
Transcriptomic profiles of consistent risk-taking behaviour across time and contexts in European sea bass

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Abstract :

Bolder individuals have greater access to food sources and reproductive partners but are also at increased risk of predation. Boldness is believed to be consistent across time and contexts, but few studies have investigated the stability of this trait across variable environments, such as varying stress loads or long periods of time. Moreover, the underlying molecular components of boldness are poorly studied. Here, we report that boldness of 1154 European sea bass, evaluated using group risk-taking tests, is consistent over seven months and for individuals subjected to multiple environments, including a chronically stressful environment. Differences in risk-taking behaviour were further supported by differences observed in the responses to a novel environment test: shy individuals displayed more group dispersion, more thigmotaxic behaviour and lower activity levels. Transcriptomic analyses performed on extreme phenotypes revealed that bold individuals display greater expression for genes involved in social and exploration behaviours, and memory in the pituitary, and genes involved in immunity and responses to stimuli in the head kidney. This study demonstrates that personality traits come with an underpinning molecular signature, especially in organs involved in the endocrine and immune systems. As such, our results help to depict state-behaviour feedback mechanisms, previously proposed as key in shaping animal personality.

Keywords : coping style, behavioural syndrome, fish, personality, anti-predator behaviour, intra-individual variability

1. Introduction

For most animals, boldness could be viewed as a risky strategy as it may increase the probability of being predated. At the same time, risk-taking behaviour of bold individuals also increases their likelihood of finding more suitable environments (e.g. for food or mating). This trade-off is known to favour bolder individuals when predation is low and shyer individuals when predation is high [1]. Risk-taking behaviour is therefore tightly linked to the eco-evolutionary dynamic of species in a given environment. Animals maximize their fitness through plasticity (i.e. variation that depends on contexts within individuals) and selection of adaptive behaviours (i.e. variation over generations within species). Indeed, it has previously been demonstrated in numerous species that the environment drives the average behaviour of a population [2,3]. The most evident examples relate to the effects of differences in predation risks between two environments, including as a consequence of human presence [4].

Nevertheless, within a given population, individuals differ in their behavioural phenotypes. Some are more prone to take risks than others, and strong heterogeneity in risk-taking has been related to global population sizes and larger spatial ranges [5]. Interestingly, these inter-individual differences in risk-taking behaviour are relatively consistent over time and contexts [6], and being bold or shy is thus considered as a personality trait in animals [7]. Personality traits have been observed for a wide range of terrestrial and aquatic species, and linked to fitness in a given environment [8]. The emergence of personalities were previously proposed to be linked to key life history traits, such as metabolism and growth, through a state-behaviour feedback [9,10]. In this framework, individuals that are more prone to take risks also exhibit lower hypothalamo-pituitary-interrenal/adrenal axis (HPI/A) responses, but stronger hypothalamo-sympathetic activity [11]. These interconnections between behaviour and stress physiology are part of the concept of coping styles [11], and might lead individuals to consistently differ in behaviour.

Yet, it remains unclear to what extent personality traits are governed by the genetic architecture of an individual and/or depend on the environment it will experience [12–14]. In addition, we still lack information regarding the molecular mechanisms underlying these risk-taking related behaviour, although some efforts have been made to elucidate the genetic component of traits, e.g. boldness: [15]. Most studies of animal personality focus on short time periods or one life stage. This focus provides a limited

view of how personality interacts with physiology, life stage, experience and the environment [16]. Understanding molecular mechanisms behind key behavioural phenotypes, such as the boldness/shyness continuum, is of primary importance in behavioural ecology.

In this study, we characterize the boldness of European sea bass (*Dicentrarchus labrax*) by assessing the consistency of risk-taking related behaviour across multiple environments, challenges and time. Then, we highlight molecular pathways in the brain, the pituitary and the head kidney associated to these divergent behavioural phenotypes. These three organs were chosen because i) the brain centralises perceptions of the environment and controls behaviour and physiology [17], ii) the pituitary secretes hormones regulating multiple processes including growth, sexual maturity, stress response and energetic balances [18], iii) the head kidney, analogous to the adrenal gland in mammals, plays a central role in the immune system and stress regulation by secreting cortisol and catecholamines, the major stress hormones in fish [19,20]. The European sea bass was used as a model species, because of its ecological and economic importance [21]. Previous work demonstrated strong inter-individual variation in metabolism [22], stress response [23], feeding behaviour [24] and coping style [25] of the species. Consistency in risk-taking behaviour over 3 months has also been demonstrated in European sea bass [26] but has never been challenged by environmental variation, especially by extreme events such as a chronic stress period. Also, within a population, correlations exist between molecular phenotypes related to behaviour and coping capacities [27], but no conserved transcriptomic signature for boldness has been shown in the sea bass whole brain [28]. The present study aims (a) to investigate the long-term consistency in risk-taking behaviour of individuals from a large sea bass population (n= 1154), (b) to demonstrate that extreme individuals (*i.e.* very shy or very bold) contrastingly respond to a different behavioural test, and then (c) to depict molecular differences in gene expression across specific organs (brain, pituitary and head kidney) in those extreme individuals.

2. Material and Methods

- (a) Consistency of group risk-taking behaviour over time and different environmental conditions

- (i) *Experimental animals*

All experiments were performed at the experimental research station of Ifremer in Palavas-Les-Flots (France). Experiments were authorized by ethics committee agreement APAFIS#10745 and all procedures involving animals were in accordance with the ethical standards of the institution and followed the recommendations of Directive 2010/63/EU.

All fish used in this study were part of a larger experiment which led to a first publication [29], providing all details on the rearing protocol. Briefly, 2053 European sea bass were individually tagged at 175 ± 3 days post fertilization (dpf), using an ISO PIT tag (8 x 1.4 mm), to ensure individual identification. They were dispatched in three 1.5m^3 tanks, supplied with water at a constant temperature of 21°C . At 255 ± 2 dpf and 358 ± 2 dpf, a total of 288 and 492 fish respectively were randomly extracted from the tank for two other studies, one being published [29]. From tagging to the end of the experiment (at 462 dpf), 119 (out of 2053, 5.8%) fish died of unknown reasons.

(ii) Group risk-taking tests

Over the whole experiment (described below) fish were evaluated in 4 group risk-taking tests (GRTs), performed following a previously described protocol [26]. All GRTs were conducted in a tank of the same size and shape as the rearing tanks, and supplied with water from the same system. The tank was vertically divided in two parts of equal volume with an opaque screen. In the middle of the screen, a 12 cm hole was surrounded by a circular RFID-reading antenna (DORSET, The Netherlands) connected to a computer. The hole was blocked with a door that could be opened without being seen by the fish.

Fish were fasted for 24h prior to the start of the experiment. They were then lightly anaesthetized (100 ppm of Benzocaine, 150 g/L, E1501, Sigma, Saint Louis, MO, USA), and transferred between 10:00 and 11:00 am on one side of the tank that we covered with a black tarp in order to get complete darkness. The other side was not covered in order to create a riskier zone. After 2 hours of habituation, the door was opened to allow the fish to move freely to the other side of the tank, PIT tags were read by the antenna and the time of first passage (*Latency to exit (s)*) was stored. In order to avoid a too long starvation period, we censored the test to 20 hours. After the test was completed, fish were anaesthetized (300 ppm of Benzocaine), and were measured for length (cm) and weight (g) and transferred back into their original rearing tank.

(iii) Experimental protocol

At 255 ± 4 dpf, the first risk-taking test (GRT1) was conducted for each rearing tank, providing a risk-taking phenotype for the 1748 fish available (Figure 1). At 309 dpf, fish from all 3 tanks were transferred in 6 tanks of 1 m^3 with equal number of fish from each initial rearing tank (Figure 1). At 336 dpf, and for a period of 3 weeks, 3 tanks were stressed as previously detailed [29], while the 3 other tanks were kept under normal rearing conditions (Figure 1). Briefly, stressors consisted in random light flashes, chasings and confinements randomly programmed over the 3 weeks.

At 358 ± 1 dpf, a subsample from each tank was extracted for the analyses published in Sadoul et al. (2021) [29]. The remaining fish ($n=1187$) were transferred to three 1 m^3 tank making sure that previously stressed and control fish were equally represented. Two weeks later, at 373 ± 1 dpf fish from each tank were evaluated in a new risk-taking test (GRT2) performed for each tank (Figure 1). A total of 1187 individuals were phenotyped at GRT2. The third GRT (GRT3) was performed 6 weeks later at 423 ± 3 dpf, on each tank for the 1181 remaining fish. At 443 dpf, all fish were then transferred in a unique 5 m^3 tank, and at 463 dpf, the last risk-taking test was performed at once in a 5 m^3 tank (GRT4) on the 1154 remaining fish. All fish were then euthanized and a random subsample ($n=606$) was sexed (Figure 1) indicating that the population was composed of 54% of females (329 females vs 277 males).

(iv) Statistical analyses of GRT data

We analysed the GRT data with hierarchical Bayesian regression with a lognormal likelihood function in Stan [30] and cmdstanr [31] in R version 4.0.5 (R Core Team, 2021). We included data from 1748 fish who participated in GRT1 and who were assigned a chronic stress group. Missing data due to fish extracted from the experiment (see previous section) were imputed in the Bayesian model rather than being discarded. Censored values were accounted for via imputation with a lower bound of 20 hours. Fish were categorized as bold or shy at the first GRT (at 255 dpf) based on whether they were above (shy) or below (bold) the median latency in their tank. We included GRT as an observation-level predictor and bold/shy (bold=875), chronic stress (yes=874) and sex (female=598, male=494) as individual-level predictors. Six-hundred-and-fifty-five fish were missing sex assignment, which was accounted for by marginalizing out of the sex variable for those fish [32].

Four models were run and compared using the expected log predictive density (ELPD) estimated via Pareto-smoothed importance sampling leave-one-out cross validation (ELPD-LOO; [33]) in the loo package [34]. Model 1 included random intercepts (*i.e.* personality) for individuals, model 2 extended model 1 with random slopes by GRT time (*i.e.* behavioural plasticity) for individuals, model 3 extended model 2 by including a random intercept of initial tank, and model 4 extended model 3 by modelling heterogeneous residual variances by individual (*i.e.* behavioural predictability). From these models, we calculated behavioural repeatability (intraclass correlation coefficients; ICCs) at each GRT time point (*i.e.* conditional repeatability; [35]) as well as the coefficient of variation for predictability as a measure of behavioural predictability effect size [36].

We summarize the parameters and results using the posterior mean and 95% highest density interval (HDI; the most probable values).

(b) Group behavioural response to novel environment and hypoxia challenge

(i) *Behavioural tests in a novel environment*

Among fish screened during GRT1, GRT2 and GRT3, two subsamples of 48 individuals displaying consistent shy (three late exits or no exit at all) or bold behavioural type (three early exits) were selected and challenged in novel environment and a hypoxia challenge at 429 ± 2 dpf (mean \pm SE, 91.0 ± 6.6 g and 194 ± 4.8 cm). This test was performed in groups of 8 fish with similar behaviour (*i.e.* $n=6$ trials*8 individuals per behavioural type, *i.e.* bold vs. shy). The trials to monitor the group behavioural response to a novel environment and hypoxia were carried out according to the protocol described in [29] and detailed in the Supplementary material (SMM1).

(ii) *Statistical analyses of behavioural data in the novel environment test*

Measured variables (time spent in the periphery, velocity and inter-individual distances) during the novel environment and hypoxia challenge were analysed independently before and after the hypoxia challenge. A linear mixed model was fitted with risk-taking behavioural phenotype (bold or shy), time (categorical) and their interaction as fixed effects and the day of experiment and trial as random effects. A

Tukey post hoc test was completed with the `glht` function from the `multcomp` package [37] to test significant differences between the levels of a significant fixed effect.

(c) Transcriptomic signatures between bold and shy individuals

(i) *Samplings*

At 478 ± 2 dpf, i.e., two weeks after GRT4 (see Figure 1), fish were caught from their rearing tank and euthanized using 1500 ppm of Benzocaine. Sampling of the whole brain, pituitary and head kidney was performed on 5 individuals per behavioural phenotype chosen among the individuals also tested in the novel environment test (i.e. 49 days after the novel environment test). The 30 samples collected (5 individuals x 2 behavioural phenotypes x 3 tissues) were flash-frozen in liquid nitrogen and kept at -80°C until further analyses. Individuals were chosen to be equally represented for each sex (2 shy males, 3 shy females, 3 bold males and 2 shy females).

(ii) *RNA sequencing and analyses*

Details on RNA extraction, sequencing and analyses are provided in the supplementary material (SMM2). Briefly, after libraries were constructed, validated and quantified, they were sequenced in equimolar amounts using a HiSeq 2500 (Illumina, San Diego, CA, USA). Reads were then aligned to the *Dicentrarchus labrax* genome (NCBI, reference GCA_000689215.1) with a set of gene model annotations [38]. Differentially expressed (DE) genes between bold and shy were identified within each organ, while the effect of sex was accounted for. Gene ontology (GO) analysis was then performed to categorize each gene within a biological process.

3. Results

(a) Consistency of group risk-taking behaviour over time and different environmental conditions

The average intraclass correlation coefficient (ICC) for between-individual differences was 0.38 (95% HDI: [0.30, 0.47]) and was highest at GRT1 (GRT1: 0.44, HDI: [0.38, 0.50]; GRT2: 0.33 (HDI: [0.29, 0.37]; GRT3: 0.41, HDI: [0.36, 0.46]; GRT4: 0.36, HDI: [0.31, 0.42]). The ICC for between-tank differences was very low (0.003, HDI: [0.000, 0.012]). The model with the highest expected log predictive density (ELPD)

was model 3 (ELPD LOO: -9832.8; model 1 = -10118.0, model 2 = -10071.8, model 4 = -9835.5) including behavioural predictability. The coefficient of variation for behavioural predictability was relatively high at 0.57 (HDI: [0.51, 0.64]).

Fish had generally lower latencies to leave the sheltered areas at GRT1 and GRT2 compared to GRTs 3 and 4, but there were interactions between GRT and fish types (Figure 2). Fish categorized as bold (see estimates in Figure 2) at GRT1 had significantly lower latencies (between 3 and 5 hours) at the following GRTs than shy fish. This was repeatable across the rearing tanks (Figure S1). Female fish had a tendency for lower latencies than male fish across the GRTs, but the difference was only significant at GRT3 and GRT4 (approximately 2-3 hours quicker). Chronic stress had no credible influence on latency to leave the shelter.

(b) Group behavioural response to novel environment and hypoxia challenge

After introduction in the novel environment, both behavioural phenotypes (bold and shy) showed first a low swimming velocity which gradually increased to reach a plateau after 30 min. Bold individuals showed a tendency for higher swimming velocity after 10 minutes compared to shy individuals ($p=0.057$, Figure 3A). At introduction in the novel environment, shy individuals showed increased dispersion in the tank (Figure 3B, $p<0.05$) and spent more time in the periphery (Figure 3C, $p<0.05$) than bold individuals. After 20min, both behavioural phenotypes showed a similar group behaviour pattern which became constant after 30 min (Figure 3C). Both behavioural phenotypes displayed a same response to the hypoxia challenge for the three behavioural measures (Figure 3).

(c) Transcriptomic signatures between bold and shy individuals

In the pituitary and the head kidney, 556 and 141 genes were differentially expressed between bold and shy individuals respectively.

In the brain, only 6 genes were differentially expressed and 4 of them were annotated (*nr2e3*, *glipr2*, *dsg2*, *pla2g1b*). In the pituitary, the biological process “Behaviour” (GO:0007610) was, out of level 2 biological processes, the most differentially represented within the genes differentially expressed (DE) between bold and shy

individuals (Table S1). Most of these behaviour-related genes were upregulated in bold individuals (Figure 4A). All DE genes related to social behaviour, exploration behaviour and memory were up-regulated in bold individuals (Figure 4 B, C and D). Only *casp3*, *trpv1* and *arrdc3* were found downregulated in shy individuals (Figure 4A).

In the head kidney, 9 biological processes out of all level 2 biological processes were overrepresented among DE genes, with “immune system process” being the most significant (GO:0002376). Most DE genes related to immune system processes were upregulated in bold individuals (Figure 5A). All DE genes related to immune system development and leukocyte activation were found to be upregulated in bold individuals except for *pawr* (Figure 5 B and C).

After adjustment of the p-values to lower false discovery rates, 6 biological processes were still significant in the head kidney, confirming the true biological signal underpinning transcriptomic differences (Table S1 and Figure S2).

4. Discussion

(a) Consistency of group risk-taking behaviour over time and different environmental conditions

Mean latency to exit the covered side of the GRT tank greatly varied over time, indicating that environment and/or age impact risk-taking behaviour. Although we are unable to identify causes of this variation, it supports that risk-taking behaviour is relatively plastic and can be affected by biotic or abiotic changes [25,39]. Nevertheless, within our studied population, individuals showed heterogeneous risk-taking behaviour which were moderately consistent over time and environmental conditions. This is particularly interesting given that repeatability was assessed over almost 7 months and was challenged by varying the rearing conditions and stress loads. Consequently, risk-taking behaviour can be considered plastic over time and environmental conditions for the whole population but, at the same time, environmental conditions are not sufficient to trigger long lasting changes in individual risk-taking within a population. In this study, a GRT was performed 2 weeks after the end of the chronic stress protocol. The absence of differences in risk-taking behaviour between the chronically stressed individuals and controls suggest that the measured behaviour is resilient (*i.e.*,

significant change but quick recovery) or resistant (little change) to different stress loads. Stress is known to affect behaviour, including risk-taking [40,41]. Behavioural measures are also generally considered as reliable markers of welfare [29,42,43]. Nevertheless, to our knowledge, no previous studies have investigated long-term post-stress differences in risk-taking behaviour in fishes; behavioural differences being observed only during the stress protocol or right after. Yet, some events occurring during fish life history impacted boldness [44,45], which was not the case in our study, suggesting that this measured behaviour, performed in group, is resilient toward stressors.

We observed moderate consistency in the latency to leave the initial area over a relatively long period of time (ICC = 0.38), and for whether fish left the initial area at all (ICC = 0.35). Previous studies have also demonstrated consistency in fish behaviour overtime within a population [15,39,46,47], but only few exceeded 45 days [26,46,48]. Long-term consistency in personality traits have, nevertheless, been studied in multiple mammal species. Multiple of these studies observed changes once puberty or sexual maturation occurs [49,50]. To our knowledge only one study investigated boldness consistency in fishes over maturation and sex change, and observed a strong loss of consistency once sea bream (*Sparus aurata*, protandric species) became adults [46]. Our study investigated behaviour from 255 to 462 dpf, while sea bass experienced puberty (at least for males), suggesting that repeatable risk-taking behaviour occurs in European sea bass over important ontogenetic transitions. Altogether, our results advocate for resilient risk-taking behaviour over time and environmental conditions, and further consolidate the bold-shy continuum as a personality trait in European Sea bass.

(b) Group behavioural response to novel environment and hypoxia

Both bold and shy fish showed similar expected behavioural responses (i.e. increasing velocity and cohesion and reducing time in the periphery) during the acclimation period in the novel environment test [29,42]. Differences between bold and shy groups were observed at the very beginning of the acclimation period. We identified higher thigmotactic behaviour for shy individuals compared to bold, classically indicative of anxiety-like behaviour [51]. Shy individuals also showed less cohesion than bold

individuals at the beginning of the acclimation period. Based on the high cohesion values observed at the end of the acclimation period for both groups, we suspect that values at the start of the experiment are a marker of fear in shy groups. The tendency for higher activity in bold animals during the acclimation period could also be characteristic of fearless individuals [52,53]. Results from the two behavioural tests are therefore consistent. In response to the hypoxia challenge, both bold and shy fish decreased their swimming activity and enhanced the distance between individuals. These behaviours allow to respectively reduce energetic costs and maximize oxygen uptake [54]. These results suggest that both phenotypes respond similarly, in their behaviour, to a life-threatening challenge.

(c) Transcriptomic signatures between bold and shy individuals

Bold and shy individuals showed only limited differences in the expression of genes in the whole brain. This contrasts with a previous study highlighting 246 DEGs between behaviours in the whole brain [28] while we only observed 6. Nevertheless, in Rey et al. (2021) [28], the deployed risk-taking test was different and involved an hypoxia condition, forcing individuals to leave the sheltered area and this was demonstrated to phenotype for a different behaviour in seabass [15].

Gene expressions in the pituitary were the most significantly different between bold and shy individuals. The pituitary gland of bony fishes is composed of 7 endocrine-involved cell types [18] under the control of the hypothalamus in the brain. They release in the circulatory system hormones playing key roles in many biological processes, such as stress response, behaviour or growth [18]. Interestingly, multiple genes related to the “behaviour” were significantly different between individuals categorized as bold or shy. Individuals categorized as bold overall showed an increased expression of genes related to social, exploration behaviours and memory.

For instance, the gene *dlg4* was upregulated in bold individuals. The knock out of this gene leads to reduced social behaviour and increased anxiety behaviour in mice [55]. This correlates well with the significant behaviour observed during the novel environment test, with bold animals showing increased grouping (lower interindividual distance) and lower anxiety (lower time spent in the periphery) than shy individuals. Shy individuals had also reduced expression of *jph3*, encoding a protein of the junctophilin family, that was previously demonstrated to have an active role in

exploratory behaviour in mice [56]. This higher exploration tendency for bold individual is consistent with behavioural responses both in GRT and novel environment test, and consistent with classic scheme of features underlying divergent coping style, i.e. proactive vs. reactive individuals [10].

Genes related to memory and learning capacities were generally down regulated in shy individuals (e.g. *egr1*, *npas4*). In some cases, bolder and faster explorers also are fast learners [57]. At the opposite, it can also be argued that shy individuals are more efficient in reverse learning, and display more elaborated memory processes [58]. Links between cognition and animal personality, including boldness, could be influenced by many factors and are still debated [58,59]. In European sea bass, previous study observed increased expression of *egr1*, a gene involved in neurogenesis, of individuals categorized as shy in an individual novel environment test [25], while the opposite has been observed in the present study. Differences may be explained by the fact that the GRT and individual novel environment are measuring different behavioural responses, potentially due the context, isolation vs. group testing [25]. These differences could also be due to difference in sampling time (after the experiment vs. control) and thus differences in fish stress state, or due to the difference in brain parts sampled. This example illustrates that multiple sampling in various context are a key to understand the inter-individual variability.

In the head kidney, many genes were also differentially expressed between the two groups. The head kidney is central in the stress axis regulation, by releasing cortisol and catecholamines into the blood. The analogue adrenal gland in mammals was previously demonstrated to show differences in the expression of genes related to glucocorticoid receptor signalling between two clear behavioural phenotypes in pigs [60]. While in our study a significant enrichment for “response to stimulus” was observed none of the differentially expressed genes were part of the gene ontology “glucocorticoid receptor signaling pathway” (GO:0042921). In teleost, the head kidney is also involved in the immune system, by producing and maturing white blood cells and hosting antibody-producing cells [19,20]. The genes part of the “immune system processes” were the most different between the two groups, with individuals categorized as bold showing overall increased gene expression. Although the link between behaviour and immune system is well documented in mammals, this link was investigated in only few fish studies reporting distinct immune capacities between behavioural phenotypes [52,61]. Whether the higher expression of immune genes

observed for bold European sea bass in our study translates in increased immune functions, later affecting responses to pathogens, still needs to be demonstrated.

5. Conclusions

Our study demonstrates that inter-individual differences in risk-taking behaviour are consistent over time and environmental conditions. Differences observed in the group risk-taking test also translated into differences in a novel environment test in smaller groups, where bold individuals adapted faster to a novel environment than shy fish. Finally, inter-individual differences also correlated with transcriptomic changes in the pituitary and head kidney, mostly through differences in behaviour- and immune-related genes. These results highlight the importance of investigating inter-individual differences in behaviour and physiology for the ecology of species as they likely explain dispersal and/or migration at the population scale [62,63], and are as such at the forefront of the response to global changes [64].

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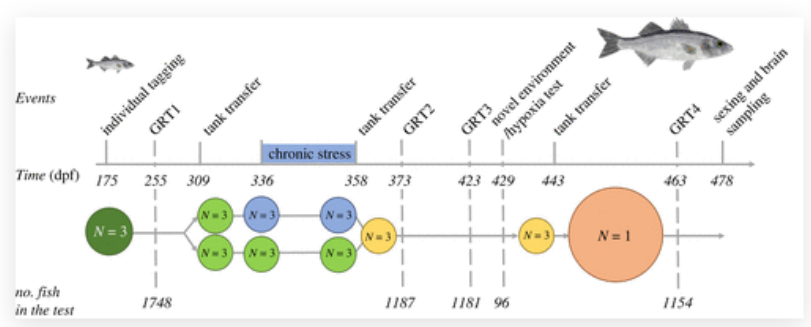


Figure 1. Experimental protocol assessing consistency of risk-taking behaviour over time and environments. Fish were reared in replicated tanks (N = number of rearing tanks). A total of four GRTs were performed on each rearing tank. Colour of the tank illustrates a different rearing condition. Until 309 dpf, juveniles were reared in 1.5 m³ tanks (dark green tank), they were then transferred in 1 m³ tanks (light green tanks) and half of the tanks were stressed (blue tanks) from 336 to 358 dpf. At the end of the stress period, fish were mixed and transferred to other 1 m³ tanks (yellow tanks) until 443 dpf when they were all transferred in a unique 5 m³ tank (orange tank). (Online version in colour.)

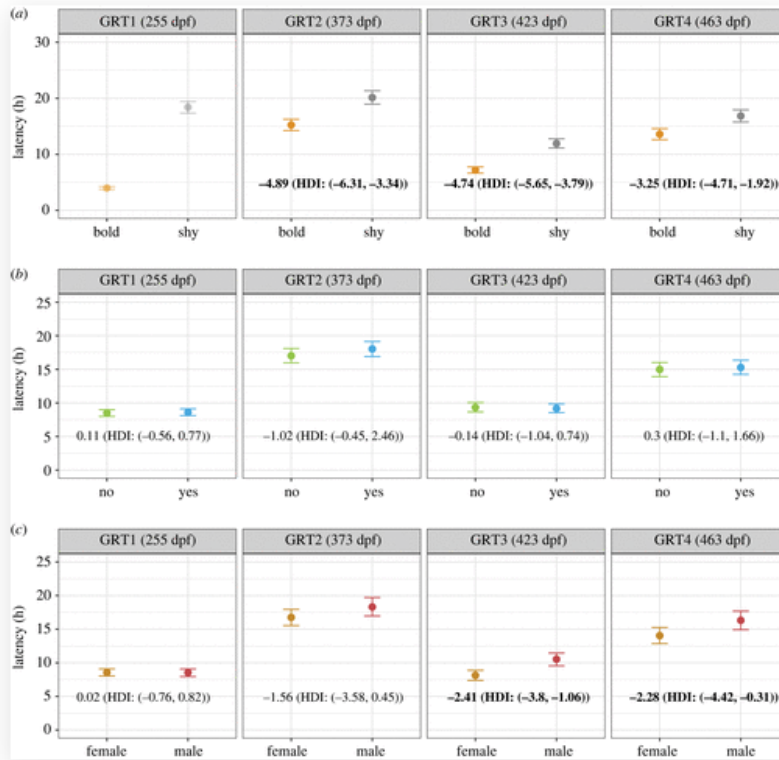


Figure 2. Latencies (in hours) at the four GRTs by 255 dpf by (a) estimates not shown for GRT1 because boldness was a direct function of latencies), (b) chronically stressed between GRT1 and GRT2, and (c) sex. Points show the posterior mean and vertical line segments represent the 95% HDI. Text annotations illustrate the posterior differences between each estimate and text in bold font highlights significant differences (i.e. the 95% HDI do not include zero). (Online version in colour.)

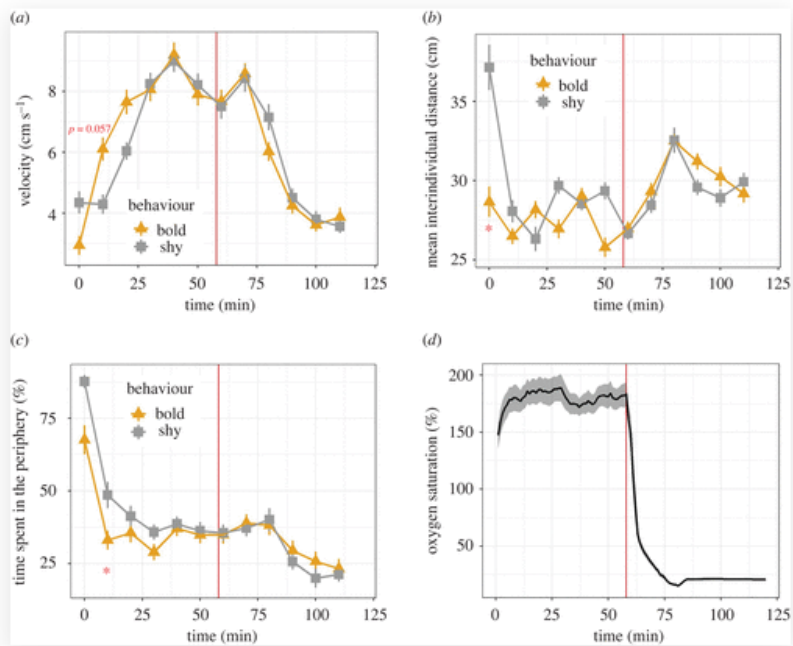


Figure 3. Group behaviour in a novel environment before and during a hypoxia challenge depending on fish behaviour in the GRTs (bold in orange, $n = 48$; shy in grey, $n = 48$). (a) Mean velocity (cm s^{-1}), (b) inter-individual distance (cm) and (c) time spent in the periphery (%) are illustrated during a 60 min acclimation period to the novel environment and during a hypoxia challenge (starting at the red line) obtained by reducing the oxygen saturation down to 20% (d). Data are represented as mean \pm s.e.m. The difference in behaviour between the two fish groups at a single time point is illustrated with an asterisk ($p < 0.05$). (Online version in colour.)

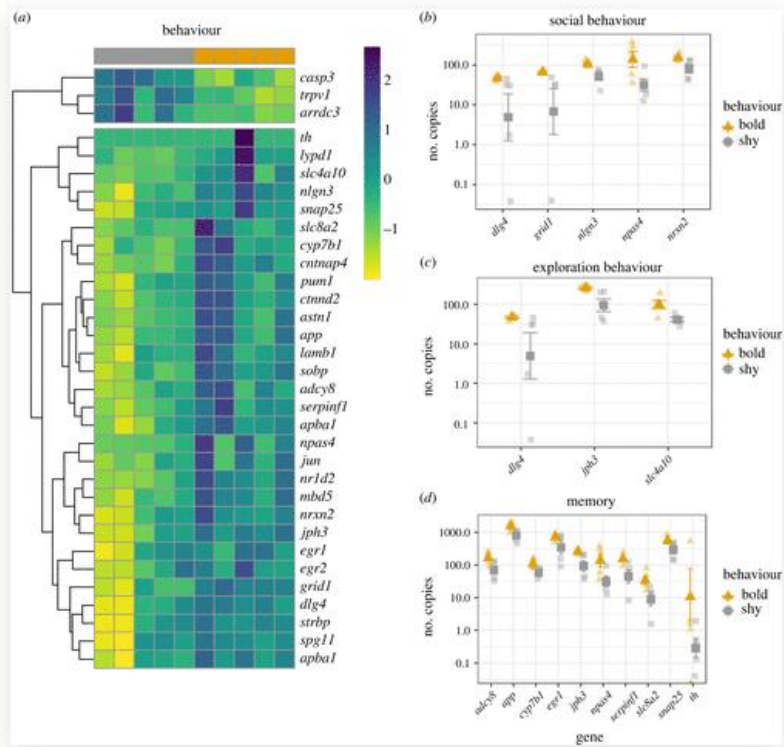


Figure 4. Differences between bold and shy individuals in the expression of genes related to the biological process 'behaviour' in the pituitary of European sea bass. (a) Heatmap of all genes DE in the GO 'behaviour'. (b–d) Raw expressions on a log-scale for the two behavioural phenotypes of genes involved in social behaviour, exploration behaviour and memory (transparent triangles or rectangles for bold or shy individuals, respectively). Mean and standard error are also illustrated. (Online version in colour.)

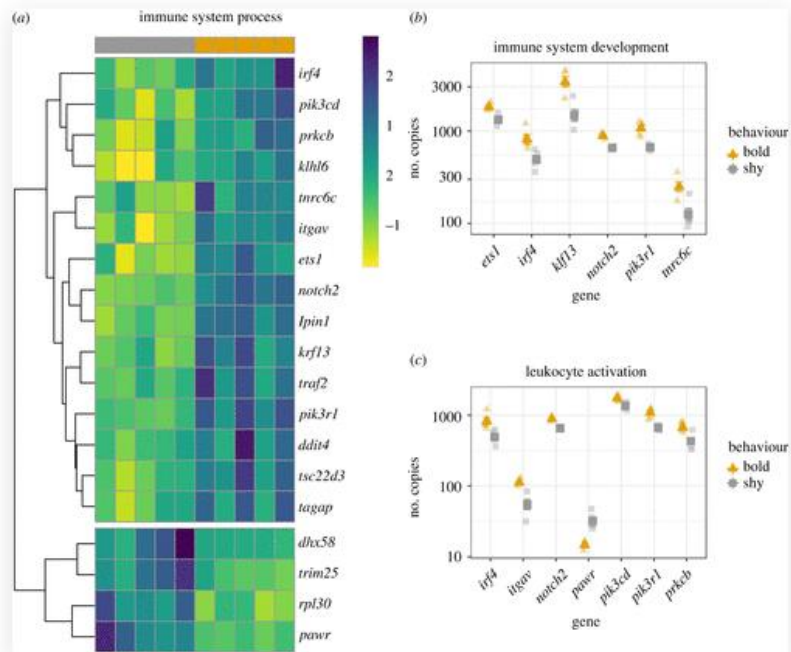


Figure 5. Differences between bold and shy individuals in the expression of genes related to the biological process 'immune system process' in the head kidney of European sea bass. (a) Heatmap of all genes DE in the GO 'immune system process'. (b,c) Raw expressions on a log-scale for the two behavioural phenotypes of genes involved in immune system development and leukocyte activation (transparent triangles or rectangles for bold or shy individuals, respectively). Mean and standard error are illustrated. (Online version in colour.)