -0.004, SE = 0.002, P < 0.05$ ).

#### *Differences of Wild-Caught Perch Captured with Artificial versus Natural Baits*

Of the 83 wild-caught perch ( $\bar{M} = 26.6 \pm 15.2$  g,  $\bar{L} = 138.6 \pm 23.1$  mm), 41 (49%) were caught using the artificial lure and 42 (51%) were caught using the natural bait from a total of 28 capture holes, with an average of three perch per hole ( $\pm 3.2$ ; range = 1–12). The age of the perch varied between 3 and 8 yr (mean =  $5.2 \pm 1$  yr,  $n = 50$ ).

Capture order, capture hole, or capture method did not explain boldness variation among these fish (table 3; GLMM III). Capture order had a significant coefficient in only one of the capture holes ( $\beta = 1.786, SE = 0.872, P < 0.05$ ), in which the boldest of all the wild-caught perch was captured first in order. The number of days spent in the laboratory (before the first boldness trial; range = 6–50 d) was barely nonsignificant in the model (table 3), suggesting that there might have been a weak association ( $\beta = 0.027 \pm 0.053$ [SE]) between laboratory confinement time and increased boldness in the wild perch, possibly due to acclimation to laboratory conditions.

If the inactive perch were excluded from the model (GLMM III<sub>A</sub>,  $n = 29$ ), the only capture hole, in which capture order was significant in the original model, turned nonsignificant ( $\beta = 1.535, SE = 1.218, P = 0.021$ ), while in other respects the model results remained very comparable to GLMM III and are not reported in detail.

In the GLM II for rSMR, none of the predictors explained rSMR: the nested terms capture order(capture hole[capture method]) ( $F_{7,38} = 0.61, n = 42, P = 0.74$ ) and capture hole (capture method) ( $F_{5,38} = 0.28, P = 0.92$ ), as well as capture method ( $F_{2,38} = 0.33, P = 0.73$ ), were all nonsignificant.

#### *Thyroxine and Relative Growth in the Wild-Caught Perch*

Total plasma T4 concentrations ranged from 12.2 to 57.4 nmol L<sup>-1</sup> ( $\bar{T4} = 26.0 \pm 12.7$  nmol L<sup>-1</sup>,  $n = 27$ ). T4 concentration did not correlate with boldness variation in the wild-caught perch (table 4) in the GLMM IV. Again, the number of days spent in the laboratory was barely nonsignificant in the model (table 4), possibly suggesting that acclimation to laboratory conditions increased boldness in the wild perch. T4 concentration did not correlate with rSMR (Pearson's  $r = 0.186, n = 27, P = 0.176$ ; fig. 3), mass-dependent SMR variation (Pearson's  $r = 0.102, P = 0.605$ ), or relative growth rate

Table 3: Explaining gamma-transformed boldness variation with capture-related predictors among wild-caught (ice fished) Eurasian perch ( $n = 44$ ) in a nested generalized linear mixed model

Explanatory variable	<i>F</i>	df numerator	df denominator	<i>P</i>
Capture order(capture hole[capture method])	.333	25	43	.998
Capture hole(capture method)	1.119	17	43	.368
Capture method <sup>a</sup>	.160	1	43	.691
Lab days	3.946	1	43	.053

Note. Lab days refers to the number of days spent in the laboratory before the first boldness trial.

<sup>a</sup>Natural bait or artificial lure.

Table 4: Results of predicting gamma-transformed boldness variation with plasma thyroxine (T4) concentration among wild-caught (ice fished) perch in a generalized linear mixed model

Explanatory variable	<i>F</i>	df numerator	df denominator	$\beta$	SE	<i>P</i>
Intercept				−.013	.315	.967
T4	.248	1	51	.004	.008	.639
Lab days	3.345	1	51	.015	.008	.073

Note. Lab days refers to the number of days spent in the laboratory before the second boldness trial;  $n = 27$ .

(Pearson's  $r = 0.033$ ,  $n = 31$ ,  $P = 0.868$ ). Last, rSMR did not correlate with relative growth rate in the wild perch (Pearson's  $r = -0.252$ ,  $n = 43$ ,  $P = 0.103$ ).

## Discussion

### POLS Concept

We found no evidence of SMR or growth rate explaining variation in individual boldness in Eurasian perch. This result does not support the central prediction of the POLS concept (Réale et al. 2010), which generally predicts positive correlations between boldness-related individual behaviors, metabolic rate, and growth rate (Careau et al. 2008; Biro and Stamps 2010). The absence of the predicted association was not due to low statistical power, as the estimated effect of growth on boldness was negative and the weak positive effect of metabolic rate was far from statistical significance ( $P = 0.546$ ).

The perch in this study showed significant and relatively high repeatability of boldness ( $ICC_{GLMM} = 0.54$ ), corroborating previous findings of consistent individual differences in behavior (i.e., personality) in this species (Magnhagen and Bunnefeld 2009; Kekäläinen et al. 2014; Härkönen et al. 2016; Vainikka et al. 2016). The average repeatability ( $ICC r$ ) of behavioral traits across animal taxa was reviewed to be 0.37 (Bell et al. 2009).

We found that the wild-caught perch were on average bolder compared to the hatchery-reared perch—possibly demonstrating the effect of environmental background on behavior (e.g., Salonen and Peuhkuri 2006; Yokota et al. 2007; Ahlbeck and Holliland 2012; fig. 2). The difference was most likely due to a higher number of perch that were inactive in the boldness trials among the hatchery perch, since excluding the inactive perch from analysis (GLMM I<sub>A</sub>) dissolved the significant difference between perch groups. Due to the between-group differences in the timing of the boldness experiments and the long transportation distance of the hatchery fish, we cannot conclude the difference in boldness to entirely result from distinct environmental backgrounds.

Having inactive perch in the data appeared not to distort the behavioral analyses or their interpretation in other respects—on the contrary, excluding these fish ended up hiding some otherwise significant associations in the behavioral models. This study serves as an example of how artificially excluding inactive individuals from data of behavioral studies may be needless and possibly misleading, when the equally meaningful information from the whole behavioral spectrum can be made

use of by using alternative data-handling methods appropriate for nonnormally distributed data.

Our data showed that SMR is a repeatable trait ( $ICC r = 0.52$ ) in perch, which is consistent with earlier studies (reviewed in Nespolo and Franco 2007) of fishes such as Atlantic salmon (*Salmo salar*,  $r = 0.68$  for rSMR; McCarthy 2000), Gulf killifish (*Fundulus grandis*,  $r = 0.68$  for SMR; Virani and Rees 2000), and spined loach (*Cobitis taenia*,  $r = 0.68$  for rSMR; Maciak and Konarzewski 2010). We found a negative association between the number of days spent in the laboratory before respirometry and rSMR levels among the hatchery-reared perch, possibly suggesting acclimation to laboratory conditions.

To date, inconsistent results have been reported for the relationship between boldness and minimal metabolism. Binder et al. (2016) found no significant difference in resting metabolic rate (RMR) between bold and shy bluegill sunfish (*Lepomis macrochirus*)—even though the bold group had higher maximum metabolic rates than the shy group. White et al. (2016) reported results comparable to our study in the Trinidadian guppy (*Poecilia reticulata*), with no covariation between metabolic or growth rate and behavior. Mell et al. (2016), on the other hand, have reported a negative correlation between SMR and activity as well as sociability—but not risk-taking behavior—in the common lizard (*Zootoca vivipara*).

Positive correlations between boldness and minimal metabolism have been found in Atlantic salmon (Finstad et al. 2007) and the common carp (*Cyprinus carpio*; Huntingford et al. 2010). In their study of great tits (*Parus major*), Mathot et al.

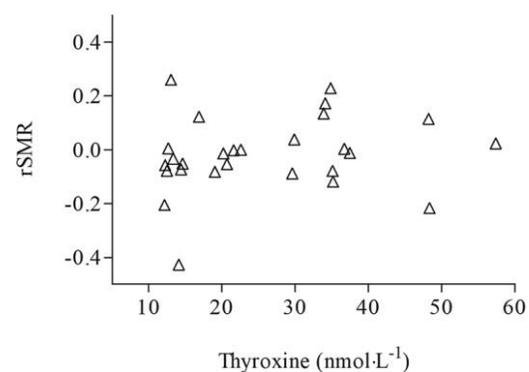


Figure 3. There was no correlation between the plasma concentration of the metabolic hormone thyroxine and the mass-corrected standard metabolic rate (rSMR) among wild-caught Eurasian perch (Pearson's  $r = 0.186$ ,  $n = 27$ ,  $P = 0.176$ ).

(2015) found that introducing a predatory risk to the boldness trial reversed the correlation between basal metabolic rate and risk-taking behavior, which was interpreted as the relationship between metabolism and behavior likely being context dependent. In our study, the wild-caught perch had presumably faced natural predation, while the hatchery-reared perch had grown in a predator-free environment. Yet the direction of correlations between rSMR and average boldness (of the two trials) did not differ between these groups in our study (Spearman's  $\rho$ : wild = 0.084,  $n = 44$ ,  $P = 0.589$  vs. hatchery = 0.047,  $n = 43$ ,  $P = 0.762$ ).

Thermal physiology has been presented as a potential driver behind behavioral variation and as necessary to be included within the POLS concept (Goulet et al. 2017; see also Mell et al. 2016; Nakayama et al. 2017). In our study, however, all the experiments were performed at the same temperature (8°C), making its inclusion in these models unfeasible.

The contradictory results obtained thus far indicate that the presence and nature of the fitness-maximizing physiological syndromes predicted by the POLS concept depend, for example, on the species, sex (Monceau et al. 2017), behavioral traits considered, predatory pressure, social environment (David et al. 2015), temperature, or other environmental conditions (Závorka et al. 2015). That is, the relationships between fundamental physiological traits, key life-history traits, and boldness-related behaviors appear to be context dependent due to variability in both genetic background and environmental conditions.

This context dependency may decrease the potential for the syndromes to become genetically fixed and significant in the context of fishing-induced evolution. Yet identifying the key traits targeted by angling-induced selection remains crucial (e.g., Arlinghaus et al. 2017). Furthermore, understanding the evolutionarily relevant links between metabolic traits, growth, and behavior in varied and variable environments requires further investigation along with a reassessment of the POLS concept.

#### *Vulnerability to Angling among Hatchery-Reared Perch*

We found that in the hatchery-reared perch group, differences in vulnerability to angling did not significantly explain boldness variation. Conversely, Diaz Pauli et al. (2015) reported that both passive and active fishing gear selected against high boldness in the guppy. However, the active gear used in their study was a trawl net and the measure for boldness was freezing-behavior time, as opposed to angling gear and emergence delay, respectively, in our study. Although the fishing gear used in our study is more relevant considering recreational fisheries, the total number of hatchery-reared perch that were assessed as vulnerable to angling and acceptably tested for both boldness and SMR was relatively low ( $n = 8$ ), preventing generalizations. With a higher number of vulnerable perch, a possible association with boldness could become more prominent, facilitating further interpretation.

An earlier study in perch suggested that fishing in general would not select against boldness (Vainikka et al. 2016). Här-

könen et al. (2016), on the other hand, showed that perch captured early in order with artificial gear were more explorative (in a seminatural shoal context) than perch caught later in order with a natural bait (with capture methods similar to this study). It would thus appear that in perch, exploration-related behavioral traits (in seminatural shoal context) are stronger predictors of vulnerability to angling than traits directly related to boldness (as measured in individual laboratory trials).

Hessenauer et al. (2015) found that unexploited largemouth bass had a 6% higher mean RMR than bass exploited by anglers and proposed angling to select against high energy demand. Likewise, Redpath et al. (2010) found that SMR was 10% higher in the group of largemouth bass vulnerable to fishing than in the nonvulnerable group. The contrasts with our results (no difference between vulnerability groups) are likely due to the long-term selection for fishing vulnerability in these studies.

The parental population of the hatchery-reared perch in this study was captured by ice fishing from the wild, excluding individuals nonvulnerable to angling, thus possibly reducing the standing genetic variation in metabolic rates and traits associated with vulnerability to angling among the study fish. However, since vulnerability to fishing has been estimated to be a moderately heritable trait ( $h^2 = 0.15$  for largemouth bass in Philipp et al. 2009), the decrease in trait variation in the first generation (in our hatchery-reared perch group) may have been very modest at most.

#### *Capture Method among the Wild-Caught Perch*

In the wild-caught perch group, neither capture order nor capture hole explained boldness variation (table 3). If each capture hole represents a random perch shoal, it is somewhat plausible to predict that bolder individuals would on average be captured before the less bold ones (Vainikka et al. 2012). In our study, this was true in only one of the capture holes. Previously, Vainikka et al. (2012) found size dependence in capture order but only in two out of 10 of the studied populations. Thus, while the prediction might hold true for some special cases, it cannot be generalized, suggesting that more factors in addition to boldness or size play a role in determining feeding order when it comes to angling.

We found no difference in boldness or rSMR between the two ice-fishing methods (artificial lure vs. natural bait) among the wild-caught perch. The results agree with the lack of such differences among the vulnerable and nonvulnerable hatchery-reared perch of this study. In an earlier study in perch, Kekäläinen et al. (2014) likewise found no difference in boldness between two angling methods (similar to those used in this study) or in relation to capture order, even though they demonstrated that boldness explained behavior in a long-term group experiment (in a seminatural shoal context, similar to Härkönen et al. 2016).

However, Wilson et al. (2015) found that large individuals of wild largemouth bass and rock bass (*Ambloplites rupestris*) caught with plastic worm baits (imitating maggots) took longer to exit a refuge (as a measure of boldness) in behavioral trials

than did individuals captured with active colorful crank baits. Nevertheless, the trend did not extend to small individuals (captured with worms), which were boldest, nor did other tested personality traits (e.g., activity; but see Cooke et al. 2017) differ between capture methods, thus complicating interpretation (Wilson et al. 2015). In light of these studies, vulnerability to angling could in fact be somewhat independent of the actual capture method used.

#### *Thyroxine in Relation to SMR and Behavior*

Contrary to our hypothesis, we found no association between plasma T4 concentration and boldness in wild-caught perch. John-Alder (1991) and Steinberg et al. (1993) have found a stimulatory effect of T4 administration on behavior in lizards. Furthermore, T4 concentration did not correlate with rSMR in our study. Previously, John-Alder (1990) found a positive correlation between T4 and SMR in lizards administered with T4. Inconclusively, Steinberg et al. (1993) found T4 deficiency to reduce SMR but T4 supplementation not to stimulate SMR in a lizard. In general, plasma T4 concentration varies greatly between fish species, developmental stages, and seasons—even times of day (Lynshiang 1998; Bau and Parent 2000; Hulbert 2000; Loter et al. 2007)—and its stimulatory effect on metabolic rate (calorigenesis) is believed to be temperature dependent in poikilotherms (Gupta and Thapliyal 1991; Lynshiang 1998; Hulbert 2000).

The lack of correlation between T4 and rSMR was not entirely unexpected, however, since the water temperature in our study (8°C) was well below what is considered as the calorogenic temperature range of THs in poikilotherms (above ~21°C; Gupta and Thapliyal 1991). Nonetheless, *in vitro* administration of THs T3 and T4 was shown to stimulate brain tissue oxygen uptake in the walking catfish (*Clarias batrachus*) at 8°C, well below their reported calorogenic temperature range (Lynshiang 1998). In Finland, water surface temperatures rarely rise above 20°C (Korhonen 2002), and the calorogenic function of THs in poikilotherms in cooler climate zones remains unclear.

The possibility of a stress response should be considered, since T4 has been identified as a stress-response hormone in fish (Bau and Parent 2000; Peter 2011). Our results indicate this not to be the case here, however, since the correlation between plasma T4 concentration and total laboratory confinement time was not statistically significant in the wild perch group (Spearman's  $\rho = 0.301$ ,  $P = 0.127$ ,  $n = 27$ ), which would have been expected in the case of prolonged stress (Peter 2011).

#### *Conclusions*

We found that rSMR was not associated with boldness in wild-caught or hatchery-reared Eurasian perch. This result does not support the predictions of the POLS concept, which expect high minimal metabolism and high energy demand to be associated with heightened boldness. According to our study, differences in vulnerability to angling (vulnerable vs. nonvulnerable) or capture method (artificial lure vs. natural bait) did not explain

SMR or boldness variation in perch. Thus, we propose that exploratory behavior may be a more relevant personality trait with respect to vulnerability to angling than boldness. Since capture order or capture hole (with different capture methods) did not explain boldness variation among the wild-caught perch, different angling methods are unlikely to cause differential selection for boldness-related traits in this species. Without conflict of interest, our results support the view that angling may not drive any significant boldness evolution in Eurasian perch, while not excluding the possibility of angling-induced selection on traits not addressed in this study.

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