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Eco-evolutionary processes in personality and spatial cognition of Arctic charr morphs (*Salvelinus alpinus*)

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FACULTY OF LIFE AND ENVIRONMENTAL SCIENCES

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Dissertation submitted in partial fulfillment of a
Philosophiae Doctor degree in Biology

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Abstract

Behaviour is a fascinatingly flexible phenotype with immense diversity of manifestations. Despite this diversity, observation of animals makes it evident that individuals show consistent differences from one another in the way they behave. However, little is known about the proximate and ultimate mechanisms leading to the emergence and maintenance of consistent among-individual behavioural differences in nature. This thesis aims at exploring the eco-evolutionary processes and developmental cues shaping two particular aspects of behaviour: personality and spatial cognition. For this, I used juveniles of five Arctic charr morphs, of which two sympatric pairs, ranging along gradients of genetic, ecological, and morphological divergence from a common ancestor. Firstly, I compared these morphs' personality profiles and their developmental plasticity in response to feeding modalities. Then, I compared their personality profiles, spatial cognitive abilities, the cognition-personality syndrome they form, their underlying neural mechanisms, and their developmental plasticity in response to structural complexity. I show that personality and spatial cognition are under strong genetic influence with low developmental plasticity, the existence of a syndrome between the two, and that genes linked to the dopaminergic pathways and to memory suppression seem particularly involved herein. The results support a model in which all these traits develop and evolve independently from each other in a "case-by-case" fashion, depending on the specific local demands of a given ecosystem. This thesis provides the very first empirical support for a co-evolution between personality and cognition, and these pioneering results encourage further research in this direction.

Útdráttur

Atferli er einstalega sveigjanlegur og margbreytilegur eiginleiki. Þrátt fyrir þennan margbreytileika er augljóst að einstaklingar innan hópa sýna ólík en mótuð hegðunarmynstur, þótt lítið sé vitað um þau ferli sem valda og viðhalda þessum mun milli einstaklinga í náttúrunni. Þessi doktorsritgerð fjallar um þá umhverfis- og þróunarferla, og þau þroskunarskilyrði sem móta tvo meginþætti hegðunar: persónuleika og rýmisskynjun. Til að rannsaka þessa þætti notaðist ég við bleikjuseiði (*Salvelinus alpinus*) af fimm ólíkum afbrigðum, þar með talin tvö pör afbrigða úr sama vatni sem eiga sér sameiginlegan uppruna en hafa aðskilist erfðafræðilega, í svip- og vistgerð. Fyrst voru persónuleikaeinkenni einstaklinga úr hinum ólíku stofnum borin saman og einnig þroskunarfræðilegur sveigjanleiki þeirra sem viðbrögð hvernig fæða var staðsett. Næst bar ég saman persónuleikaeinkenni þeirra, rýmisgreind, skynjunar-persónuleika-svipgerð, undirliggjandi taugalífeðlisfræðilega ferla, og sveigjanleika í svipgerð sem svar við margbreytileika umhverfis. Niðurstöðurnar sýna að persónuleiki og rúmskynjun eru undir sterkum áhrifum erfða, en hafa lítinn þroskunarfræðilegan sveigjanleika. Þær leiða einnig í ljós tilvist heilkennis þessara tveggja þátta, og að gen tengd dópamínörvuðum taugaferlum og minnisbælingu gegna sérlega miklvægu hlutverki hvað þetta varðar. Niðurstöðurnar styðja líkan þar sem allir þessir eiginleikar þroskast og þróast óháð hver öðrum, háð því hvers hvert vistkerfi krefst. Í rigterðinni eru ályktanir um samþróun persónuleika og skynjunar settar fram og rökstuddar. Niðurstöðurar leggja grunn að og sýna fram á mikilvægi frekari rannsókna á sviðinu.

Résumé

Le comportement animal est fascinant de flexibilité et montre une immense diversité de manifestations. Malgré cette diversité, la simple observation montre indéniablement que les individus diffèrent systématiquement les uns des autres dans leur manière de se comporter. Cependant, on en sait peu sur les mécanismes proximaux et ultimes qui sous-tendent l'émergence et le maintien de ces différences de comportement entre individus. Cette thèse vise à explorer les processus éco-évolutifs et les signaux développementaux façonnant deux aspects particuliers du comportement : la personnalité et la cognition spatiale. Pour cela, j'ai utilisé des juvéniles de cinq morphs d'ombles chevaliers, dont deux paires sympatriques, variant selon des gradients de divergence génétique, écologique et morphologique par rapport à leur ancêtre commun. Tout d'abord, j'ai comparé ces morphs en termes de profils de personnalité et leur plasticité développementale en réponse aux modalités d'alimentation. Ensuite, j'ai comparé leurs profils de personnalité, leurs capacités cognitives spatiales, le syndrome cognition-personnalité qu'ils forment, leurs mécanismes neuronaux sous-jacents et leur plasticité développementale en réponse à la complexité structurelle de l'environnement. Je montre que la personnalité et la cognition spatiale sont sous forte influence génétique avec une faible plasticité développementale, l'existence d'un syndrome entre les deux, et que les gènes liés aux voies dopaminergiques et à la suppression de la mémoire semblent ici particulièrement impliqués. Les résultats soutiennent un modèle dans lequel tous ces traits se développent et évoluent indépendamment les uns des autres, « au cas par cas », en fonction des demandes locales spécifiques d'un écosystème donné. Cette thèse apporte le tout premier support empirique en faveur d'une co-évolution entre personnalité et cognition, et ces résultats pionniers encouragent la poursuite des recherches dans cette direction.

This is who we are,

These are the roads we paved,

The strength we found in the mistakes we made along the way.

— Matty Mullins

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List of Papers

This thesis is a collection of three scientific papers and one short communication, to which I will henceforth refer to as follows:

Paper I – Dellinger, M., Steele, S. E., Sprockel, E., Philip, J., Pálsson, A., & Benhaïm, D. (2023). Variation in personality shaped by evolutionary history, genotype and developmental plasticity in response to feeding modalities in the Arctic charr. *Proceedings of the Royal Society B*, *290*(2013), 20232302. https://doi.org/10.1098/rspb.2023.2302

Paper II – Dellinger, M., Caperaa, M., Le Clech, R., Quéru, J., Philip, J., & Benhaïm, D. (n.d.). Do animal personality components independently evolve and develop in response to environmental complexity? *Animal Behaviour. Accepted.*

Paper III – Dellinger, M., Suret, L., Choblet, M., Jozet-Alves, C., & Benhaïm, D. (2024). Methodological approach to investigating spatial cognition in fishes: a case study on Arctic charr (*Salvelinus alpinus*). *Journal of Fish Biology*, 1-5. https://doi.org/10.1111/jfb.15936

Paper IV – Dellinger, M., Suret, L., Choblet, M., Ladurée, G., Vandroux., M., Lobligeois, S., Vanhussel, M., Steeley, I., Cousin, X., Jozet-Alves, C., Jónsson, Z.O., & Benhaïm, D. (manuscript). Cognition-personality syndrome and underlying neural mechanisms shaped by eco-evolutionary background and developmental plasticity in response to environmental complexity in the Arctic charr.

During the course of my PhD, as part of a wider project or as part of parallel studies, I also contributed to the following publications, which are not included in the thesis:

Philip, J., Dellinger, M., & Benhaïm, D. (2022). Among-individual variation of risk-taking behaviour in group and solitary context is uncorrelated but independently repeatable in a juvenile Arctic charr (Salvelinus alpinus) aquaculture strain. *Applied Animal Behaviour Science*, *249*, 105596. https://doi.org/10.1016/j.applanim.2022.105596

Roque d'orbcastel, E., Bettarel, Y., Dellinger, M., Sadoul, B., Bouvier, T., Monin Amandé, J., Dagorn, L., & Geffroy, B. (2021). Measuring cortisol in fish scales to study stress in wild tropical tuna. *Environmental Biology of Fishes*. https://doi.org/10.1007/s10641-021-01107-6

Dellinger, M. (2019). Le cortisol comme indicateur de bien-être chez les poissons marins : applications sur un modèle halieutique, les thons albacore (Thunnus albacares) et listao (Katsuwonus pelamis) et dans la mise en évidence de l'axe cerveau-intestin-microbiome d'un modèle aquacole, le bar commun (Dicentrarchus labrax) en condition de stress chronique. Thèse de doctorat vétérinaire, Oniris - Ecole Nationale Vétérinaire, de l'Agroalimentaire et de l'Alimentation Nantes Atlantique, Faculté de médecine de Nantes.

Authors contributions

Table 1 – CRediT Contributor Roles Taxonomy for the four Papers (I-IV) included in this thesis. MD: Marion Dellinger (Thesis Author). SS: Sarah E. Steele. ES: Evert Srockel. JP: Joris Philip. MCa: Maud Caperaa. RLC: Renée Le Clech. JQ: Julia Quéru. LS: Léo Suret. MCh: Maxime Choblet. GL: Gabrielle Ladurée. MVx: Marie Vandroux. SL: Sabine Lobligeois. MVh: Margaux Vanhussel. IS: Isabella Steeley. XC: Xavier Cousin. AP: Arnar Pálsson. CJA: Christelle Jozet-Alves. ZJ: Zophonías O. Jónsson (PhD Advisor). DB: David Benhaïm (PhD Supervisor).

Author	**Conceptualisation**	**Data Curation**	**Formal Analysis**	**Funding Acquisition**	**Investigation**	**Methodology**	**Project Administration**	**Resources**	**Software**	**Supervision**	**Validation**	**Visualization**	**Writing – Original Draft**	**Writing – Review & Editing**
MD	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	II-IV		I-IV	II-IV	I-IV	I-IV	I-IV	I-IV
SS	I	I			I	I	I							I
ES		I			I									I
JP	I				I-II	I-II								I-II
MCa					II									II
RLC					II									II
JQ					II									II
LS					III-IV	III-IV								III-IV
MCh					III-IV	III-IV								III-IV
GL					IV									IV
MVx		IV			IV	IV			IV					IV
SL					IV									IV
MVh					IV									IV
IS					IV									IV
XC	IV					IV								IV
AP	I			I		I	I	I		I	I			I
CJA	III-IV					III-IV					III-IV			III-IV
ZJ	IV					IV		IV		IV	IV			IV
DB	I-IV			I-IV	II	I-IV	I-IV	I-IV		I-IV	I-IV		III	I-IV

Abbreviations

actb — Beta-actin (qPCR reference gene).

AN — Anadromous morph from Fljótaá River.

bdnf — Brain-Derived Neurotrophic Factor.

cDNA — Complementary Desoxyribonucleic Acid (also called copy DNA).

c-fos — Cellular Fos proto-oncogene.

CI — Credible Interval (Bayesian) or Confidence Interval (frequentist).

Cq — Quantification Cycle in qPCR.

dbh — Dopamine Beta-Hydroxilase.

crebbp — Cyclic Adenosine Monophosphate-Responsive Element Binding protein-Binding Protein.

DNA — Desoxyribonucleic Acid.

dNTP — Deoxynucleotide triphosphate.

egr1 — Early Growth Response protein.

etbr2 — Endothelin B receptor-like protein 2 (sexing PCR positive control).

gr — Glucocorticoid Receptor.

HPDI — Highest Posterior Density Interval.

IEG — Immediate Early Genes.

KMO — Kayser-Mayer-Olkin index.

LB — Large Benthic Arctic charr morph from Lake Þingvallavatn.

mao — Monoamine Oxidase.

milliQ water — Deionized water purified through filters/resins/membranes of which the resistivity is 18.2 MΩ·cm (Millipore Corporation).

mr — Mineralocorticoid Receptor.

mRNA — Messenger Ribonucleic Acid.

neurod — Neurogenic Differentiation factor.

OFT — Open Field Test (with shelter, in the context of this thesis).

PC1 — First axis (*i.e.*, first component) of a PCA.

PCA — Principal Component Analysis.

pcna — Proliferation Cell Nuclear Antigen.

PCR — Polymerase Chain Reaction.

PD — Probability of Direction.

PL — Planktivorous Arctic charr morph from Lake Þingvallavatn.

pp1 — Serine/threonine Protein Phosphatase 1.

qPCR — Quantitative real-time Polymerase Chain Reaction.

RNA — Ribonucleic Acid.

RT — Reverse Transcription.

SdY — Sexually Dimorphic on the Y-chromosome gene.

TAE — Tris Acetate Ethylenediaminetetraacetic acid.

ub2l3 — Ubiquitin-conjugating enzyme E2 L3 (qPCR reference gene).

VB — Brown Arctic charr morph from Lake Vatnshliðarvatn.

VS — Silver Arctic charr morph from Lake Vatnshliðarvatn.

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1 General Introduction

Behaviour has always carried a particular status among biologists because it fascinatingly is an extremely flexible phenotype with immense diversity of manifestations: it plays a fundamental role in the interaction between the organism and all aspects of its environment, determining where, when, and how animals live, exploit resources, regulate homeostasis and interact with other organisms (preys and predators, competitors, potential mates, progeny). Behaviour is both genetically determined and highly sensitive to environmental inputs. It is the most immediate response by which organisms can attempt to accommodate to changes in their environment and cope with new conditions. Behavioural change can consequently happen faster than adapting physiological and morphological traits and therefore should be taking the lead in evolution (West-Eberhard, 2003). In this sense, behaviour can be viewed at once as an object, *i.e.,* submitted to and shaped by evolutionary forces (evolutionary consequence), and as a subject, *i.e.,* actively participating in evolutionary processes (evolutionary cause) (Bateson, 2004; Bogert, 1949; Levins & Lewotin, 1985; Lewotin, 1983; Mayr, 1963). This manuscript is going to develop this concept and emphasize how it can be linked to the evolution of two particular aspects of behaviour: cognition* and personality.

1.1 Animal Cognition

Ulric Neisser (1967) defines cognition as "*all the processes by which the sensory input is transformed, reduced, elaborated, stored, recovered, and used*". Organisms endowed with cognitive abilities can assimilate sensory information from their environment which are internally computed, possibly retained on shorter or longer term. This processed information can then be reused to guide the said organisms' in choosing and implementing actions directed towards a specific goal (R. A. Wilson & Keil, 1999). According to Tim Bayne (Bayne et al., 2019), for a state or a process to be cognitive, it must necessarily involve the use of concepts. He describes how concepts hold two central features: firstly, stimulus-independence, which means that the organism must be capable of decoupling the concepts from the perceptual world, withholding representations of a concept in its perceptual absence (Bayne gives the example of the ability to represent a lion independently from currently experiencing one); and secondly, concepts can be systematically recombined with each other to form new information (he exemplifies it through the concepts of "lion", "me", "eat", and "want", that can be combined into "the lion wants to eat me" or recombined into "I want to eat the lion"). Cognition enables to form new information by combining information, collected in separate events or through spontaneous processes like insight (Lars Chittka, in Bayne et al., 2019). Hence, cognition includes a wide variety of mental processes (Shettleworth, 2010), such as perception (transformation of raw sensory data into interpretable information), attention (selective information processing), thinking (manipulation of information, *e.g.*, reasoning, decision-making, problem-solving, creating),

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* As described in the next section, cognitive processes are purely mental mechanisms. Nevertheless, as these mechanisms are manifested and measured through behavioural proxies, for simplification purposes, I use the term behaviour as encompassing cognition.

or memory (encoding, storage and retrieval of information). Learning is a cognitive process that involves several other cognitive processes., *e.g.*, perception and memory, usually defined as a change in behaviour resulting from information stored from previous experience (Breed & Moore, 2016a). Cognitive styles refer to the ways individuals consistently display differences in how they acquire, process store and use information. For example, differences in the speed and accuracy with which different types of information are learned, or preference in relying on new rather than past information for problem solving (Sih & Del Giudice, 2012). I will now present the aspects of animal cognition that are of interest in this thesis.

1.1.1 Associative Learning

Also coined conditioning, associative learning is a primitive form of causal induction resulting from experiencing relations between events, or between actions and outcomes, in other words, it is the learning of cause-effect associations (Breed & Moore, 2016a; R. A. Wilson & Keil, 1999). During associative learning, organisms integrate an internal representation of the causal structure of their environment and, if there is divergence between this representation and the experienced reality, they adjust it to match the actual causal structure of the world (R. A. Wilson & Keil, 1999). Conditioning processes are important determinants of fitness for animals, as it allows them to learn and act efficiently upon all sorts of vital contingencies in their environment, such as which cues reliably signal food availability, which actions ensure effective resource collection, or which events indicate a danger. Associative learning is divided into two major forms of conditioning: classical and operant conditioning.

Classical conditioning is also known as Pavlovian conditioning, due to Ivan Pavlov's famous experiment in which a dog salivates at the sight of food, but also salivates at the sound of a bell even in the absence of food, provided that this bell sound had previously been paired repeatedly with food delivery (Pavlov, 1927). Classical conditioning occurs when a reflexive, involuntary response (salivation in Pavlov's experiment), unconditionally triggered by a relevant stimulus (the unconditioned stimulus, here the food), can also be automatically triggered by another neutral or irrelevant stimulus (the conditioned stimulus, here the bell sound) conditionally signalling the unconditional stimulus (Gottlieb & Begej, 2014; R. A. Wilson & Keil, 1999). More generally, classical conditioning occurs when individuals adjust their response after experiencing temporal association between environmental or proprioceptive stimuli (Gottlieb & Begej, 2014).

In operant conditioning (Skinner, 1953), also called instrumental conditioning, the animal learns to voluntarily operate on its environment to produce a consequence (Murphy & Lupfer, 2014). This form of learning involves a change in the frequency of a behaviour according to its consequence (Figure 1): consequences increasing the likelihood of a behaviour are called reinforcements while those decreasing the likelihood of a behaviour are called punishments (Breed & Moore, 2016a; Murphy & Lupfer, 2014). Positive reinforcements are the addition of pleasant stimuli following a targeted behaviour, which increases the behaviour's rate (classically called a reward), while in the case of negative reinforcements, the targeted behaviour terminates (escape procedures) or prevents (avoidance procedures) aversive stimuli, which increases the probability of the behaviour. Positive punishments are noxious stimuli following the behaviour, which decreases the behaviour's frequency, while negative punishment consist in the removal of a desired stimulus following the behaviour, which decreases the frequency of this behaviour (Breed & Moore, 2016a; Murphy & Lupfer, 2014).

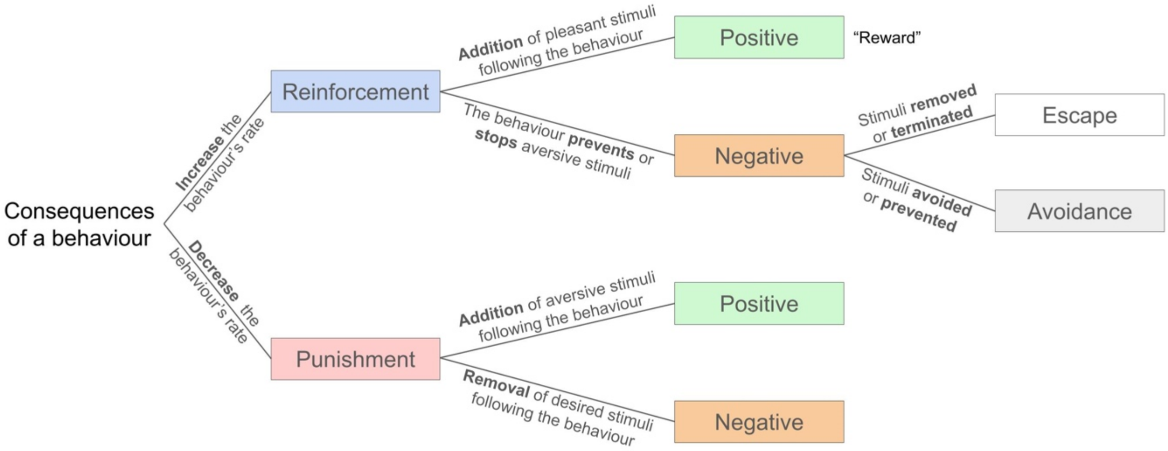


Figure 1 – In associative learning, a behaviour is associated with consequences. Depending on whether the consequence increases or decreases the frequency of this behaviour, the consequencial procedure is called a reinforcement or a punishment. Procedures consisting of the addition of stimuli are called positive (e.g., a positive reinforcement, often called "a reward"), while procedures consisting of the removal or prevention of stimuli are called negative (e.g, negative reinforcement where the behaviour either stops (escape procedure) or prevents (avoidance procedure) the occurrence of an aversive stimulus).

1.1.2 Spatial Cognition

Spatial cognition is defined by David Waller and Lynn Nadel as "*a branch of cognitive science that seeks to understand how humans and other animals perceive, interpret, mentally represent, and interact with the spatial characteristics of their environment. These characteristics include object and scene properties, such as size, shape, and scale, as well as relations among objects, such as distance, direction, orientation, and location.*" (Waller & Nadel, 2013). An organism uses spatial cognition to determine where it is, where (not) to go, and how to find its way orienting and navigating from one departure point to a goal point. Orientation is the alignment of the body in a given direction (Capaldi et al., 1999). Navigation is the process of using cues to determine and maintain a goal-directed trajectory from one location to another (Breed & Moore, 2016b; Capaldi et al., 1999; Darmaillacq et al., 2018). Specifically, spatial learning refers to the formation of memories allowing later place discrimination by reference to the surroundings, and position discrimination as defined by the learner's relative orientation (Capaldi et al., 1999). In other words, spatial learning is the ability for an animal to learn and remember the locations of places, objects, and itself, in the environment. The evolution of spatial cognition is of particular interest because all animals rely on orientational and navigational skills to find food or mates, retrieve the nest, escape predators, and, most markedly, for migration in some animals. Hence, spatial cognitive abilities have a crucial impact on individuals' fitness and are consequently subject to natural selection (Morand-Ferron, 2017; Morand-Ferron et al., 2016) (see section 1.6 for further development). The identification of individual variations in cognitive traits is therefore essential to investigate their evolution (Lucon-Xiccato & Bisazza, 2017b; Rowe & Healy, 2014).

Types of Spatial Information & Reference Frames

The information cues used for navigation can be of different sensory nature. Idiothetic information are internal information directly generated by the organism's movements (*e.g.*, proprioceptive, kinesthetic or vestibular signals), informing about positions and changes of positions of the body (Breed & Moore, 2016b; Darmaillacq et al., 2018; Tommasi et al., 2012). Allothetic information are external information stemming from the environment, which are independent from the organism's movements, and can be visual, auditive, olfactory, tactile, or even magnetic (Breed & Moore, 2016b; Darmaillacq et al., 2018; Tommasi et al., 2012). Among allothetic information, one can find landmarks and compasses. A landmark is a significant feature in the environment with a stable location (*e.g.*, a particular tree or a scent marking), while a compass is a biological mechanism allowing to assess angular directions (*e.g.*, light polarization, geomagnetic field inclination, stars movements) (Breed & Moore, 2016b).

The system of reference in which the subject represents and uses these cues for navigation are called reference frames (Figure 2). The egocentric reference frame is a self-centred reference, where spatial information is encoded relative to the body and its different axes (Darmaillacq et al., 2018; Trullier et al., 1997): "I need to go to that tree to reach my goal" (allothetic); "The goal is on my left" (idiothetic). The allocentric reference frame is fully independent of the animal's position in space: the location of a place or an object is encoded relative to other objects or landmarks in the environment (Darmaillacq et al., 2018; Trullier et al., 1997): "The goal is between the tree and the rock" (allothetic). In this manuscript, we will focus on the egocentric reference frame, which itself entails different possible navigation strategies (Arleo & Rondi-Reig, 2007; Darmaillacq et al., 2018; Trullier et al., 1997): path integration (also called dead reckoning), beaconing (also called guidance), and motor response (also called route-based) strategies.

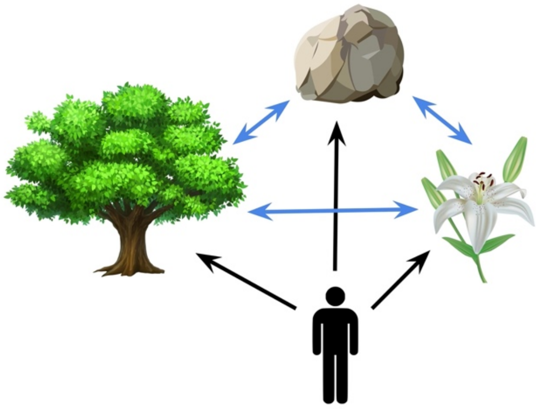


Figure 2 – Reference frames. In the egocentric reference frame, the position of objects in space is encoded relative to the axes of the observer's body (black single-sided arrows). In the allocentric reference frame, the position of obejcts in space is encoded relative to other objects, places or landmarks (blue double-sided arrows).

Path Integration Strategy

The path integration strategy (Figure 3) only involves internal estimations of direction and distance between the animal and its start point. The animal constantly updates its knowledge of its current location by integrating the path created by its movements, speed, trajectories, and time elapsed since the start point. It is then able to calculate the shortest direction and distance vector to return directly to the start point in a straight line. This strategy is hence based fully on idiothetic information and is useful in the absence of environmental cues, the animal relying solely on its odometry (ability to estimate distances, for example by counting steps (pedometry), by the energy expended during travel, or through the speed at which images go by the eye while moving (optical flow)) (Breed & Moore, 2016b; Darmaillacq et al., 2018). However, this strategy can be very sensitive to computation errors (Darmaillacq et al., 2018).

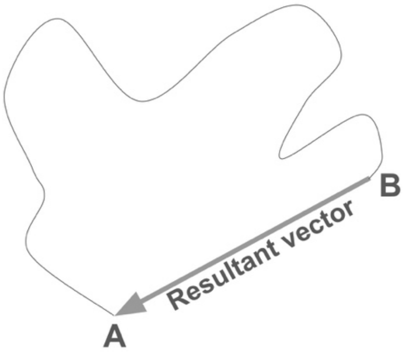


Figure 3 – Path integration strategy: integration of movements and trajectories from point A to point B and generation of a resultant vector returning directly to point A.

Beaconing Strategy

The beaconing strategy uses a single salient landmark in the environment, called a beacon, that is either the goal itself, or that signals the goal. The animal simply orients and moves towards (or away from) the landmark to reach its goal, necessitating the animal to learn a unique egocentric stimulus-response association ("I move towards the tree"). This strategy relies entirely on allothetic information (a visual cue, a scent, a sound, etc) and requires only limited cognitive resources (Arleo & Rondi-Reig, 2007; Darmaillacq et al., 2018). However, it requires the beacon to be availably perceived by the animal to be used (which can be thwarted by distance; light, noise or chemical pollution; or weather conditions for example), and the beacon must remain stable in location and aspect to be reliable.

Motor Response Strategy

In the motor response strategy, the animal learns a sequence of Pavlovian stimulus-locomotor response associations where reorientations of the body need to be performed in a precise order to reach the goal. This strategy is appropriate in situations where the goal is not directly perceivable or signalled, and one or an ensemble of cues can be associated to changes in direction (choice points) towards the goal. These cues can be either idiothetic (where the cue is the movement itself: "After I turn left, I turn right"), or allothetic ("I turn right at the tree, and then left at the rock") (Darmaillacq et al., 2018). Like the beaconing strategy, the motor response strategy requires only limited cognitive processing, and the more the path is repeated, the more the sequence is reinforced, which progressively reduces the level of attention needed to navigate. This strategy is however inflexible and easily disturbed by unforeseen obstacles occurring in between learnt choice points, necessitating learning a new motor sequence (Arleo & Rondi-Reig, 2007; Darmaillacq et al., 2018).

1.1.3 Caveats and Biases in Cognitive Testing

Many confounding factors can bias the interpretation of results originating from cognitive tests. These include stress, personality traits, sex, motivation (willingness to perform a task), differences in perception, brain lateralization, previous experiences and unlearnt preference biases of individuals tested (Dunlap et al., 2019; Herrero et al., 2006; Lucon-Xiccato & Bisazza, 2017b; Morand-Ferron et al., 2016; Oitzl et al., 2010; Rowe & Healy, 2014; Salena et al., 2021), variables that have to be controlled for during cognitive experiments. Exposure to stressful events or conditions is known to reduce participation in cognitive tasks, impair behaviour, cognitive abilities and neural plasticity (Galhardo & Oliveira, 2009; Salena et al., 2021; Sørensen et al., 2013). Consequently, special attention should be carefully paid to avoiding stressful procedures in methodological designs in order to accurately assess cognitive abilities, but also behavioural traits in general (Salena et al., 2021). The different levels of attractiveness for the reward between individuals can also bias the response patterns, as some individuals might lack motivation to obtain it compared to others (Van Der Harst et al., 2003; Wood et al., 2011). In the guppy (*Poecilia reticulata*) for example, access to food is more attractive for females than for males that hence do not participate much in cognitive tasks rewarded with food, while access to females is a more ecologically relevant reward for male guppies engendering greater participation (A. Kotrschal et al., 2015). Further, lateralisation that induces a preference for a particular side in animals (Vallortigara & Rogers, 2005) can bias orientation choices in cognitive mazes (Alves et al., 2007; Andrade et al., 2001; Brown & Braithwaite, 2005). Hence the need to test, ahead of cognitive experiments, for an adequate and ecologically-relevant reward among tested individuals, as well as for individual side-turning preference. The personality of an individual (see section 1.2) is described as differences in behaviours among individuals that are consistent across time and/or contexts (Réale et al., 2007; Sih, Bell, Johnson, et al., 2004). Cognitive abilities are usually measured through behavioural proxies, but by definition, the behaviour of an individual is strongly influenced by its personality; conversely, the way an individual perceives and processes information extracted from its environment may influence its systematic behavioural reactions to its surroundings, *i.e.*, the personality of an individual is also dependent on its cognitive abilities (Adolphs & Xu, 2024; Guillette et al., 2017). This intrinsic interdependence between personality and cognition implies that those two aspects cannot be decoupled (Lucon-Xiccato & Bisazza, 2017b; Morand-Ferron et al., 2016) and must be assessed simultaneously (see further development in section 1.3).

1.2 Animal Personality

Animal personality, also referred to as temperament, or behavioural individuality (Bierbach et al., 2017; Gosling, 2001; Koolhaas et al., 1999; Réale et al., 2007; Sih, Bell, Johnson, et al., 2004), is an exponentially growing field of research since the last two decades. It has been recognized that individual variations in behaviour cannot merely be considered noise around and adaptive mean (Dall et al., 2004). Animal personality is defined as among-individual differences in behaviour that remain consistent across time and/or contexts (Réale et al., 2007; Sih, Bell, Johnson, et al., 2004). Although any repeatable behaviour across time or context (see next section) can technically be considered a personality trait (Dingemanse & Wright, 2020), there are currently five axes of personality, or personality traits, widely recognized in animals (Réale et al., 2007): exploration-avoidance is the individual's willingness to investigate a new object or environment; activity refers to the general level of activity in a familiar environment; aggressiveness represents the agonistic reaction towards conspecifics; sociability is the individual's reaction to the presence or absence of conspecifics, excluding aggressive behaviours. Finally, shyness-boldness is defined by Réale and colleagues (2007) as the individual's reaction to any risky but not novel situation, while Toms et al. (2010) argues that tests involving novelty are best suited to capture boldness (empirically supported by Perals et al., 2017). I will hence consider a general definition of boldness—that does not pertain to the notion of novelty—as the individual propensity to take risks (D. S. Wilson et al., 1994).

The bold-shyness personality trait is especially linked to the coping style theory (Koolhaas et al., 1999), a concept closely related to personality. Coping styles correspond to a suit of correlated behavioural, cognitive and physiological traits (see also behavioural syndromes in section 1.3) along a continuum of reactive to proactive styles that are adapted for dealing with environmental changes: proactive individuals are bolder, more explorative and aggressive, create inflexible routines, are quick but inaccurate learners, and respond to stressors in a fight-or-flight manner with elevated circulating catecholamines (predicted to perform better in stable and predictable environments), while on the contrary, reactive individuals are shyer, slow but accurate learners with flexible behaviours, and use freeze-and-hide strategy with elevated corticoid response when subjected to stressors (predicted to be favoured in unpredictable and changing environments) (Koolhaas et al., 1999; Sørensen et al., 2013).

Elucidating the emergence and ontogeny of personality is currently a pressing question in the field, as it is a paramount step towards the understanding of proximate and ultimate implications of animal personality, yet remaining understudied (Cabrera et al., 2021; Stamps & Groothuis, 2010). Personality traits have been shown to be partially genetically determined and sometimes highly heritable (Bell et al., 2009; Dochtermann et al., 2015). However, they are also plastic traits that can be shaped by environmental factors (*e.g.*, Xu et al., 2021). Several theoretical models suggest proximate and ultimate mechanisms for how different personality traits appear and coexist among individuals within and between populations (reviewed in Dingemanse & Wolf, 2010; Laskowski et al., 2022; Wolf & Weissing, 2010). For example (see section 1.5.1 for further development), personality differences could arise from individual differences in life-history strategies (Réale et al., 2010), or from individual differences in state that can be either directly due to heterogeneous environmental factors or indirectly due to differences in parental contributions (Reddon, 2012). Positive or negative feedbacks between an individual's behaviour and its state (somatic, information, social rank, etc) could engender progressive and consistent divergence (fanning-out) or convergence (fanning-in) of behavioural trajectories across individuals (Ehlman et al., 2022; Sih et al., 2015; Stamps & Biro, 2016). It has even been shown that personality emerges in individuals even when drastically controlling for the absence of any genetic and environmental differences in clonal species, suggesting that personality could also arise from stochastic chaos dynamics during development (Bierbach et al., 2017; Fisher et al., 2018; Polverino et al., 2016).

1.2.1 Personality Components

At the heart of the concept of personality, is the notion of behavioural consistency, which applies both within and among individuals (Sih, Bell, Johnson, et al., 2004). Within-individual consistency is the idea that a given individual will display similar behaviour across observations (also called individual stability, Stamps & Groothuis, 2010). Among-individual consistency implies the idea of stably ranked behaviour among individuals in a group across observations/contexts (*e.g.*, the most aggressive individual in a safe context becoming less agonistic in the presence of predators, but still exhibiting the highest number of aggressions when compared to the others). This point entails that, despite the idea of within-individual consistency, personality traits can still be plastic over time and/or contexts. It appears that a single personality trait in a group of individuals implies several different aspects, that can be broken down into several personality components: mean, within- and among-individual variabilities, repeatability, and plasticity (Figure 4).

The mean component represents the average behaviour over the group and across behavioural measurements. To assess behavioural consistency—the prerequisite qualifying a personality trait by definition—it is necessary to measure behaviours for each individual in the group not only once, but repeatedly (Dingemanse & Wright, 2020). This gives access to among- and within-individual variances components ($Vamong$ and $Vwithin$ respectively) (Royauté & Dochtermann, 2021). $Vamong$ represents the group-level variance in behaviour, *i.e.*, how much individuals behave differently from each other, within and across behavioural measurements. $Vwithin$ represents the variance in behaviour at the individual level, *i.e.*, how much an individual behaves differently from itself across repeated behavioural measurements, representing within-individual consistency. Hence, $Vwithin$ is itself composed of individual plasticity (systematic individual changes in behaviour across contextual gradients or time), and predictability (residual variance after accounting for $Vamong$, individual plasticity, and covariates and confounding factors that could systematically change individuals' behaviour) (see Stamps et al., 2012, Biro & Adriaenssens, 2013, and Jolles et al., 2019 for more details on individual plasticity and predictability). $Vamong$ and $Vwithin$ are needed for the calculation of the behaviour's repeatability. Repeatability (R) evaluates the proportion of the total variance that is due to among-individual variance in the behaviour (Dochtermann et al., 2015), corresponding to the behavioural consistency at the group level over repeated measures. In other words:

$$R = \frac{Vamong}{Total\,Variance} = \frac{Vamong}{Vamong + Vwithin}$$

Phenotypic plasticity is the capacity of a single genotype to produce different phenotypes in response to varying environmental conditions (Whitman & Agrawal, 2009). This is observed through differences in average traits across different conditions, known as reaction norms. The plasticity component of personality could itself be broken down into several subtypes of plasticity (Stamps, 2016), but in this thesis, I will focus on developmental plasticity of personality (see also section 1.5.1), which is the variation in personality across individuals within a given genotype, at a same age and time, as a function of the conditions in which these individuals developed (Stamps & Groothuis, 2010). In other words, developmental plasticity of personality corresponds to differences in personality between same-age individuals from the same genotype that experienced different past conditions (Stamps & Biro, 2016). Personality plasticity might sound like an oxymoron, since a fundamental attribute of personality is behavioural consistency, while plasticity implies variation. However, a behaviour being plastic does not prevent it from being consistent among individuals across time and/or contexts: Figure 4 illustrates how individuals (or genotypes) globally increase their behavioural score across measurements (personality plasticity either across time or contexts), but also individuals from a same genotype globally differ across environments they were raised in (personality developmental plasticity), but individuals still conserve their behavioural ranking among each other (repeatable personality trait). Developmental plasticity can even extend to variabilities (Akhund-Zade et al., 2019), as shown in Figure 4, where within- and among-individual variances were increased in environment II compared to environment I.

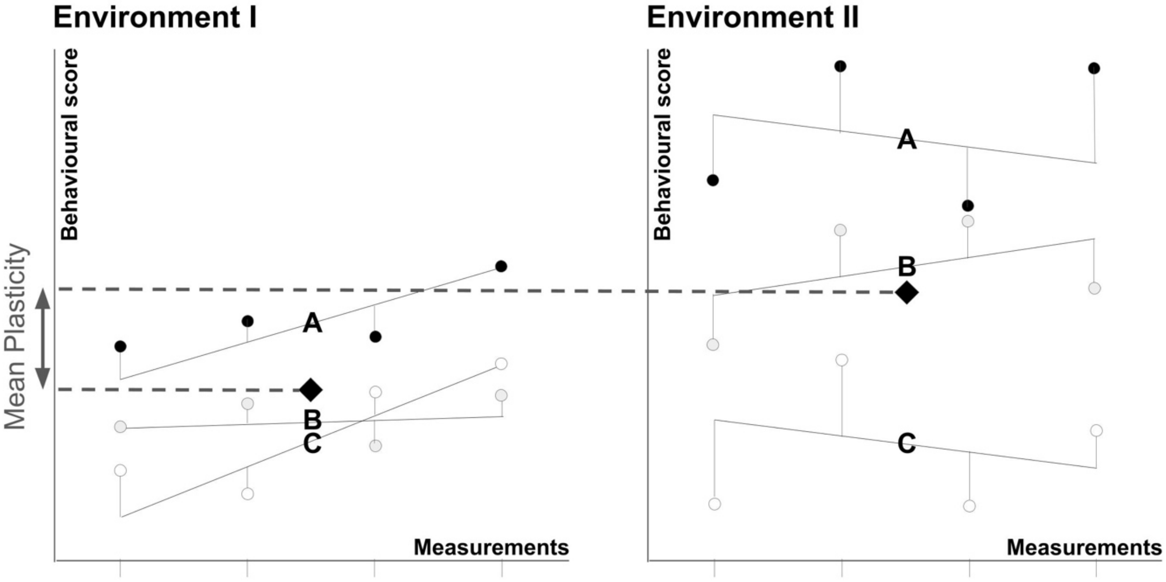


Figure 4 – Reaction norms for the behavioural score repeatedly measured (either over time or in different contexts), in three hypothetical individuals or genotypes (A black; B grey; C white; each dot being a behavioural data point) that developed either in the environment I or in the environment II. The letters represents the mean personality trait for each respective individual or genotype. The space between the letters hence represents the among-individual variance (Vamong). The slopes represent the plasticity over the measurements: e.g., individual/genotype B in environment I does not show much plasticity compared to A and C that both increase their behavioural score over measurements (positive slopes). The slope and the vertical lines departing from the data points of an individual/genotype represent together the within-individual variance (Vwithin). The behaviour is more repeatable in individuals/genotypes that developed in environment II, as individuals/genotypes rank order is more conserved over measurements than in environment I. The diamond represents the overall mean personality score over all individuals/genotypes and measurements. This overall score, Vamong, and Vwithin, are higher when individuals/genotypes developed in environment II than in environment I, i.e., the mean, Vamong, Vwithin and the repeatability components of this personality trait are developmentally plastic. All individual/genotypes differ in their developmental plasticity: for example, individual/genotype C has a constant mean personality over the two environments, hence the mean component for this individual/genotype is not developmentally plastic, compared to A and B that display increased mean personality when they developed in environment II compared to environment I.

1.2.2 Personality Profiles

Different groups of individuals can display varying combinations of values for each component of a given personality trait. For example, in Figure 4, the group that developed in environment I shows low the mean, variances and repeatability components of the personality trait, with an intermediate level of plasticity (individual/genotype B showing constant behaviour across measurements). Contrastingly, the group that developed in environment II shows high values for all these personality components. One could envision a third group characterized by a high mean personality trait, low *Vamong* variance, intermediate *Vwithin* and repeatability, and no plasticity. A particular combination of values for each component of a given personality trait forms what I term a personality profile. Investigating the developmental trajectories of all personality components—in other words, the ontogeny of personality profiles—most likely offers a more exhaustive and comprehensive picture of how personality emerges, develops, and evolves (Akhund-Zade et al., 2019), than focussing on the mean component only—as is the usual approach. For instance, high *Vwithin* might suggest behavioural flexibility, enabling populations to respond to environmental disturbances within generations, while high *Vamong* offers wider opportunities for adaptive evolution across generations (Dingemanse et al., 2022). Indeed, natural selection occurs when traits differences among individuals causes fitness differences. Hence, studying variation in personality profiles within and between species may bring new insight into animals' adaptability to environmental change. Personality mean, variabilities, repeatability, and plasticity might be developing and evolving independently from each other. This could grant populations with the ability to come up with different combinations of those components, providing a wide diversity of behavioural phenotypes to adapt more accurately to subtleties of a broad array of environmental demands (Lande, 2015)

1.3 Behavioural and Cognitive Syndromes

When two or more personality traits correlate with each other (either positively or negatively), they form what is called a behavioural syndrome (Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, et al., 2004). For example, individuals exhibiting higher boldness often are also more aggressive, more active, and less social (Luchiari & Maximino, 2023). Behavioural syndromes are most likely of eco-evolutionary relevance (developed in sections 1.5 and 1.6), as Dingemanse and colleagues (2007), found that exploration, activity and aggressiveness were all positively correlated in some populations of three-spined sticklebacks (*Gasterosteus aculeatus*) from the North Wales, but not in all 12 populations tested, as a result of differential exposure to predation risk; while Bell & Aubin-Horth (2010) in a Canadian population of the same species, found that boldness positively correlated with aggressiveness but not with exploration and activity, the latter two positively correlating together.

In section 1.1.3, I mentioned the interdependence between personality and cognition. This is actually not a new concept, as Pavlov, back in the early 1900's, had already described four personality types related to excitability in dogs that highly influenced associative learning styles (*e.g.*, behaviourally inhibited dogs showing difficulties to learn compared to "lively" dogs, Carere & Locurto, 2011). More recently, and in line with the coping style theory predictions (see section 1.2, Koolhaas et al., 1999), a link between cognitive styles and personality was described, leading to speed-accuracy trade-off where more "hasty" and "impulsive" individuals (bolder, more active, and more aggressive) learn or make decisions faster but with lower accuracy than "careful" subjects (Chittka et al., 2009; Mettke-Hofmann, 2014; Sih & Del Giudice, 2012). This syndrome has so far received mixed support (Dougherty & Guillette, 2018). For instance, exploration in red junglefowls (*Gallus gallus*) was either negatively or positively correlated with the acquisition of a reversal learning task, depending on the age class (Zidar et al., 2018). In male broiler chickens (*Gallus gallus domesticus*), more explorative individuals had exhibited poorer motor regulation capacity and poorer spatial memory (Ferreira et al., 2019, 2020). Shy three-spined sticklebacks took longer to learn to discriminate colours, but did not differ from bold fish in reversal learning (Bensky et al., 2017). This intrinsic interdependence between personality and cognition is currently raising scientists' attention (*e.g.*, quite recent special issue by Guillette et al. (2017), meta-analysis by Dougherty & Guillette (2018), doctoral theses (Mazza, 2018; Prentice, 2021), or brand-new theoretical mechanisms proposed (Buenhombre et al., 2024) on the subject). More and more evidence implies that cognition and personality cannot be decoupled (Lucon-Xiccato & Bisazza, 2017b; Morand-Ferron et al., 2016), to such an extent that those two aspects are thought to have co-evolved (Carere & Locurto, 2011). In that sense, personality traits and cognitive abilities could form a behavioural syndrome as well.

The relationship between personality traits and spatial behaviours (such as movement, dispersal and migration patterns) is progressively galvanizing research in ecology (e.g., Beukeboom, 2023). For example, it has been shown in European hares (*Lepus europeaus*, Stiegler et al., 2022) and in bank voles (*Myodes glareolus*, Schirmer et al., 2019) that shy and bold individuals differed in their space use and home ranges. Stuber and colleagues (2022) recently put forward that spatial behaviours can exhibit a high among-individual consistency in animals, leading the authors to coin the term "spatial personality". Since spatial behaviours inherently rely on spatial cognitive abilities, this concept also implies a link between spatial cognition and personality. More specifically, given the mentioned examples linking boldness and movement patterns, links between boldness personality trait and spatial cognitive performances emerge as correlations worth of interest. To my knowledge, these correlations have so far been investigated in only a handful of empirical studies per taxonomic groups: eight in rodents (Brust & Guenther, 2015, 2017; Delacoux & Guenther, 2023; Guenther et al., 2014; Guenther & Brust, 2017; Kazlauckas et al., 2005; Mazza et al., 2019; Schuster et al., 2017); seven in birds (Bousquet et al., 2015; Chen et al., 2022; Garnham et al., 2019; Gibelli & Dubois, 2017; Medina-García et al., 2017; Titulaer et al., 2012; Watrobska et al., 2023); ten in fishes (Bannier et al., 2017; Bensky & Bell, 2020; Daniel & Bhat, 2020, 2023; Gibelli et al., 2019; Kareklas et al., 2017, 2018; Liu et al., 2022; Sommer-Trembo et al., 2017; S. L. White et al., 2017); three in reptiles (Carazo et al., 2014; De Meester et al., 2022; Goulet et al., 2018); and one in insects (Doria et al., 2019). Moreover, these studies provided contradictory results, reporting positive, negative, or no spatial cognition-boldness correlations. These inconsistencies are probably due to intrinsic inter- and intra-specific differences in biology, variations in cognitive abilities considered across studies, but also most likely due to wide differences in the methodologies used to measure both boldness and spatial cognitive traits (Gibelli et al., 2019). As a result, a clear need for harmonisation and standardisation to allow comparisons is highlighted. All in all, this makes spatial cognitive styles-boldness a particularly promising syndrome to explore (Dougherty & Guillette, 2018).

1.4 Neural Bases of Spatial Cognition and Boldness Personality Trait: a Focus on Fishes

1.4.1 Brain Regions Involved in Spatial Cognition

The brain is extremely responsive to all kinds of environmental stimuli experience by the individual (Kolb, 2018), which is manifested by two major kinds of plastic responses: structural reorganisation of the neural networks, and biochemical switching involving neuromodulators (see Sørensen et al. (2013) for a review).

In fishes particularly, one of the major neurogenic zones in the brain (Figure 5) is located in the telencephalon (part of the forebrain, or prosencephalon, A. Kotrschal & Kotrschal, 2020), specifically in the lateral division of the *area dorsalis telencephali* (Dl), also called lateral pallium (Øverli & Sørensen, 2016). This zone is homologous to the hippocampus which is the nervous centre controlling mood, learning and memory (Wingenfeld & Wolf, 2014) and especially the centre of spatial cognition in mammalians (Calvo & Schluessel, 2021; Rodríguez et al., 2021). The telencephalon is involved in more complex spatial information processing such as allocentric map-like representation of space (Ebbesson & Braithwaite, 2012). Interestingly, the size of the lateral pallium correlates with environmental complexity (Park & Bell, 2010; Shumway, 2008), supposedly because a structurally complex environment requires encoding of more spatial information. Accordingly, cell proliferation in the telencephalon is significantly increased in zebrafish (*Danio rerio*) reared in structurally enriched tanks compared to plain tanks (von Krogh et al., 2010). Even more precisely, the ventral subdivision of the Dl (Dlv) might be the substratum of cognitive mapping abilities, while its dorsal subdivision (Dld) might be homologous to the mammalian isocortex, responsible for allocentric and relational strategies (Durán et al., 2010; Rodríguez et al., 2021; Salas et al., 2017). The medial division of the dorsal telencephalon (Dm) is believed to be homologous to the mammalian amygdala, potentially important in personality and spatial cognition due to its implication in emotional and motivational aspects of learning, memory, and behaviour (Broglio et al., 2003; Salas et al., 2006, 2017).

The cerebellum is another brain region responsible for spatial cognition (part of the hindbrain, or rhombencephalon, A. Kotrschal & Kotrschal, 2020), involved in simpler spatial information processes such as associative spatial learning and egocentric reference abilities (beaconing and motor response strategies) (Calvo & Schluessel, 2021; Salas et al., 2017; Thompson & Steinmetz, 2009).

The optic tectum in teleost fishes (part of the midbrain, or mesencephalon, A. Kotrschal & Kotrschal, 2020), equivalent to the superior colliculus in mammals, is also an important actor processing beaconing and motor response egocentric navigation strategies. This region garners and integrates sensory information from many sensory organs (*e.g.*, visual, auditory, somatosensory, lateral line) and is directly connected to the pre-motor cells in the brain stem activating motoneurons (Rodríguez et al., 2021). The optic tectum is responsible for coordinating vision, postures, and movements, governing orientation towards—or avoidance of—a stimulus, and is hence an essential centre for generating egocentrically referenced actions in space (Rodríguez et al., 2021). Interestingly, guppies (*Poecilia reticulata*) subjected to a spatial learning task had larger relative size of brains and optic tecta than untrained fish or fish trained in a reversal learning task (Fong et al., 2019). In the same species, the size of the optic tectum was shown to positively correlate with colour discrimination learning performances (a task closely related to beaconing navigation), while the size of the telencephalon was positively correlated to reversal learning—a sign of cognitive flexibility (Triki et al., 2022). Similarly, during learning in a task also assimilable to a beaconing navigation learning task rewarded with food, notable brain zones activated in the zebra mbuna cichlid fish (*Pseudotropheus zebra*) were the optic tectum and the ventral subdivision of the central division of the dorsal telencephalon (cerebellum was however not tested) (Calvo, Hofmann, et al., 2023).

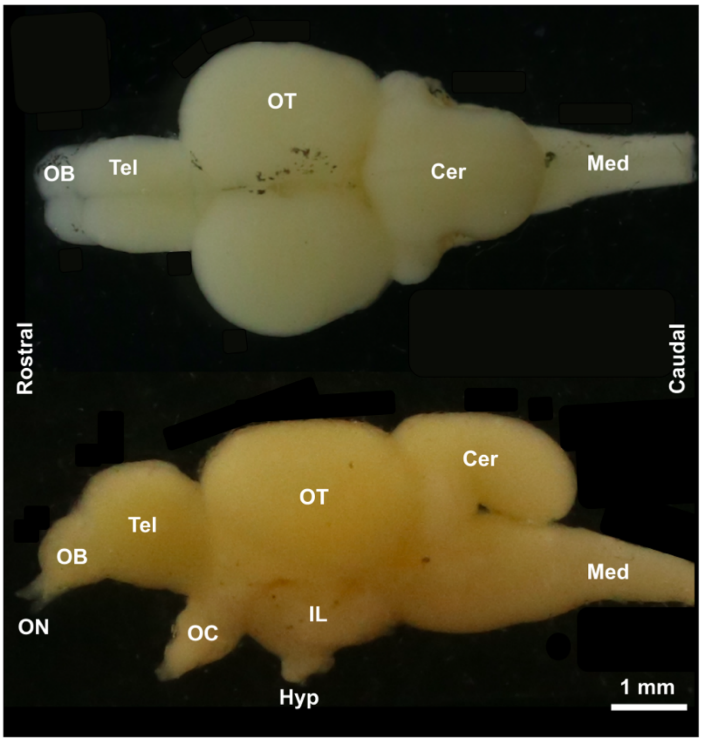


Figure 5 – Photographs of juvenile Arctic charr brains (approx. 500 days post hatching) fixed in formaldehyde baths, in dorsal (top) and lateral left (bottom) views. The white line in the bottom right corner gives the scale. Cer: Cerebellum. Hyp: Hypophysis. IL: Inferior lobes of the hypothalamus (paired). Med: Medulla. OB: Olfactory bulbs (paired). OC: Optic chiasm. ON: Olfactory nerve (paired). OT: Optic tectum (paired). Tel: Telencephalon (paired).

1.4.2 Molecular Markers Linked with Cognition and Personality

Cognition-personality associations correlate with drastic differences in brain neurogenesis patterns between personalities. For instance, shy individuals display a higher neural plasticity (Alfonso et al., 2019; Sørensen et al., 2013). Consequently, shyer individuals exhibit higher learning performances than bold individuals (Benhaïm et al., 2017; Ferrari et al., 2014). In this section, I will expose molecular markers that can be associated with spatial cognition and boldness personality trait in fishes, using examples from other taxa when relevant studies on teleosts are, to the best of my knowledge, lacking in the literature.

Markers Linked with Neurogenesis and Neural Plasticity

We can estimate brain plasticity through the gene expression quantification of some markers of neurogenesis, neuronal activity, and neuronal development (Sørensen et al., 2013).

The proliferating cell nuclear antigen (*pcna* gene, Mandyam et al., 2007) characterizing newborn cells is a marker of neurogenesis. It showed increased expression levels in the telencephalon of fishes reared in structurally enriched tanks compared to plain tanks, both in terms of more numerous nuclei of PCNA-marked cells in the zebrafish (von Krogh et al., 2010), and in terms of higher mRNA levels of *pcna* in the rainbow trout Salmonid (*Oncorhynchus mykiss*, Cardona et al., 2022). On the contrary, *pcna* expression was reduced in the telencephalon of zebrafish reared in enriched conditions, which was also associated with lower spatial avoidance learning, potentially due to a different appraisal of negative stimuli relative to barren-reared fish (Manuel et al., 2015). *Pcna* has also been linked to differences in personality traits (see further) (Johansen et al., 2012; Sørensen et al., 2013).

The neurogenic differentiation factors (n*eurod*, Korzh et al., 1998) genes are implicated in neural differentiation and survival and in synaptic maturation, and in the case of mammals, play an important role in hippocampal neurogenesis (Ince-Dunn et al., 2006; Steiner et al., 2006). In guppies (*Poecilia reticulata*), an increased cerebral expression of *neurod2* was linked to bolder, more aggressive and more social individuals depending on the context (Pauli et al., 2023). Atlantic salmon (*Salmo salar*) reared in enriched conditions displayed higher levels of *neurod1* mRNA in the telencephalon, as well as faster spatial learning (Salvanes et al., 2013). In the above-mentioned study of Manuel and colleagues (2015), enriched-reared zebrafish, that showed lower spatial avoidance learning, also had lower expression of *neurod1* in the telencephalon.

The immediate early genes (IEG) whose regulation occurs immediately after external simulation, are markers of neuronal activity (Calvo & Schluessel, 2021; Lara Aparicio et al., 2022), and code for transcription factors such as the early growth response protein (*egr1* gene, also sometimes called *zif268*, *zenk* or *krox-24*, Burmeister & Fernald, 2005) and the cellular Fos proto-oncogene (*c-fos*, Lara Aparicio et al., 2022; Watson & Tsai, 2017). *Egr1* and *c-fos* are both implicated in cell differentiation and proliferation, holding pivotal functions in synaptic plasticity, and more particularly in cognitive functions such as learning and memory (Calvo & Schluessel, 2021). For example, *c-fos* expression in rats' thalamus and cortex increases with the difficulty of spatial tasks to solve (Vann et al., 2000). In zebrafish (*Danio rerio*), structural enrichment of the tanks induced an increase in the cerebellum size and in the number of c-Fos-marked cerebellar neurons (Flores-Prieto et al., 2024). In Wistar rats, spatial memory is thought to be consolidated by both c-Fos and Egr1 protein translation patterns over time (an initial high increase over basal levels 90 minutes after spatial training, and a later drop below basal levels 8 hours post training) in the cortex and hippocampus (Barry & Commins, 2017). Hence, several clues point towards implications of those genes in personality and spatial cognitive abilities, but little is known about their implications in those domains regarding teleost fishes.

Also, the gene encoding the brain-derived neurotrophic factor (*bdnf)*, a neurotrophin and effector IEG marker of neural plasticity, implicated in neuronal survival, growth, and differentiation, is also involved in the regulation of learning and memory, as well as cognitive and emotional behaviours (Bergami et al., 2008; Cunha et al., 2010; Forsythe & Kunze, 2013; Leal et al., 2017; Yamada et al., 2002). Knowledge about *bdnf* expression and pathways in the fishes' brains is scarce (de Girolamo & D'Angelo, 2021) and mostly pertains to the zebrafish (*Danio rerio*) (Anand & Mondal, 2019; Cacialli et al., 2016; Lucini et al., 2018; Sahu et al., 2019). For example, zebrafish individuals with higher cerebral *bdnf* expression were faster learners in a colour discrimination task (Lucon-Xiccato et al., 2022). Also, *bdnf* knocked-out fish showed greatly impaired learning in colour discrimination, habituation, and spatial tasks, but learning was rescued by injection of BDNF agonists (Lucon-Xiccato et al., 2022). BDNF has also been shown to be a lead factor in neurogenesis in response to environmental enrichment: mice reared in enriched conditions demonstrated twice as much hippocampal neurogenesis in comparison to standard-reared mice, but this increase was absent when *bdnf* gene was partially knocked out from enriched-reared mice, as survival of new-born cells in the brain was drastically impaired (Rossi et al., 2006). Black rockfish (*Sebastes schlegelii*) reared in structurally complex conditions demonstrated faster spatial learning and higher BDNF protein levels in the telencephalon when compared to fish reared in barren conditions (Shen et al., 2023). On the contrary, in the telencephalon of Atlantic salmon parr (*Salmo salar*) reared in tanks enriched with rocks and artificial plants for seven weeks, fewer BDNF-marked cells were counted in the Dlv (tendency in the Dld, and no difference in the Dm) relative to parr raised in standard conditions, while no difference in *bdnf* mRNA were found between the two groups (Mes et al., 2019). Similarly, hatchery-reared salmon parr also showed higher number of BDNF-marked cells in the telencephalon (Dlv, Dm) than wild-caught salmon parr (Mes et al., 2018), the latter allegedly experiencing a more complex environment than the former. Interestingly, these two studies also showed that *c-fos* is not differentially expressed, and no differences in c-Fos-marked cells are found, in any part of the telencephalon of enriched-reared *versus* control fish (Mes et al., 2019), while c-Fos-marked cells are more numerous in the telencephalon overall (especially Dlv and Dld) in hatchery-reared fish than in wild-caught ones, and this difference is even amplified after exposure to acute stress (Mes et al., 2018). The Dm c-Fos-marked cells number increased after acute stress regardless of the fish origin (Mes et al., 2018).

In fishes trained in spatial tasks, metabolic rates are higher in the Dlv (Uceda et al., 2015), *egr1* expression is increased in the whole telencephalon during active learning phases of a new task (Emmanuvel Rajan et al., 2011), and transcription of *bdnf* and *egr1* (but not *c-fos*, even though its expression positively correlated overall with those of *egr1* and *bdnf* in the whole brain) is increased in the Dl according to learning capacity (Wood et al., 2011).

Markers Linked with Memory

Other epigenetic markers were also described as associated with cognitive abilities (Day & Sweatt, 2011), such as the genes coding the histone acetyl-transferase CREB binding protein (*crebbp*) implicated in acquisition and storage of memories (Alarcón et al., 2004; Guan et al., 2002), or memory-associated genes epigenetically modified in response to experience (Miller & Sweatt, 2007): *reelin*, a gene promoting memory, responsible for neural migration during cerebral development and also controlling synaptic transmissions and long-term potentiation of neurons (Weeber et al., 2002), and the serine/threonine protein phosphatase 1 (*pp1*, Munton et al., 2004). Increased PP1 expression and activity in the hippocampus of Wistar rats slowed learning down and impaired long-term memory in the Morris water maze spatial task (Rahman et al., 2012). Particularly, PP1 entails several catalytic subunits (alpha, beta and gamma), and higher cerebral expression of alpha and beta subunits is thought to lead to deficient long-term memory in mandarin fish (*Siniperca chuatsi*), although not in a spatial task (Cheng et al., 2015). During context learning in rats, DNA methylation is decreased for reelin and increased for pp1 (Miller & Sweatt, 2007).

Markers of Stress Response and Monoamine Metabolism

Genes such as *gr* and *mr* (coding for glucocorticoid and mineralocorticoid receptors respectively, binding notably with the stress hormone cortisol (Kiilerich et al., 2018; Mommsen et al., 1999)), as well as genes such as *mao* and *dbh* (respectively, monoamine oxidase degrading monoamines, and dopamine beta-hydroxylase that converts dopamine into noradrenaline) encoding enzymes involved in monoamines neurotransmitters metabolism (serotonin, dopamine, noradrenaline and adrenaline), are major actors implicated in stress response and have also been shown to play a role in behaviour regulation, memory, and learning processes (Harley, 2004; Herrero et al., 2006; Martorell-Ribera et al., 2020; Oitzl et al., 2010; Øverli et al., 2007; Sørensen et al., 2013). Depending on the species, teleosts can present one or several paralogs of glucocorticoid receptors (even within Salmonids for example, one in the Arctic charr *Salvelinus alpinus* (Vindas et al., 2017) and the brown trout Salmo trutta (Stolte et al., 2006), two in the rainbow trout *Oncorhynchus mykiss* (Johansen et al., 2011; Stolte et al., 2006)), but for the sake of simplicity, we will use "*gr*" to circumvent all glucocorticoid receptors.

Gluco- and mineralocorticoid receptors ratios have been shown to be modified according to age in the telencephalon of zebrafish reared in structurally enriched conditions, associated with lower spatial avoidance learning, compared to fish reared in plain conditions (Manuel et al., 2015). Interestingly, the expression of *gr* and *mr* was shown to positively correlate with the expression of *pcna* and *bdnf* in several fish species, further suggesting that sensitivity to cortisol might enhance neurogenesis (Sadoul et al., 2018). In the Arctic charr (*Salvelinus alpinus*) subjected to acute restraint and confinement stress, proactive individuals displayed lower serotoninergic activity in the brain stem as well as higher expression of *gr* and *mr* in the hypothalamus but not in the telencephalon, when compared to reactive individuals (Vindas et al., 2017). However, the behavioural tests used to determine coping style in these individuals were not repeated, hence personality involvement cannot be certified (Dingemanse & Wright, 2020).

The dopaminergic pathway is especially interesting to explore in cases of cognitive studies using reinforcements and punishments training, as this pathway is fundamentally implicated in the mesolimbic reward system, which controls the motivation for experiencing stimuli that produce desirable positive outcome, or the aversion for negative stimuli (Lewis et al., 2021; Matsumoto & Hikosaka, 2009). Levels of dopamine metabolites have been shown to strongly correlate positively with spatial cognitive performances in the zebrafish (Levin, 2011). Interestingly, *bdnf* has been shown to be a key factor implicated in the development and maintenance of serotoninergic and dopaminergic neuronal populations of the zebrafish (Sahu et al., 2019), suggesting potential interdependent roles of neural plasticity and monoamine pathways in cognition. Dopamine could notably enhance spatial memory in the Caribbean bicolour damselfish (*Stegastes partitus*) by amplifying salience and recognition of visual landmarks (Hamilton et al., 2017). Indeed, dopaminergic cells were shown to be activated in the brain of zebra mbuna cichlids during learning in a task assimilable to a beaconing navigation learning task (Calvo, Schluessel, et al., 2023); in the zebrafish, finely balanced activation of dopamine receptors was shown to be implicated in the acquisition and consolidation of spatial learning and spatial memory (Naderi, Jamwal, Chivers, et al., 2016; Naderi, Jamwal, Ferrari, et al., 2016); still in the zebrafish, a nicotine-induced increase in brain dopamine metabolites was linked with higher spatial accuracy (Eddins et al., 2009); in the cleaner fish *Labroides dimidiatus*, activation of dopamine receptors enhanced learning speed in cue discrimination and spatial tasks (Messias et al., 2016).

Also, as serotonin is implicated in risk assessment, anxiety, and fear-like behaviours (e.g., Lima-Maximino et al., 2020), among-individual differences in monoamines physiology are suspected to participate in among-individual differences in personality traits including boldness (Luchiari & Maximino, 2023). In strains of rainbow trout selected for reactive and proactive coping styles, usually assimilable to shy and bold personalities respectively, reactive individuals displayed higher levels of monoamines in several brain regions (notably in the telencephalon and optic tectum) after exposure to stress compared to proactive individuals (Øverli et al., 2001). Proactive individuals, on the contrary, showed higher levels of monoamines metabolites than reactive ones, presumably in part due to higher MAO activity (Øverli et al., 2001). Interestingly, in the same selected strains, when compared to proactive trout, reactive trout showed no differential expression of *bdnf* in any brain region but showed higher gene expression in the telencephalon: of *pcna* after short-term stress, and of *neurod* after long-term stress (Johansen et al., 2012). Finally, in yet another study on these same selection lines, *mr* expression was found to be upregulated in the telencephalon, the optic tectum, the cerebellum, and the hypothalamus of proactive trout relative to reactive ones, which was proposed as an explanation for the increased propensity of proactive fish to longer retain learnt routines and information about stressful events compared to reactive fish (Johansen et al., 2011); meanwhile, one *gr* paralog did not show any differential expression between proactive and reactive trout, the second *gr* paralog being significantly more expressed by reactive fish than proactive fish in the cerebellum only (Johansen et al., 2011). Alfonso and colleagues demonstrated that bold European sea bass (*Dicentrarchus labrax*) displayed lower cerebral noradrenaline levels and lower brain plasticity, as bolder fish express less *mr, gr, egr1* and *neurod2* in the whole brain compared to shy fish, while no differences in terms of *neurod1* and *pcna* were found (Alfonso et al., 2019). On the contrary, in the hypothalamus of the same species under chronic stress conditions, n*eurod2* was upregulated in bold fish compared to shy fish, but there were no differences in terms of *bdnf*, *egr1*, *neurod1*, *pcna*, *mr* and *gr* (Dellinger, 2019). Cerebral expressions of *mr* and *gr* also did not differ between bold and shy zebrafish (Tudorache et al., 2013), however the group risk-taking test used to assess boldness was not repeated, hence it cannot confidently be considered a personality trait in this study (Dingemanse & Wright, 2020). Whole brain expression of *gr* was positively correlated with the boldness-aggressiveness syndrome in three-spined sticklebacks, but not the expression of *mr* (Aubin-Horth et al., 2012). In a transcriptomic screening between bold and shy European sea bass also tested in a (repeated) group risk-taking test, the vast majority of genes linked with social and exploratory behaviour as well as memory, including *egr1* notably, were found to be upregulated in the brain of bold individuals, but no differences were found between shy and bold fish regarding the other genes mentioned here (Sadoul et al., 2022). In the olive flounder (*Paralichthyus olivaceus*), reactive (shy) fish displayed higher mRNA levels of both *gr* and serotonin receptors in several parts of their central nervous system, notably in the telencephalon (and in the optic tectum in the case of *gr*), in comparison to proactive (bold) or intermediate fish (Rupia et al., 2023). In the latter study, the authors had primarily investigated these genes as they are involved in central appetite control, as shy flounders did not recover eating during post-stress feeding trials (Rupia et al., 2023), which interestingly also links personality and its underpinning neural mechanisms to individual feeding habits.

Also, a recent study (Cardona et al., 2022) on the brain plasticity mechanisms involved in the behavioural changes occurring due to structural enrichment in the rainbow trout (*Oncorhynchus mykiss*), is worth mentioning here: this study shows that the transcription of *pcna*, *creb* and two genes related to dopamine and serotonin pathways was increased in the forebrain of trout reared in structurally enriched conditions when compared to trout reared in barren tanks. This was not the case in the hypothalamus or the hindbrain where groups did not differ in expression levels of those genes. Also, *bdnf*, *c-fos*, *egr1* and *neurod1* genes were not differentially expressed between the two rearing groups in any of these three brain regions (Cardona et al., 2022). All the differences in gene expression highlighted in this study between enriched- and barren-reared fish are centred on the forebrain, where emotional and cognitive processes are mainly controlled (Cardona et al., 2022), which leads to hypothesize potential links between structural environmental enrichment, brain regional plasticity, personality, and cognition.

All in all, I exposed here that there is relatively little knowledge about the neural processes involved in personality and spatial cognition in teleosts, and the limited literature on this subject often presents contradicting results. When considering the results obtained by Rey and her team (2021) through comparative transcriptomics studies showing that gene expression patterns underpinning personality seem to be essentially species-specific in fishes (Rey et al., 2021), there is an exciting array of uncharted underlying neural processes to elucidate regarding fishes' personality, spatial cognition, and their interplay.

1.5 Behavioural Patterns as an Evolutionary Consequence

A simple observation of our surroundings, make it undeniable that behaviours are extremely variable between species, within species, between individuals and even within individuals, yet they can be very consistent across time and contexts (Gosling, 2001). One question then comes to mind: how can evolution favour both variation and consistency in behaviour at the same time (Bell, 2007a, 2007b)?

1.5.1 The Evolutionary Origin of Consistent yet Variable Behavioural Phenotypes

The variability in behaviour can be explained through its high plasticity, which is the capacity of a genotype to produce different phenotypes in different environments (see reviewed definitions in Debat & David, 2001). Snell-Rood (2013) describes two types of behavioural plasticity occurring simultaneously: developmental behavioural plasticity and activational behavioural plasticity.

In developmental behavioural plasticity, different environments trigger different developmental trajectories for a genotype, ultimately producing different behavioural phenotypes. It comprises all phenomena leading to the development of the behavioural repertoire of the individual, such as learning, neural plasticity and formation of morphological and physiological traits relevant to behaviours. Hence it also controls the development of neural networks available in activational behavioural plasticity.

Indeed, activational behavioural plasticity refers to the ability of an individual to display different behaviours, or different levels of a behaviour, in different situations by differentially activating pre-existing neural and muscle networks.

Developmental behavioural plasticity is a process that is slow but results in potentially highly integrated behavioural phenotypes. This mechanism could explain the emergence of personality traits and behavioural syndromes: indeed certain environmental cues in early development might favour the formation of sets of particular neural networks and morphological or physiological features for behaviours relevant to those early cues, which once formed, would be too expensive to redesign (*e.g.* metabolic cost for resorbing and re-developing neurons and associated muscles, trial-and-error costs and risks associated with sampling different behavioural phenotypes against later conditions). Hence, different behavioural profiles between individuals and correlated behaviours within individuals can be formed in different developmental contexts, crystallized, and maintained consistent temporally and contextually (Koolhaas et al., 1999; Sih, Bell, Johnson, et al., 2004).

Activational behavioural plasticity allows immediate response to the environment, but also bears the cost of maintaining a wide range of circuits necessary to respond accurately to each situation. This mechanism could be at the basis of personality plasticity (Dingemanse et al., 2010), allowing individuals to adjust the level of their behavioural response in a particular situation, even though it is channelled by their personality traits (*e.g.,* an aggressive individual displaying fewer attacks towards conspecifics when in the presence of predators). Developmental behavioural plasticity would progressively prune and refine the pertinent neural networks over time to reduce the cost of activational behavioural plasticity (Stamps & Groothuis, 2010). This would explain why we observe differences in plasticity of personality even within species (Jolles et al., 2019). Such a diversity in personalities and their plasticity between individuals can then be maintained in the population through frequency-dependent mechanisms (Dall et al., 2004), or dependence on life-history strategies (Réale et al., 2010; Wolf et al., 2007). In the latter case, the pace-of-life syndrome hypothesis (POLS) predicts that behavioural differences are maintained among individuals as part of a suite of coevolving traits (life-history, morphological, physiological, behavioural), as factors favouring a certain life-history strategy would also favour the whole set of traits (Réale et al., 2010). The POLS hence suggests that behavioural phenotypes are restricted to relevant behaviours for a given life-history strategy: slow-growing individuals reproducing late will have to adopt a consistently more careful way of life in order to survive until they can accomplish their reproductive expectations; in contrast, fast-growing individuals that reproduce early should take all risks necessary to reproduce as soon as possible before they die. Consequently, any behaviour implicated in pace-of-life strategy will be kept consistent and stable across time and contexts within an individual adopting a particular strategy, but also consistently differ from another individual adopting an opposite strategy (Bell, 2007a; Wolf et al., 2007). However, conflicting results were found so far in empirical studies investigating the POLS and there is currently a lack of formal modelling for this hypothesis, such that Laskowski et al. (2022) recently pointed the need for a revision of the POLS hypothesis and predictions.

The life-history strategies view was also linked to the evolution of learning (Mettke-Hofmann, 2014; Snell-Rood, 2013): long-lived species are likely to experience a high number of different conditions, which should favour activational behavioural plasticity. As a consequence, we can also expect these species to show higher neural investment in learning and memory, as they need to develop and maintain wider ranges of neural information networks during life. Neural investment has been shown to trade-off with reproduction, hence explaining later reproduction in long-lived species (Barrickman et al., 2008). We can consequently expect long-lived species to display a large range of different behaviours, and/or different levels within a behaviour, as well as better cognitive performances. Accordingly, when comparing a species of bat (*Myoris nattereri*) and a shrew (*Sorex araneus*), two mammals that are very similar in habitat, diet, nocturnal activity, body size and brain weight but differing in there pace-of-life (bats being long-lived, shrews being short-lived), Page and colleagues (2012) found that bats could use spatial learning and spatial memory to locate a food reward in a maze, while shrews used systematic exploration to locate food but were almost incapable of learning the location-reward spatial association (the authors nevertheless acknowledge that other factors than pace-of-life dramatically differ between the two species, such as sociality and ability to fly, and might play a role in those differences). There are also numerous and salient exceptions to this pattern (e.g., *Octopus* spp., with usual lifetime of one year but presenting remarkable cognitive capacities (Mather & Dickel, 2017)), which has it clear that pace-of-life might be one, but not the only factor driving the evolution of learning.

I presented how diversity and consistency in behaviours can be formed and maintained, notably through environmental factors influencing developmental behavioural plasticity, or pace-of-life strategy influencing activational behavioural plasticity. I will now develop the directions in which environmental factors can pull the evolution of behavioural phenotypes.

1.5.2 Environmental Factors as Moulders of Behavioural Patterns

Cognitive traits are subject to trade-offs, as they imply metabolic costs for the development and maintenance of neural machinery necessary to acquire, analyse, store, and recall information (Dukas, 1999; Mery & Kawecki, 2003). For instance, it would not be much beneficial for a tapeworm, living almost its entire life attached to the gut wall, to allocate as much energy to the same spatial cognitive abilities as a male tiger striding across a 100 km^2 territory. Even more precisely, spatial cognitive styles in each population within a species have been shown to be locally adapted to its particular ecological conditions (Mettke-Hofmann, 2014; Rowe & Healy, 2014). Supporting this idea, comparisons of different populations of cichlids within African lakes proved that both the physical and social environment shaped their brains and cognition, while between-lakes comparisons revealed convergent evolution of species occupying similar niches (Bshary & Brown, 2014). Furthermore, when comparing chickadees (*Poecile atricapillus*) originating from Alaska and Kansas and raised in common gardens, Kansas birds had a lower number of hippocampal neurons, less accurate spatial memory, and slower innovative problem-solving capacities compared to Alaska birds, where winter conditions are harsher and retrieval of hoarded food is absolutely critical for survival (Pravosudov & Roth, 2013).

Morand-Ferron (2017) lists the possible selective agents that can act on cognition: seasonality, variation in food quality, predation risk, social interactions, interspecific interactions, and intra- and intersexual selection. The flag model from Dunlap and colleagues (2019) states that learning should evolve as a function of certainty of actions and reliability of cues used to learn, certainty favouring unlearnt preferences and reliability favouring learning. In other words, highly variable environments with highly reliable cues will favour reliance on learning whereas highly stable environment and/or offering unreliable cues would favour unlearnt preferences. Many environmental factors can modulate certainty and reliability and hence lead to particular cognitive styles and personality profiles, as reviewed by Mettke-Hofmann (2014). As an example, she develops how spatiotemporal instability and unpredictability of resources leads to extensively exploratory personality traits and low cognitive innovation rate as individuals simply need to find and follow resource-abundant spots in a nomadic way. They also display poor learning and long-term memorization abilities as unpredictability makes learning cues unreliable and memories useless.

Nevertheless, memory on shorter time is often well developed in order to remember already visited sites (Mettke-Hofmann, 2014). Differences in ecology can also favour equal learning abilities but differential cognitive navigation strategy use: three-spined stickleback fish from Putah creek, characterized by low water movement and denser vegetation, rely preferably on visual cues to locate food rewards, while sticklebacks from Navarro river, where dramatic seasonal variations in water flow might render visual cues unreliable over time, rely preferably on self-centred side orientation (Bensky & Bell, 2018); Other studies found similar results between pond *vs.* fast-flowing river sticklebacks (Girvan & Braithwaite, 1998; Odling-Smee et al., 2008) and climbing perches (Sheenaja & Thomas, 2011). Additionally, consistently with the environmental complexity thesis (Godfrey-Smith, 2002), environmental complexity in nature, or environmental enrichment in captivity, has been proven to influence behaviour and cognitive traits in several taxa (*e.g.,* rats in Bredy et al. (2004); fishes in Carbia & Brown (2019), Finstad et al. (2007), White & Brown (2015); seals in Hunter et al. (2002)). I already mentioned in section 1.4.2 how environmental complexity or structural enrichment could influence brain plasticity (Cardona et al., 2022; Flores-Prieto et al., 2024; Mes et al., 2018, 2019; Rossi et al., 2006; Shen et al., 2023). Environmental enrichment even provokes epigenetic DNA methylations modifications in the brain of rainbow trout right from egg and alevin stages (Reiser et al., 2021). Salvanes and colleagues showed that neural plasticity promoted by environmental enrichment also correlates with enhanced cognitive capacities in *Salmo salar* (Salvanes et al., 2013). Guppies reared in enriched conditions (structurally, socially and feeding-wise) were faster learners compared to animals reared in barren condition, but the two groups did not differ in cognitive flexibility (Montalbano et al., 2022), while rainbow trout raised in structurally enrichment tanks were both faster and more flexible learners than plain-reared fish (Brunet et al., 2023). Congruently, comparisons between populations within species of teleost fishes differing in ecological conditions in the wild highlighted variations in spatial cognition: bishop fish (*Brachyrhaphis episcopi*) from low predation contexts display shorter learning time and a superior spatial memory accuracy compared to bishop fish from high predation contexts (Beri et al., 2014; Brown & Braithwaite, 2005). Benthic sticklebacks, experiencing more structurally complex habitats, are faster learners and are more accurate regarding maze learning task compared to pelagic sticklebacks, living in the stimuli-poor water column (Odling-Smee et al., 2008). In addition, we might expect the degree of complexity in the environment to influence the evolution of personality traits, as complexity might elicit cautious behaviours (shyness, neophobia) to be able to pay attention to a lot of different cues that can happen simultaneously and potentially be harmful, while simpler environment might favour boldness and routine behaviours. Accordingly, Mettke-Hofmann (2014) summarizes that animals usually adopt fast-inaccurate exploration strategies in simple environments vs. increased slow-accurate exploration strategy in complex environments (Sih & Del Giudice, 2012). This is consistent with stream sticklebacks found to be bolder than pond sticklebacks (Álvarez & Bell, 2007). Also, the behavioural correlations in behavioural syndromes were shown to be stronger in populations of sticklebacks experiencing predation risks compared to predator-free populations, most likely being an adaptive behavioural evolution (Dingemanse et al., 2007).

The various behavioural phenotypes hence appear as a consequence of external environmental forces shaping them. However, individuals can express behaviours that would either enhance the number and the strength of the evolutionary forces applied, or shield themselves from those forces, hence either accelerating or slowing evolutionary rates. It is worth quoting Waddington (1959) here writing "*Animals [...] themselves select the* *particular habitat in which their life will be passed. Thus, the animal by its behaviour contributes in a most important way to determining the nature and intensity of selective pressures which will be exerted on it. Natural selection is very far from being as external a force as the conventional picture might lead us to believe.*". Hence, I will now present how behaviour can be a conductor regulating the tempo in the symphony of evolution.

1.6 Behaviour as a Conductor of Evolution

Traditionally, evolutionary rates are determined by the strength of selective pressures sorting out mutation-driven variations. Though, the current rate of environmental change exceeds by far such evolutionary potential for many populations, that however do not reach extinction (Hoffmann & Sgró, 2011). Consequently, there must be more immediate adaptive mechanisms at play, and behaviour might be at the core of those mechanisms (Bateson, 2004; Bogert, 1949; Levins & Lewotin, 1985; Lewotin, 1983; Mayr, 1963). In this section, I will develop thoughts around a slightly modified framework (Figure 6) of Duckworth's proposition of the role of behaviour in evolution (Duckworth, 2009).

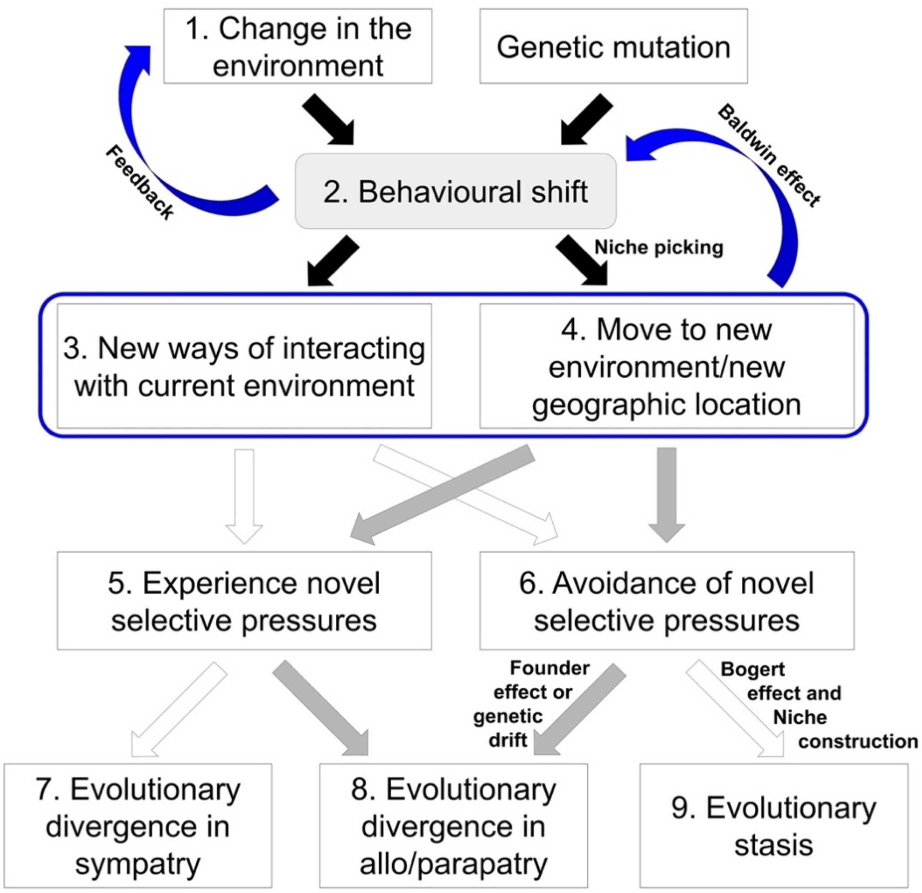


Figure 6 – Illustration of a unified framework of behaviour as both a motor and a brake of evolutionary changes. Modified from Duckworth (2009). *In this conceptual scheme, a change in the environment usually precedes behavioural shifts (with random mutation being the only exception), after which evolutionary divergence and stasis can occur simultaneously. For example, a behavioural shift leading to Box 3 can simultaneously cause an animal to experience novel selection pressure on one trait (Box 3 to 5) while avoiding selection on another (Box 3 to 6). Gray arrows indicate continuation of paths originating from Box 4, white ones from Box 3. Note that new selection pressures are not necessary for evolutionary diversification in the case of a behavioural shift causing relocation to a new environment (Boxes 2-4-6-8 path). The blue curved arrows indicate potential feedbacks between environmental and behavioural changes.*

1.6.1 Vivace: Behaviour as a Motor of Evolution

Conditions for a Behavioural Shift to Trigger Population-Level Evolution

Just like any phenotypic trait, certain conditions must be met for a behavioural shift to originate evolutionary change in a population (Duckworth, 2009) :

Firstly, the behavioural shift must occur in a sufficiently large portion of the population for novel selective pressure to act upon. This can happen through four different situations: when a similar developmental behavioural plastic response occurs in several individuals in the population that undergo an environmental variation; when a behaviour is learnt and transmitted, culturally or by extensive social learning, to a larger part of the population; when selection is shifting the entire population's behavioural phenotype; when founder effect induces a general reduction in the behavioural repertoire in the entire population.

Secondly, the behavioural shift has to be maintained over several generations in order to make other traits evolve. This can happen either through cultural transmission of learnt behaviours to next generations (Sih et al., 2011), or through a genetic change that is actually the origin of the behavioural shift (rarer case), or through a genetic fixation of the new behavioural accommodation acquired after behavioural plasticity, *i.e.*, a Baldwin effect (Baldwin, 1896; Bateson, 2004; Robinson & Dukas, 1999) allowing individuals to survive until genetic assimilation (Waddington, 1953). This latter case notably resonates with the "plasticity-first hypothesis" (Levis & Pfennig, 2016), stating that ancestral phenotypic plasticity induced by environmental perturbations leads to developmental reorganization and uncovers cryptic genetic variation, producing novel trait variants on which selection can act. These new trait variants undergo phenotypic accommodation and ultimately genetic accommodation (*i.e.*, Baldwin effect), being progressively canalized. Canalization is the property of organisms to produce standard phenotypes despite genetic and environmental perturbations, reducing variance around the mean trait value (Loison, 2019; Stearns, 1989). In this sense, the new behavioural accommodations are acquired through ancestral behavioural plasticity and decanalization when the environment changes or when invading a new environment, and the costs of maintaining behavioural plasticity is then reduced over evolutionary time, when the optimal behavioural trait value for the new conditions has been reached, by genetically fixing and canalizing this phenotype in the population. In a nutshell, the plasticity-first hypothesis suggests that plasticity in ancestral populations provides opportunity for adaptive divergence when colonising a new environment, describing ancestral plasticity as a key for rapid evolution, predicted to be reduced after acclimation to the new habitat (Levis & Pfennig, 2016).

If those two conditions are fulfilled, behavioural shifts then have the potential to engender different evolutionary diversification patterns, accordingly to whether these shifts lead individuals to cope with the current environment or to move to a new one.

Behavioural Shifts Leading to Evolutionary Diversification

Phylogenetic clades with greater behavioural plasticity were shown to display the highest species richness (Nicolakakis et al., 2003; Sol et al., 2005), underlining the importance of behavioural plasticity in the evolutionary processes of diversification (Pfennig et al., 2010). Figure 6 shows the routes by which switches in behaviour can lead to different diversification outcomes. Indeed, animals can respond to environmental changes in two ways: either (1) they stay put and have to develop new ways of interacting and coping with their new conditions (which can include behavioural, physiological and morphological shifts), or (2) they move towards another location to avoid dealing with those changes. Nevertheless, the choice of one response or the other ultimately leads to different evolutionary outcomes in terms of diversification patterns.

- Mechanisms Towards Sympatric Divergence (Figure 6, Box 7)

In this case, a behavioural shift within a given environment induces a change in how individuals interact with their environment, hence creating a niche shift. This way, these individuals will start to experience new selective pressures within their new niche (Figure 6, Box 3 to 5), which will eventually lead to divergence from the individuals in the niche of origin, *i.e.*, sympatric diversification (Figure 6, Box 5 to 7). An example of this can be found in the Arctic charr (*Salvelinus alpinus*) in Lake Þingvallavatn in Iceland, where several sympatric morphs can be found, all deriving from a common ancestor but having each specialized in a particular foraging niche (Jonsson & Jonsson, 2001; Kristjánsson et al., 2011). This behavioural shift in learning how to exploit different food items led the charr to a sympatric evolutionary within-species diversification of morphological and behavioural adaptations fitted to each type of feed, improving the lake system productivity (Franklin, 2017; Franklin et al., 2018; Sandlund et al., 1992). This mechanism is especially interesting regarding cognitive abilities, as higher cognitive innovations and learning performances can trigger a variety of new niche openings (Kerr, 2007), creating various ecological opportunities within the given environment. Also, Stamps & Groothuis (2010) posited that personalities could be at the origin of differential niche-picking or niche construction between individuals differing in personality types, hence virtually separating behavioural profiles in particular sympatric preferred niches, which we can hypothesize to initiate a sympatric divergence process between behavioural profiles (Dall et al., 2012; Holtmann et al., 2017; Ingley, 2018).

- Mechanisms Towards Allopatric and Parapatric Divergence (Figure 6, Box 8)

In this case, the behavioural shift is at the origin of a relocation of the concerned individuals, and either this relocation causes this population to experience different selective pressures in the new location (Figure 6, Box 4 to 5), or not if the environment they moved to is similar to the place of origin (Figure 6, Box 4 to 6). In both cases, the moved population and the population that remained at the initial location will diverge from each other due to geographical isolation, leading to allopatric or parapatric divergence. Nevertheless, mechanisms differ, as in the first case, new selective pressure might select for some genotypes and hence change the genetic structure in this population compared to the population of origin (Figure 6, Box 5 to 8). But in the second case, selective pressures are not necessary for the population to evolve, as stochastic events like founder effect or genetic drift are sufficient to be the origin of the change in the genetic structure (Figure 6, Box 6 to 8). An example of this is found in the flycatcher bird (*Tyrannus savana*) in which a change in the migratory behaviour (migration drop-off) promoted speciation through changes in reproductive times and grounds between the migratory and the "neo-sendentary" lineages (Gómez-Bahamón et al., 2020). Cognitive abilities and personality traits are of particular interest in this mechanism of diversification, as individuals with the highest behavioural flexibility and learning capabilities are also the ones most likely to survive in newly colonised environment (Sih et al., 2011). (Mettke-Hofmann, 2014) accordingly summarizes that bird and primate populations with a successful invasive history display less neophobia, higher innovative rates, are faster learners, and have larger brains than non-invading populations. A study showed that disperser sticklebacks populations were bolder, while recently established populations were more behaviourally flexible, those two traits being negatively correlated within populations (Bensky & Bell, 2022). The authors propose an adaptive behavioural shift from boldness to flexibility between invasion phase and establishment phase. This example illustrates how different personalities could lead to the relocation of subsets of the population (Figure 6, Box 2 to 3), and how this relocation might then alter behavioural and cognitive patterns in return (Figure 6, feedback loop towards Box 2).

Behavioural syndromes might even foster rapid evolution: when changes in environmental conditions necessitate a coordinated shift in a whole set of phenotypic traits, correlated traits already co-varying within a syndrome will allow the evolution of the new adapted integrated phenotype to occur much faster than if one had to wait for the right mutation to happen independently for each trait (Wolf & Weissing, 2012). For example, it has been shown that selection for boldness personality trait parallelly selected for caudal width and swimming performances in the zebrafish (*Danio rerio)*, a correlation that could allow rapid adaptation in response to predation pressure, when bold-fast individuals could gain fitness benefits from efficiently escaping predators (Kern et al., 2016).

But for the same reason, correlated behaviours can also slow evolution down (Dochtermann & Dingemanse, 2013), as opposite selective pressures can be applied on each trait in the syndrome, hence potentially annulling their effects and leading to evolutionary stasis. Hence, in the next section, we will discuss how behaviour can also be a brake for evolutionary processes.

1.6.2 Adagio: Behaviour as a brake for Evolution

Bateson (2004) emphasizes that animals can bypass evolutionary pressures by actively choosing (niche picking) and modifying (niche construction) their habitat, allowing them to find or build an environment to which they are best suited for (Figure 6, Box 6 to 9 and Box 2 to 4). This phenomenon is part of what is called niche construction (recapitulated in Laland et al., 2016). An intuitive and spectacular illustration of this phenomenon is the impact of beavers engineering, building their dams and modifying the whole fate of a valley in order to reproduce at ease. The animals' ability to shield their physiological traits from evolution through behavioural buffer of environmental variations is known as Bogert effect, or behavioural inertia (Bogert, 1949). It enables animals to enlarge the breadth of their climatic fundamental niche and mitigates the negative impacts of chronic high-amplitude disturbances, such as climate change. A striking example is found in the *Anolis* lizards in Hispaniola Island (Muñoz, 2014), in which heat tolerance physiology evolves considerably slower compared to cold tolerance physiology, as lizards would actively retreat to shade from sun but cannot thermoregulate that efficiently at night when temperatures drop under incapacitation threshold. As there is no warm refuge available at night, populations must evolve physiological cold tolerance. In contrast, as behavioural modifications are considered easier to evolve than physiological changes (Mayr, 1963; West-Eberhard, 2003), behavioural thermoregulation might be sufficient for heat tolerance.

Learning can also be a brake on the evolvability of species (Sih et al., 2011), in the sense that learning related to dangerous situations, such as taste aversion conditioning, is very efficient, but learned responses to those dangers are often generalized to analogous food items, which induces a loss of opportunity to create a new niche by avoiding the exploitation of a harmless potential food source. Personality can also be a brake on evolution, as shy individuals were shown to take more time to adapt to new environments compared to bold individuals (Sadoul et al., 2022), which we can imagine might impact their capacity to efficiently claim and exploit new beneficial niches. On the contrary, we can also imagine that bold individuals too prompt to resume usual behaviours in new environments of unknown danger status might also risk their survival, hence not letting enough time for evolution to act before drastic negative selection and extinction of bold individuals.

As mentioned earlier, behavioural syndromes can also be a brake in evolutionary processes, as correlated behaviours are a constraint by which one trait cannot evolve without applying pressures on another trait to evolve as well (Wolf & Weissing, 2012). A positive correlation between one behavioural trait positively selected and another trait (either behavioural, physiological or morphological) that is conversely selected against, or the other way around, induces evolutionary trade-offs where both traits are then sub-optimally expressed at what we could call an evolutionary unstable equilibrium state, which leads to seemingly maladaptive phenotypes. This is exemplified in the salamander larvae (*Ambystoma barbouri*) displaying positive correlations of activity rates across situations (in the presence and absence of predators), revealing that neither highly active nor less active individuals live at an optimal state. Indeed, less active individual access less resources while active individuals are more exposed to predators and vice versa, but their correlated behaviours prevent them from switching to the optimal behaviour for the given circumstances (Sih et al., 2003). A meta-analysis by Dochtermann & Dingemanse (2013) even suggests that correlations between behaviours within behavioural syndromes might impose greater evolutionary constraints than correlations between morphological or life-history traits.

1.6.3 Levins & Lewonin's Hypothesis: Multidimensional Behavioural Guidance of Evolution for Multidimensional Organismal Traits

As we just exposed, behaviours can be both brakes and motors of evolution. But these two mechanisms are not mutually exclusive, as a phenotype and the niche in which it lives are composed of virtually an infinity of traits that are each under differential selective pressures, which might conflict and strengthen each other in a multidimensional way. The idea of behaviour being at once a drive and an inhibitor of evolution was introduced by Levins & Lewotin in 1985 and later modeled by Huey and colleagues (2003). Their idea is that a single behaviour simultaneously impacts multiple dimensions of a species niche, that might lead to evolutionary trade-offs: buffering evolution in one dimension/one set of traits can occur at the expense of exposing other traits to new evolutionary forces and hence instead accelerate evolution in this dimension. This is represented in Figure 6 by the multiple routes possibly taken simultaneously after a behavioural shift, but also the feedback loops symbolizing the inevitable interdependence of all traits of the organism and its ecological conditions. Again, an example can be found in the *Anolis* lizard, in which a shift in thermoregulatory perching behaviours (from trees at low elevations to rocks at high elevation) resulting in a physiological stasis, was concomitant to the divergence in head shape and hind limbs length adapted to the new substrate use in those lizards (Muñoz, 2014). As previously mentioned (section 1.3), the same interdependence pattern is thought to apply to the link between cognitive and personality traits within individuals (Guillette et al., 2017), two behavioural traits mutually influencing each other so much that they could be closely co-evolving (Carere & Locurto, 2011; Sih & Del Giudice, 2012).

1.7 Exploring Eco-Evo-Devo Interplay of Spatial Cognition and Personality

What becomes apparent after developing this first chapter, is that animals' behaviour cannot be decoupled from the environment they live in, as they constitute an indivisible package of instruments necessary for a coherent orchestra. Behaviours are either a consequence of the individuals' adaptation to their environment, or the cause of changes in their environment through niche construction, or even both at once in an endless Red Queen dynamic circle of feedbacks. The last two decades have seen the fields of animal personality and cognitive ecology flourish. Yet, despite the acknowledged implication of behaviour in diversification processes and speciation (Duckworth, 2009; Snell-Rood, 2013), there is a lack of clear empirical evidence about the role of cognition and personality in the picture of divergence processes. The scientific knowledge remains incomplete regarding how specific cognitive abilities or personality traits might differ between and within species, and what the eco-evolutionary factors involved in such differences are. As we have seen, studying the evolution of a behavioural trait alone can be misleading. Consequently, and as also advocated by Mettke-Hofmann (2014) or Bell (2007b), future research should focus on several behavioural traits in parallel (for instance several cognitive abilities, or the association between cognition and personality), alongside the study of associated brain structures and ecological conditions involved. Only such a multi-instruments association can allow us to appraise the multidimensional symphony of evolutionary interplays between organisms and their environments.

I previously exposed how spatial cognition-boldness is a behavioural syndrome that is particularly worth exploring (section 1.3). Carere and Locurto (2011) recommend three steps to empirically assess cognition-personality syndromes: firstly, parallelly characterising within- and among-individual variation in cognitive styles and personality profiles; secondly, testing the predictivity of personality profiles over cognitive abilities; and finally, testing whether differences in early-life ontogeny influencing personality are accompanied by differences in cognitive performances as well. According to Mettke-Hofmann (2014), cognitive ecology studies should ideally associate three research axes simultaneously in a comparative approach: cognitive performances, associated brain structures, and ecological conditions. However, current studies usually associate only two of those aspects (cognition with ecology, or cognition with brain structures), and studies linking the three axes are scarce (*e.g.*, Fong et al., 2019; Salvanes et al., 2013; Zhang et al., 2021). Moreover, the relationships between brain architecture, the level of cognitive performances and personality are only starting to be established in fishes (*e.g.*, Fong et al., 2019; A. Kotrschal et al., 2013, 2015; Liu et al., 2022; Philip, 2021; Triki et al., 2022), where a lack of empirical studies needs to be addressed. Hence, studies following those empirical recommendations in the investigation of ecology, evolution and development (eco-evo-devo) mechanisms involved in the interplay between cognitive and personality traits in a thorough overview should bring significant answers to these gapping interrogations.

2 Aims of the Doctoral Project

This doctoral project investigates the above-mentioned knowledge gaps by simultaneously investigating the eco-evo-devo interplay in shaping spatial cognition alongside boldness personality trait, exploring the extent to which those traits are influenced by the environment in which the organism develops, as well as the neurological mechanisms involved in such traits (Figure 7). This study addresses the three-way association between cognitive performances, ecology and neural mechanisms advocated by Mettke-Hofmann (2014), as well as their links with personality following Carere and Locurto's (2011) empirical recommendations.

Comparative studies focusing on interspecific or inter-populations variations between and within species, occupying different niches, can allow for the identification of ecological factors driving the evolution of cognitive and personality traits. Nevertheless, many studies are (1) limited to comparisons between two species or populations, which is hardly enough to infer general eco-evolutionary mechanisms. It also implies some pitfalls which can make comparisons hazardous: (2) intrinsic inter-species differences in behaviour and cognitive skills and (3) geographical confounding effect, as ecological conditions differ from one site to another. In this project, we aim at partly by-passing those biases by using (1) five morphs (2) of the same species, the Icelandic Arctic charr (*Salvelinus alpinus*), a teleost fish from the Salmonids family. Different isolated populations of Arctic charr can be found in separated Icelandic freshwater lakes and rivers, most of those populations being divided in up to four distinct morphs (3) living in sympatry within a same lake. The particular interest of this species (see section 3.1.2) lies in the fact that it displays an evolutionary gradient of morphological, ecological, and genetic divergence between and within populations throughout Iceland (Brachmann et al., 2021; Gíslason et al., 1999), which represents an elegant model to study how different degrees of divergence might impact spatial cognition, personality, and their plasticity. Working with sympatric morphs, hence genetically closely related, also allows to investigate how differences in ecological niches within pairs of morphs might influence cognitive styles and personality traits. I investigate the eco-evo-devo mechanisms involved by manipulating environmental conditions during early life in common gardens of five morphs of Arctic charr (one ancestral proxy, two moderately diverged sympatric morphs, and two highly diverged sympatric morphs), to better understand the causes and consequences of gene-environment interactions in the development of cognition and personality.

Research Hypotheses

H1- Early environment as a trigger for the expression of different behavioural phenotypes: the features of the early environment in which animals develop shape spatial cognition and personality traits within wild Arctic charr morphs (developmental behavioural plasticity).

H2- Ecological niches as a factor of behavioural differentiation between populations: between sympatric morphs within a population, *i.e.,* genetically closely related individuals with equal divergence time from the ancestral form, spatial cognitive abilities and personality traits will be shaped differently accordingly to the niche's features. This implies a heritable component selected for in the wild.

H3- Evolutionary divergence governing behavioural variability and plasticity within populations: the degree of divergence from the common ancestor influences the degrees of developmental plasticity and variability of personality and spatial cognitive abilities between morphs, potentially via progressive canalization.

H4- Cognition-Personality interactions: correlations can be found between individual cognitive abilities and personality traits, potentially influenced by all factors mentioned in the previous hypotheses.

H5- Mechanisms underlying such behavioural differences involve brain plasticity: differences in cognitive abilities and personality traits between individuals are linked to differential brain gene expression patterns, modulated by all factors mentioned in the previous hypotheses.

This innovative project is the very first to study spatial cognition in Arctic charr, and importantly also the first to address cognition in combination with personality along gradients of evolutionary and ecological divergence in sympatric morphs of the same species. This project develops a highly original and interdisciplinary approach, merging ethology, neurology, molecular biology and evolutionary ecology. This multidisciplinary approach, in extensive common-garden laboratory experiments, on such a remarkable biological system in which young populations (~10Kyr) diverge in sympatry in the wild, is essential to understand at fine scale which eco-evolutionary and cerebral mechanisms act on the emergence of behavioural plasticity, a strong driver of speciation processes (Pfennig et al., 2010; Snell-Rood, 2013). Current human-driven disturbances in nature apply constant pressures on wildlife to adapt to rapid modifications of the environment. Hence, as rapid habitat changes might literally disorient animals, determining spatial cognitive abilities, their interdependencies with other traits, and their plasticity, in threatened species is an important step to assess their adaptability, and implement adapted conservation policies. This is of particular concern for the Arctic charr, one of the only seven species of freshwater fish in Iceland, that represents an emblematic species of the country's cultural and natural heritage.

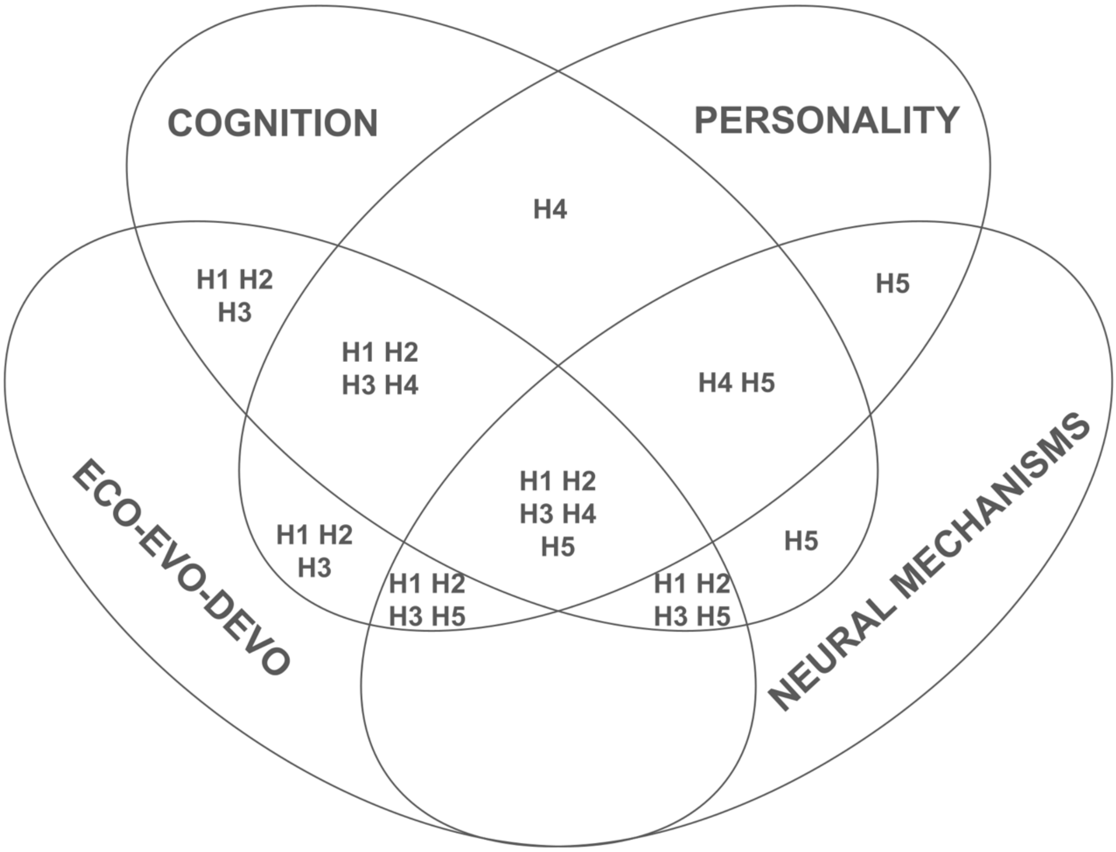


Figure 7 – Overall framework of the doctoral project and associated research hypotheses.

3 Experimental Design and Methods

All experiments were run in the facilities of the Department of Aquaculture and Fish Biology at Hólar University in Sauðarkrókur, where a centre for behavioural studies had been recently created with high-tech equipment for behavioural tests and analysis. Molecular analyses—brain gene expression quantification and Polymerase Chain Reaction (PCR) sexing—were conducted in the facilities of Askja at the University of Iceland in Reykjavík. This section will describe an overview of the methods. Fine scale methodological details can be found in the associated Papers and/or their supplements.

3.1 Study System

3.1.1 Fishes as Model Species for the Evolution of Behaviour

Contrary to mammals showing neurogenesis mostly limited to the juvenile developmental period, fishes experience neurogenesis throughout their entire lifetime (Øverli & Sørensen, 2016). Consequently, differences in brain organisation and functioning might reflect lifelong neural plasticity in response to particular experiences and ecological conditions (K. Kotrschal et al., 1998). The use of fishes as model taxa is garnering increasing interest in the study of personality (Luchiari & Maximino, 2023) and cognition (Bshary & Brown, 2014; Salena et al., 2021). With more than 30,000 characterized species of fishes characterized on Earth, exhibiting various degrees of phylogenetic closeness, their amazing diversity in life history and ecology ranging from polar streams to coral reefs, a wide variety of sociality and hierarchical structures, access to senses unknown to humans such as the lateral line sensitivity, fishes offer a plethora of opportunity to study the eco-evo-devo mechanisms underlying personality and cognition (Bshary et al., 2002). This is particularly the case in the field of spatial cognition, as fish species, like flying animals, evolve in a three-dimensional environment allowing six degrees of freedom—contrarily to extensively studied ground-bounded animals like rodents and primates, including humans, constrained to three degrees of freedom in their two-dimensional terrestrial environment (Holbrook & Burt de Perera, 2011b). In fishes, spatial cognition is the most studied aspect among cognitive fields (Salena et al., 2021). Current knowledge about spatial cognition in fishes revealed that they are able to solve complex mazes and spatial problems (Burt de Perera et al., 2016; Lucon-Xiccato & Bisazza, 2017a), can use different types of navigational cues concomitantly (beaconing and motor response egocentric strategies), and even build internal cognitive maps (complex allocentric strategy) to orient through space (see Ebbesson & Braithwaite, 2012, Rodríguez et al., 2021 and Salas et al., 2017 for reviews). Fishes can accurately evaluate distances in the three dimensions, and they are able to encode both horizontal and vertical components of space with equal accuracy, but seem to prioritise the vertical axis when both components are set in conflict (Davis et al., 2014; Holbrook & Burt de Perera, 2009, 2013). This priority may stem from the swim bladder reacting to differential hydrostatic pressure, which could provide a reliable cue for the fish to assess its absolute depth in the water column (Holbrook & Burt de Perera, 2011a).

Ebbesson & Braithwaite (2012) as well as Noakes & Jones (2016) argue that the *Salmonidae* is an especially relevant Family to use for studying the environmental effects on fishes' cognition, given the extensive knowledge of their physiology, natural history and behaviour provided in the literature. Particularly, the life and evolutionary histories of the Icelandic Arctic charr (*Salvelinus alpinus*), a highly polymorphic Salmonid species, have been deeply studied. Repeated evolution of coexisting sympatric morphs within lakes is not a rare occurrence in freshwater fishes, as the differential use of the benthic and the pelagic habitats has driven intraspecific ecological specialisation and evolutionary divergence (Cooper et al., 2010; Parsons et al., 2020; Skúlason et al., 2019), notably through resource polymorphism (Skúlason & Smith, 1995). Resource polymorphism is a process of continuous divergence, in which intraspecific competition is reduced as sympatric populations diverge in their exploitation of new trophic resources and niche use. Phenotypic diversification is then progressively enhanced through divergent selection for the specific resources exploited. Polymorphism increasingly stabilises in the population as gene flow is progressively reduced between adapted morphs. This high evolvability is believed to be due to a high plastic potential, notably in morphology of feeding apparatus structures linked with different diets (Adams et al., 2003). Such plastic changes are suspected to originate from behavioural shifts between sympatric sub-populations (Snorrason & Skúlason, 2012). Behaviour being the most immediate adaptive mechanism available for animals against environmental change (Bateson, 2004; Bogert, 1949; Duckworth, 2009; Levins & Lewotin, 1985; Lewotin, 1983; Mayr, 1963), Wilson & McLaughlin (2010) posited that studying populations exhibiting behavioural divergence, yet low morphological divergence, is relevant to understand the initial stages of resource polymorphism (Skúlason & Smith, 1995), which makes the Arctic charr an excellent model candidate for studying the eco-evo-devo mechanisms underlying cognition and personality.

3.1.2 The Arctic Charr (*Salvelinus alpinus*): A Peculiar and Uncanny Polymorphic Species

The Arctic charr is the northernmost circumpolar freshwater species, considered the most cold-adapted Salmonid (Bolgan et al., 2016; Jonsson & Jonsson, 2001), and usually thrives in species-poor and oligotrophic lakes and rivers (Klemetsen et al., 2003). All Arctic charr in Iceland have been shown to descend from the single ancestral Atlantic Lineage that colonised Iceland at the end of the Pleistocene era (Brunner et al., 2001; A. J. Wilson et al., 2004). This species displays an evolutionary gradient of morphological and genetic divergence between and within populations across Iceland (Brachmann et al., 2021; Gíslason et al., 1999), from anadromous populations close to the ancestral forms, to lake populations that diverged from the ancestral populations after being landlocked at the end of the last glaciation. Although broader sampling and more genetic data are needed to estimate the origin and history of each population, the degree of divergence from the ancestral form is consequently assumed to depend on the age of the lakes' formation (Brachmann et al., 2021, 2022). The ancestral population is putatively represented contemporarily by extant anadromous populations, like the monomorphic anadromous population in Fljótaá River (AN) retaining higher genetic diversity (Brachmann et al., 2021). This represents an elegant model to study how different degrees of divergence might impact cognition and personality. Indeed, phenotypic differentiation between charr morphs seems to develop under extensive inter- and intra-specific competition for feeding resources during growth (resource polymorphism, (Skúlason & Smith, 1995). This strong selective pressure, stable through time, triggers the development of different morphological traits adapted to specific foraging strategies in each morph (reviewed in Jonsson & Jonsson, 2001, Jónsson & Skúlason, 2000 and Kristjánsson et al., 2011), allowing individuals to share resources by exploiting different niches and hence increase the productivity of the ecosystem (Jónsson & Skúlason, 2000).

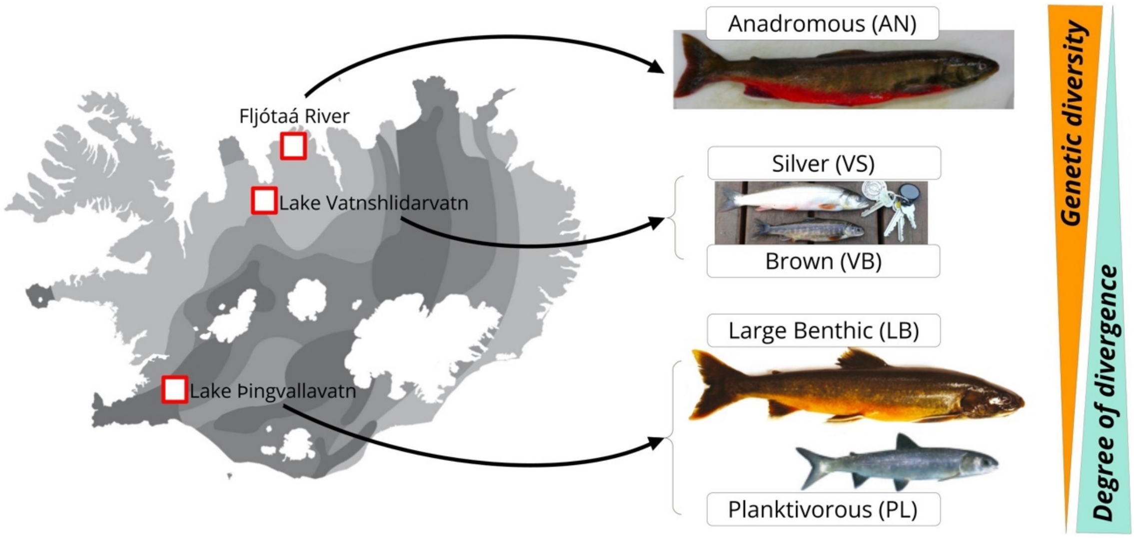


Figure 8 – Map of Iceland (modified from Kristjánsson et al. (2011)*) indicating the location of the three water systems sampled for this study (red-sided squares) with their associated Arctic charr morphs (Fljótaá population composed of the monomorphic Anadromous (AN); Vatnshlíðarvatn population composed of the Brown (VB) and Silver (VS) sympatric morphs; Þingvallavatn population composed of four sympatric morphs, of which the Large Benthic (LB) and the Planktivorous (PL) morphs are studied here). The two gradients on the right highlights the varying degrees of divergence and genetic diversity existing between the three populations. On the map, white areas delimit ice caps and the different shades of grey indicate the age of the bedrock: lightest grey all the way to east and west periphery indicates upper Tertiary (above 3.3 Myr); medium-grey indicates upper Pliocene and lower Pleistocene (0.8 to 3.3 Myr); dark grey indicates upper Pleistocene (0.01 to 0.8 Myr); and darkest grey mostly in the center, indicates postglacial terrain (0 to 0.01 Myr). NB: the age of the lakes formations does not necessarily correspond to the age of the bedrock: Lake Þingvallavatn was formed about 10 000 years ago, and Lake Vatnshlíðarvatn 6000 to 8000 years ago.*

In Icelandic lakes, Arctic charr morphs tend to be either benthic, *i.e.*, feeding on the bottom with stocky dark-brown bodies and subterminal lower jaw seemingly adapted to benthic prey exploitation, or pelagic, *i.e.*, feeding in the water column with fusiform silvery bodies and pointy snouts with terminal mouth seemingly adapted to plankton filtration or even to piscivory (Gíslason et al., 1999; Malmquist et al., 1992; Snorrason et al., 1994). The morphs studied here (Figure 8, Figure S1 in Paper I) display various degrees of genetic, ecological and morphological divergence in the wild, due to partial reproductive isolation and differential niche-use in lakes (Gíslason et al., 1999; Guðbrandsson et al., 2019; Skúlason, Snorrason, et al., 1989). Consequently, morphs ultimately thrive in fundamentally different types of environments in term of structural complexity of the habitat as well. The large and deep spring-fed Lake Þingvallavatn (84km^2, 34m mean depth, 114m max. depth, Figure 8) hosts four sympatric morphs of Arctic charr, of which the Large Benthic (LB, benthic) and the Planktivorous (PL, pelagic) are an extreme example of such divergence, with obvious morphological and coloration differences, distinct specialized diets and spatio-temporally separated reproduction (Jonsson et al., 1988; Malmquist et al., 1992; Sandlund et al., 1987, 1992; Skúlason, Snorrason, et al., 1989; Snorrason et al., 1994). The LB experience complex features amid volcanic boulders beds, crevasses, and algae characterizing the benthic zone of the lake, while the PL live in open waters where visual landmarks and stimuli are scarce (Jónasson, 1992; Malmquist et al., 2000). Lake Vatnshlíðarvatn, a small, shallow (0,7km^2, 2-3 mean depth, 6m max. depth, Figure 8), and structurally simple lake composed in majority of a muddy bottom with gravels on the shores, hosts two sympatric morphs, the Brown (VB) and the Silver (VS), displaying more subtle morphological and ecological differences (Jónsson & Skúlason, 2000): while VB are closer to the previous description of a benthic morph and VS are closer to the description of a pelagic morph, morphological divergence is not as pronounced as between LB and PL (Brachmann et al., 2021). VB specialise on one prey-type and spend their whole life cycle within the lake, reproducing in the littoral area close to the shores of the lake. On the contrary, VS are generalists that alternate prey according to seasonal abundance, their diet overlapping the VB's in the summer, and that migrate to adjacent streams to spawn (Brachmann et al., 2021; Jónsson & Skúlason, 2000).

The Arctic charr development is punctuated with several major ontogenetic shifts happening at different ages according to the morph considered (Klemetsen et al., 2003). Firstly, the fry switch from endogenous feeding relying on their yolk sac reserves, to exogeneous feeding a few weeks after hatching (Wallace & Aasjord, 1984). Secondly, PL, VS and AN morphs undergo habitat shifts, accompanied by different degrees of morphological, physiological, coloration, and behavioural changes linked with smoltification: from spawning grounds to the pelagic areas at around 6 months of age for the PL (Sandlund et al., 1992), from inlets and outlets to the lake between one and two years of age for the VS (Jónsson & Skúlason, 2000), and from the river to the sea for the AN. Very little is actually known about the biology of the AN morph from Fljótaá River specifically, but in other Arctic charr systems, generally both juveniles and sexually mature adults migrate yearly to and from the sea, and first-time migrants are between three and seven years old (Klemetsen et al., 2003). Finally, sexual maturity occurs at around height years of age for the LB and four to five years of age for the PL (Jonsson et al., 1988), two years of age for the VB and 7 years of age for the VS (Jónsson & Skúlason, 2000). Again, mean age at maturation is unknown for the AN morph from Fljótaá River, but other anadromous Salmonids usually mature after one to three seasons at sea (Klemetsen et al., 2003). The non-migratory benthic morphs VB and LB are considered the most derived from the ancestral form due to retention of paedomorphic traits (Skúlason, Noakes, et al., 1989), while the VS morph has retained numerous ancestral features: body and head morphology, colourations, migration (Jónsson & Skúlason, 2000), resource use (Brachmann et al., 2021) and potentially even ancestral shy personality (Benhaïm et al., 2023).

To study the evolution of cognition and personality in this project, I used five morphs of Arctic charr ranging along a gradient of evolutionary divergence from the common ancestor: the monomorphic anadromous population in Fljótaá River (AN) as the putative ancestral form, sympatric VS and VB morphs from Lake Vatnshlíðarvatn as moderately diverged from the ancestor (landlocked 6000–8000 years ago (Jónsson & Skúlason, 2000)), and sympatric LB and PL morphs from Lake Þingvallavatn as highly diverged forms (landlocked approximately 10 000 years ago (Saemundsson, 1992)). Consistently, genetic analyses show strong genetic divergence between Lake Þingvallavatn sympatric morphs (LB and PL, in advanced sympatric divergence state), but weaker genetic separation between Lake Vatnshlíðarvatn sympatric morphs (VB and VS, in early sympatric divergence process) (Brachmann et al., 2021; Gíslason et al., 1999; Guðbrandsson et al., 2019; Kapralova et al., 2011; Steele et al., n.d.).

3.2 Biological Material and Housing Conditions

To address all hypotheses (**H1** to **H5**), studying the eco-evolutionary factors influencing the ontongeny of personality profiles and spatial cognitive abilities, their interactions, and their underlying neural mechanisms, I used the five morphs presented here above raised in common garden experiments. Common garden designs are regularly used in wild morphs of Arctic charr to study the genetic bases of variation in life history (Beck et al., 2022; Skúlason et al., 1996), morphology (*e.g.*, Parsons et al., 2010, 2011), foraging behaviour (Skúlason et al., 1993), gene expression and DNA methylation (Ahi et al., 2015; Matlosz et al., 2022), and recently personality (Benhaïm et al., 2023; Horta-Lacueva et al., 2021). The general workflow of this doctoral project is presented in Figure 9.

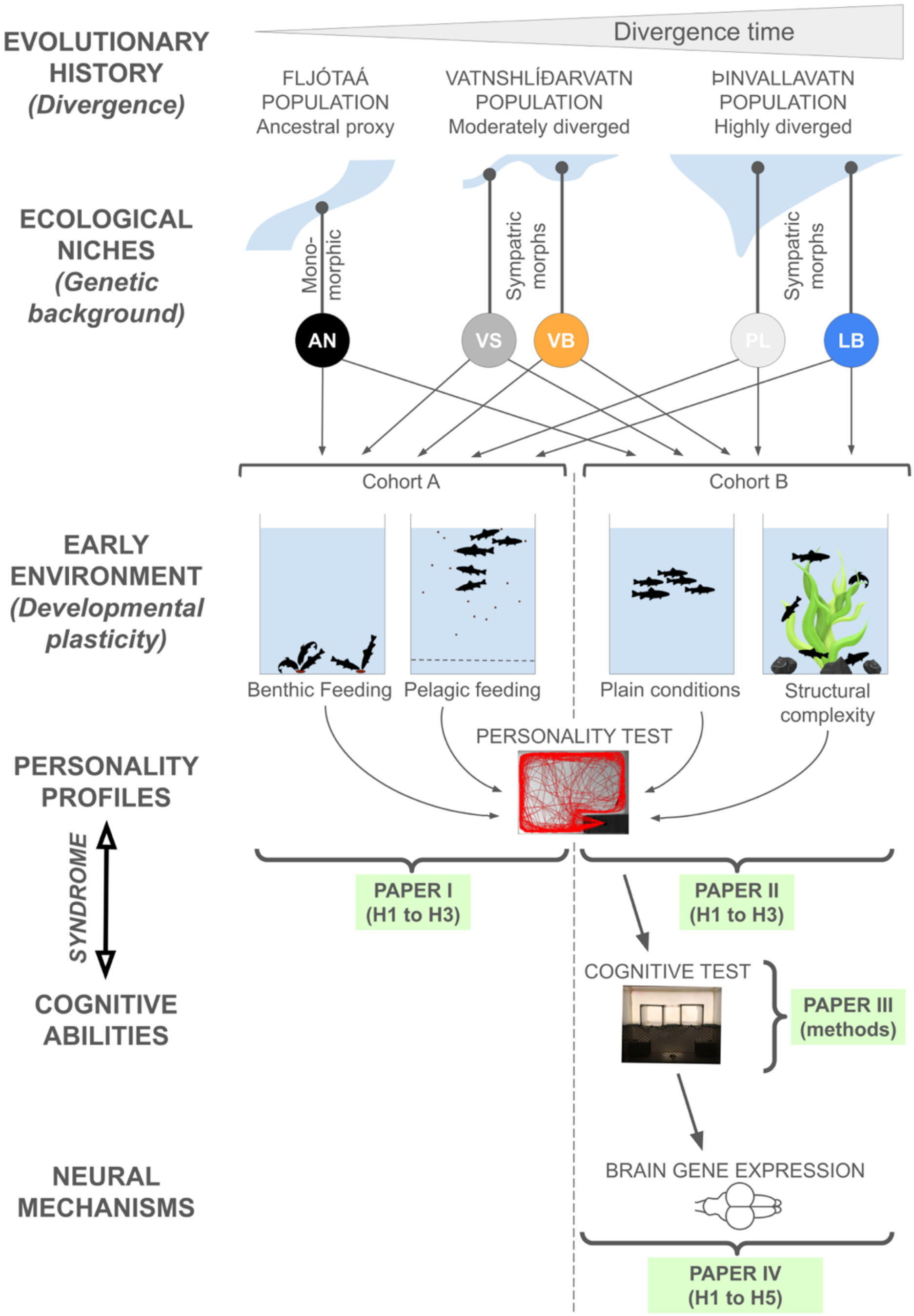


Figure 9 – General workflow of the doctoral project's experiments and the related Papers, along with the hypotheses tackled in each Paper. I used populations from three different freshwaters systems. Each water system is represented in light blue: Vatnshlíðarvatn and Þingvallavatn Lakes as depth profiles, Fljótaá River and streams adjacent to Vatnshlíðarvatn as weaving strings. Fljótaá population consist of the monomorphic Anadromous (AN), extant representative of the ancestral Arctic charr. Vatnshlíðarvatn population is composed of the Brown (VB) and Silver (VS) sympatric morphs, VB being a specialist spending its whole life cycle within the small, shallow and simple lake, while VS is a generalists that migrates to and from adjacent streams to spawn. Þingvallavatn population is composed of four sympatric morphs, of which two are studied here: the Planktivorous (PL), a pelagic morph dwelling in open waters, and the Large Benthic (LB), dwelling amongst complex lava rocks and crevasses of the lake's bottom.

3.2.1 Cohort A: Manipulation of Feeding Modalities

I first wanted to address hypotheses **H1**, **H2** and **H3** focussing on the impact of feeding modalities as the environmental cue for personality development and evolution (Paper I).

For this, I used a first cohort of juvenile fish from the five morphs presented above (Cohort A), from a previous common garden experiment (Steele et al., n.d.). This cohort was obtained through pure crosses of wild-caught Arctic charr, captured at their respective spawning grounds in 2018. This crossing design created three families of full sibs per morph, which were split between two treatments manipulating the fish's feeding modality (following Parsons et al. (2010, 2011), Figure 10). Hence, ten different batches (5 morphs x 2 treatments) were obtained. Food quantity and quality were strictly equal between treatments (aquaculture pellets and bloodworms): only the distribution modality differed, mimicking prey shape and accessibility in natural habitats (Figure 11): pelagic vs. benthic. In the pelagic treatment, food was minced and chopped in small particles, and spread homogeneously through the water column with a mesh placed above the bottom of the tanks to prevent benthic feeding, forcing the fish to catch or filter drifting items like pelagic feeders would in the wild. In the benthic treatment, whole food was accessible exclusively at the bottom of the tank, distributed through a funnel and in a food trap placed at the bottom to encourage scraping of food from the substrate like benthic feeders would. The fish were reared under these feeding modalities for 120 days, after which boldness personality trait was individually measured for 18 fish per family, per treatment and per morph (*i.e.*, 108 fish per morph, N = 540) thanks to an Open Field Test with shelter as described below (section 3.3.1), giving way to individual boldness scores for each fish, and to a group-level proxy of boldness.

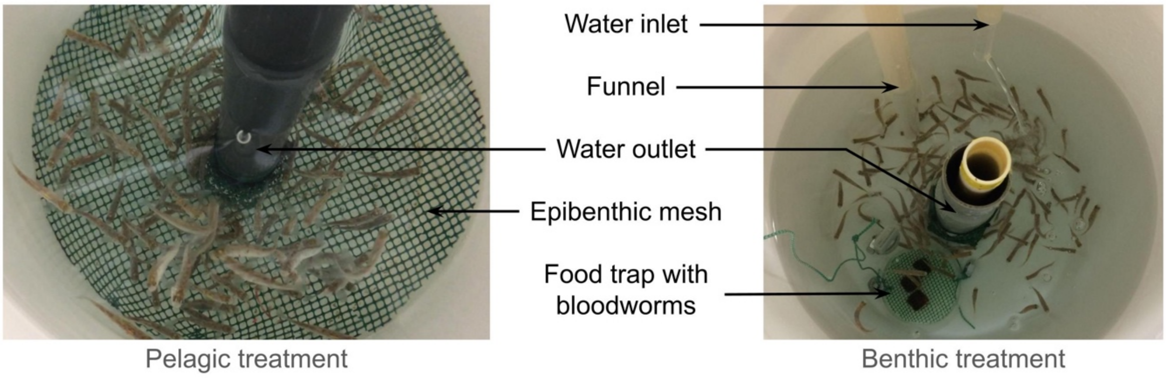


Figure 10 – Photographs of juvenile Arctic charr of Cohort A raised in the pelagic feeding treatment (left) versus the benthic feeding treatment (right). Photograph courtesy: Sarah. S. Steele.

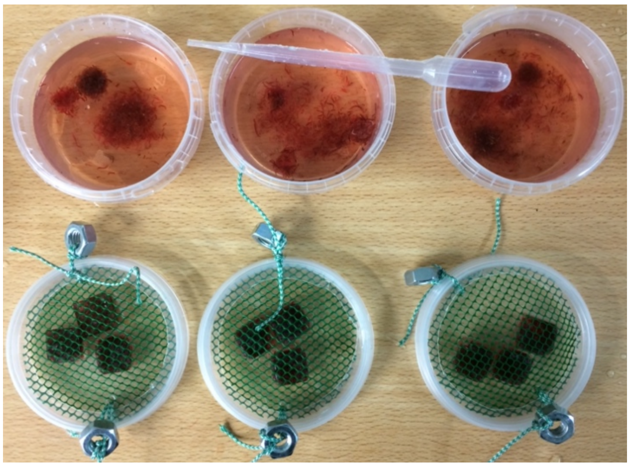
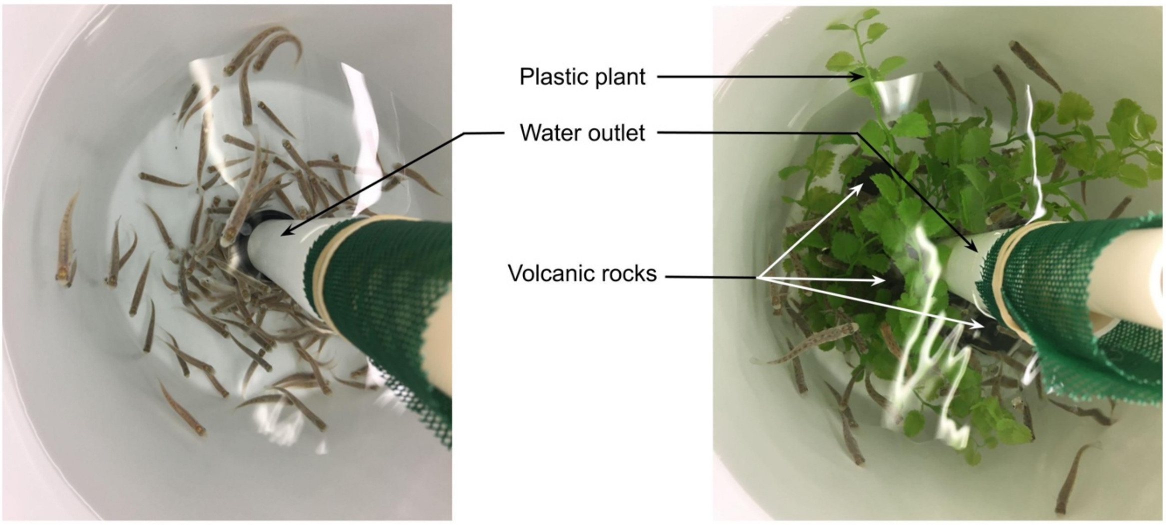


Figure 11 – Bloodworm distribution modalities between feeding treatments in Cohort A. In the pelagic treatment, bloodworms portions were chopped in small particles, distributed with a pipette at the surface, and homogeneously spread through the water column thanks to the current (top pots); in the benthic treatment, whole bloodworms portions were place in a netted food trap sunken at the bottom of the tank (see Figure 10) encouraging scraping (bottom pots). Photograph courtesy: Sarah. S. Steele.

3.2.2 Cohort B: Manipulation of Structural Complexity

Subsequently, I addressed **H1** to **H5**, focussing on the impact of structural complexity as the environmental cue for the development and evolution of spatial cognition and personality, how they relate to each other, as well as the neural mechanisms involved (Papers II to IV).

For this, I used a second cohort of juveniles from wild-caught Arctic charr (Cohort B), from the same five morphs as previously described and as Cohort A. To obtain Cohort B, for each morph, eggs and sperm from wild adult fish were collected at their respective spawning grounds and pooled together in order to enhance genetic variability. After hatching, fish were distributed within a morph over two treatments modulating the structural complexity of the rearing environment (Figure 12, Philip et al., 2022): plain treatment (bare tanks) vs. enriched treatment (with one plastic plant and five volcanic rocks at the bottom of each tank, mimicking natural features of the Arctic charr environment and providing different colours and textures, and structural heterogeneity both horizontally and vertically to the tank). Here again, we obtained 10 different batches (5 Morphs x 2 Treatments), with three replicate tanks per treatment (see details in Paper II). After having experienced those rearing treatments for about 11 months, 52 fish per tank (N=1508 in total) were chosen at random to undergo the Open Field Test with shelter as described previously to characterize personality profiles.



Plain treatment

Enriched treatment

Figure 12 – Photographs of juvenile Arctic charr of Cohort B raised in the plain treatment (left) versus the structurally enriched treatment (right) with a plastic plant and five volcanic rocks.

3.3 Data Collection

3.3.1 Measuring Boldness Personality Trait

I chose to specifically focus on the most widely studied aspect of animal personality (Conrad et al., 2011): boldness – the individual propensity to take risks (D. S. Wilson et al., 1994) – that has recently been reported as a personality trait in the Arctic charr (Benhaïm et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022). For this project, I chose to use the Open Field Test (OFT) as my boldness test. This test was developed in rats by Hall & Ballachey (1932) to measure locomotion and explorative behaviours. Since, the method has undergone substantial modifications and improvements to be used in the field of animal personality, notably with the addition of a shelter (OFT with shelter) to allow easy and rapid assessment of boldness (Perals et al., 2017). The OFT with shelter, also sometimes coined "Emergence Test" (Carter et al., 2013), has been efficiently used to measure boldness in all sorts of taxa including fishes (*e.g.*, Black-chinned tilapia (Benhaïm et al., 2017); European sea bass (Alfonso et al., 2019; Ferrari et al., 2016); Guppy (Burns, 2008); Panamian bishop (Brown et al., 2007); see also the overview of Toms and colleagues (2010) for other fish species), and notably the Arctic charr (Benhaïm et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022).

Open Field Test with Shelter

Four adjacent OFT arenas (Philip et al., 2022) each had a shelter in a corner, and a camera placed above the arenas recorded each trial (Figure 13, Figure 14). At the beginning of a trial, a fish was introduced in each closed shelter. After a 5-minute acclimation, the shelters doors were simultaneously opened, so the fish could willingly emerge from the shelter and were free to explore their respective arenas for 20 minutes. To assess boldness repeatability, the OFT was repeated twice for each individual, with a 7-day interval between the two replications (short-term repeatability). We used the software Ethovision XT (Noldus Information Technology, v. 14 in Paper I, v. 15 in Paper II) to track the behaviour of each fish on the obtained videos (Figure 14). This allowed to quantify a total of 12 risk-taking or risk-aversion behaviours, such as time needed for the fish to dare out of the safe shelter, or thigmotactic behaviours. Thigmotaxis is the propensity to remain close to the enclosure walls and is commonly associated with shyness (Dahlbom et al., 2014; Simon et al., 1994), exposed central areas being considered riskier. Individuals not exiting the shelter within 20 minutes in either replication were considered non-responsive to the test and were excluded from the subsequent Principal Component Analysis (PCA) (see justification in Figure S2 from Paper I).

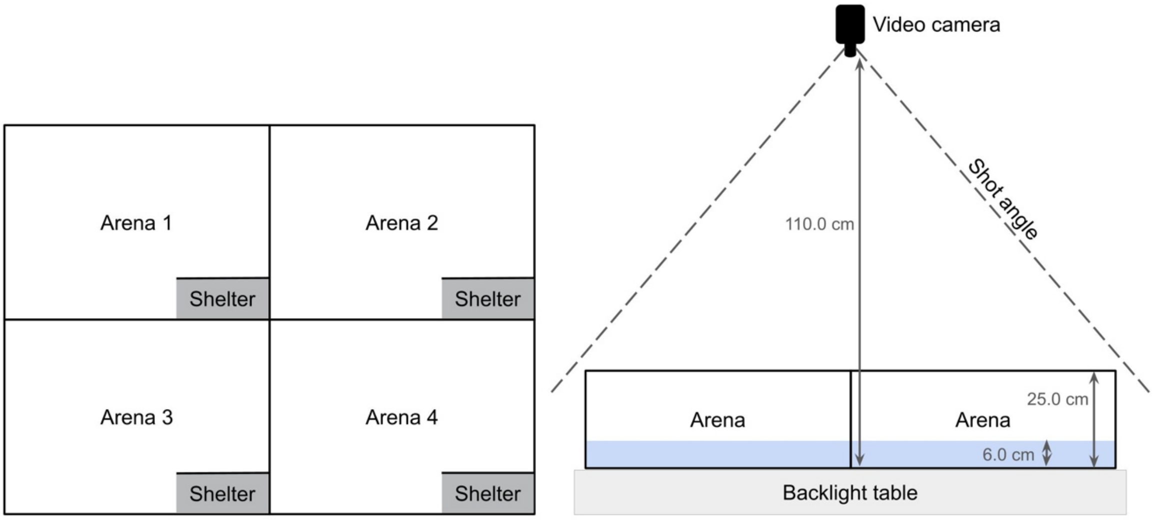


Figure 13 – Top view of the four adjacent Open Field Test with shelter (OFT) arenas (left) and side view of the OFT setup (right), placed on a backlight table (Noldus Technology) providing homogeneously lighting frow the underside for contrast.

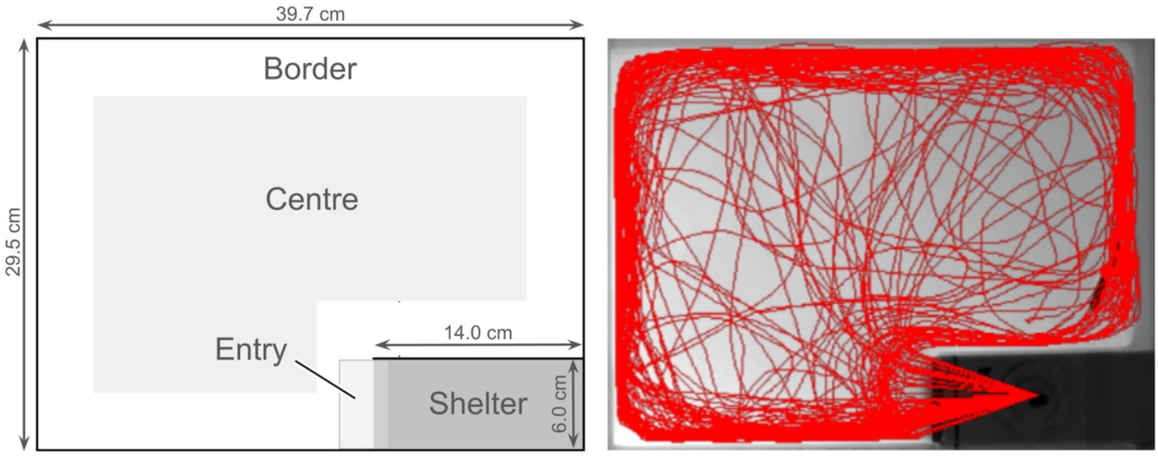


Figure 14 – Top view of the layout of an Open Field Test with shelter (OFT) arena and its virtual zones (left) with a corresponding snapshot of fish movements trackings using Ethovision XT (Noldus Technology) on a video recorded during an OFT trial (right).

Boldness Score: a Multiple-Behaviour Individual-Level Boldness Proxy

Methods using PCA to calculate personality scores have been advocated against, as such designs do not take repeated measures into account, which constitutes a pseudoreplication violation (Budaev, 2010; Dingemanse & Wright, 2020). It is sometimes recommended to use a single behavioural variable instead of a multivariate score to reduce ambiguity (Dingemanse & Wright, 2020). I personally think that using only one behavioural variable, for example the time spent in the shelter, does not reflect the complexity of risk-taking. Fish A could be spending X amount of time in the shelter, but be rather conspicuously explorative of the new risky environment when out of the shelter, while fish B could be spending the same X amount of time in the shelter, but freezing in a corner while out, which would not be reflected if we would only take the single variable of time spent in the shelter into account. Consequently, I chose to keep on creating a boldness score with a PCA method, conserving the multiple-behaviours complexity of risk-taking, but improved the approach to take the repeated measures into account. All behavioural variables recorded during the first OFT replication were reduced into a primary axis of behavioural variation with a PCA, removing variables showing high correlation with others on the factor map (see respective Papers I and II). Both KMO index and Bartlett's sphericity test revealed that the set of variables used to build these PCA were suitable for PCA analyses (Budaev, 2010). The first axis of this PCA (PC1) represented a gradient from shyest (negative values) to boldest individuals (positive values), as low PC1 coordinates usually indicated that the fish remained hiding in the shelter instead of being active in the open field (see details in the respective Papers). We then used the weights of this first PCA as a template applied to the whole dataset. Each individual was hence assigned a boldness score for each replication, corresponding to its coordinate on PC1, the boldness scores at the second OFT replication being predicted based on the PCA parameters calculated with the first replication, which accounts for repeated measures.

Proportions of Non-Responding Individuals: a Group-Level Boldness Proxy

The boldness score, as calculated above, corresponds to an individual-level indicator of boldness. As a major component of shyness in the PCA was the time spent in the shelter, we considered proportions of non-responding individuals—technically spending the maximum time in the shelter—as a complementary group-level indicator of boldness.

3.3.2 Measuring Spatial Cognitive Abilities

Spatial cognitive testing was carried out on individuals from Cohort B (section 3.2.2) and started for a given morph about five weeks after that morph had completed the OFT experiment (see section 3.3.1 above). A minimum of 4 fish per tank (*i.e.*, 12 fish per treatment and per morph, *i.e.*, 24 fish per morph, N=120) were chosen at random among the individuals that had previously been screened for personality with the OFT. These individuals were then subjected to a spatial cognitive task in a T-maze (Paper III). This allowed the characterization of individual spatial learning performances as well as navigation strategies prioritized by the fish, and investigating the extent of mutual influences between spatial cognitive abilities and boldness personality profiles (Paper IV). In order to maximize the sample size, we also tested additional subjects within morphs (N > 24) when time allowed it or when a fish did not respond to the cognitive test (non-responding individuals defined below). Hence, a total of N=145 fish (see Table S1 from Paper IV for details) were submitted to the cognitive test, which was divided into six phases (transfer, familiarisation, laterality test, training, probes and reinforcements), each specifically designed to minimize as much as possible any source of stress for the fish subjects, as well as other caveats (Paper III) that might potentially bias cognitive tests results (section 1.1.3). A brief overview of the cognitive experiment is described hereafter, as extensive technical details can be found in Papers III and IV).

Experimental Set Up and Phases

Spatial cognitive abilities of each fish were individually tested in cognitive arenas (Paper III and IV), made up of a living area connected directly to a T-maze by a starting box, while both left and right arms of the T-maze departing from the starting box directly led back to the living area. Each arena was surrounded by a wooden box, lined with black plastic sheets and with a video-camera slot on top, to prevent the use of extra-maze cues for navigation and to ensure the subject's complete tranquillity (Figure 15, Figure 16). This experiment consisted for the subjects in learning the association between solving the T-maze and obtaining a reward (place-reward association), the reward being returning to the safe living area containing two shelters, lower light exposure, and food. When a subject was to be introduced in an arena (see Methods S1 in paper IV for randomisation details), the transfer from home tanks to the arenas was designed to be as gentle and stress-free as possible (Paper III). The subject then underwent a three-day familiarisation phase, during which the fish would get used to the new living conditions. At the end of the last familiarisation day, the subject performed a laterality test, where the first side of the T-maze (left or right arm) it chose when leaving the starting box was recorded as its individual side-turning preference (Alves et al., 2007). The rewarded arm during the following training and reinforcement phases (left or right) was determined for each subject as the opposite of its side-turning preference, to ensure the fish would actually learn to navigate during training instead of only relying on unlearnt side biases (section 1.1.3). The goal of the following training phase was to train the subjects six times a day, every day, until they learned the rewarded arm's location. In the T-maze, a target-shaped landmark was placed on the side indicating the correct arm (rewarded). To exit the maze, the fish could choose a side through a turn (orienting left or right, motor response strategy) and/or by following the landmark (beaconing strategy). During a training trial, the fish was free to emerge into the T-maze and choose a side. If the fish chose the correct arm, it could swim back to the living area on its own to enjoy its positive reinforcements: peace, shelters, low light, and a maximal food reward of three food pellets. If the fish chose the wrong choice, it would encounter a cul-de-sac blocking access to the living area, received a positive punishment of being guided back to the living area through the correct arm, and a negative punishment of being fed a minimal food reward of only one food pellet. Each subject repeated training trials until it would reach a learning criterion of nine correct choices out of ten consecutive trials. After reaching the 9/10 learning criterion, the subject underwent three probe trials, separated by reinforcement phases of regular training trials to reinforce learning until a reinforcement criterion of four correct choices out of five consecutive trials was reached. During each probe trial, orientation cues were set into conflict by displacing the landmark in the opposite arm compared to training, and the choice of the fish would therefor reveal which navigation strategy was prioritised by the subject: if the fish would choose the side of the landmark, it learnt to associate the landmark with the direction of the reward during training, *i.e.*, beaconing strategy; if the fish would choose the arm previously rewarded during training, it learnt to associate a particular turning direction with the reward during training, *i.e.*, motor response strategy.

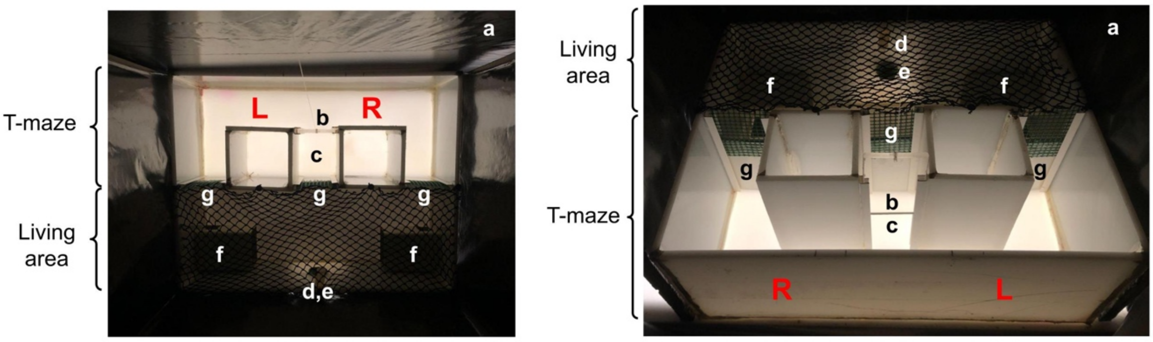


Figure 15 – Photographs of a cognitive arena (see Figure 16 for measurements) in top view from the video camera slot of the closed black box (left) and in high-angle view from the black box open door (right). a: black box. b: transparent pulley-operated door liftable thanks to a transparent string. c: starting box connecting the living area and the T-maze. d: water inlet. e: water outlet. f: shelters. g: opaque removable doors separating the living area and the T-maze. L: left arm of the T-maze. R: right arm of the T-maze. Note that the setup is placed on a backlight table (Noldus Technology) providing homogeneous lighting from the underside, and that the light exposure is softened in the living area. A net is also covering the living area to prevent Salmonids' natural propensity to jump.

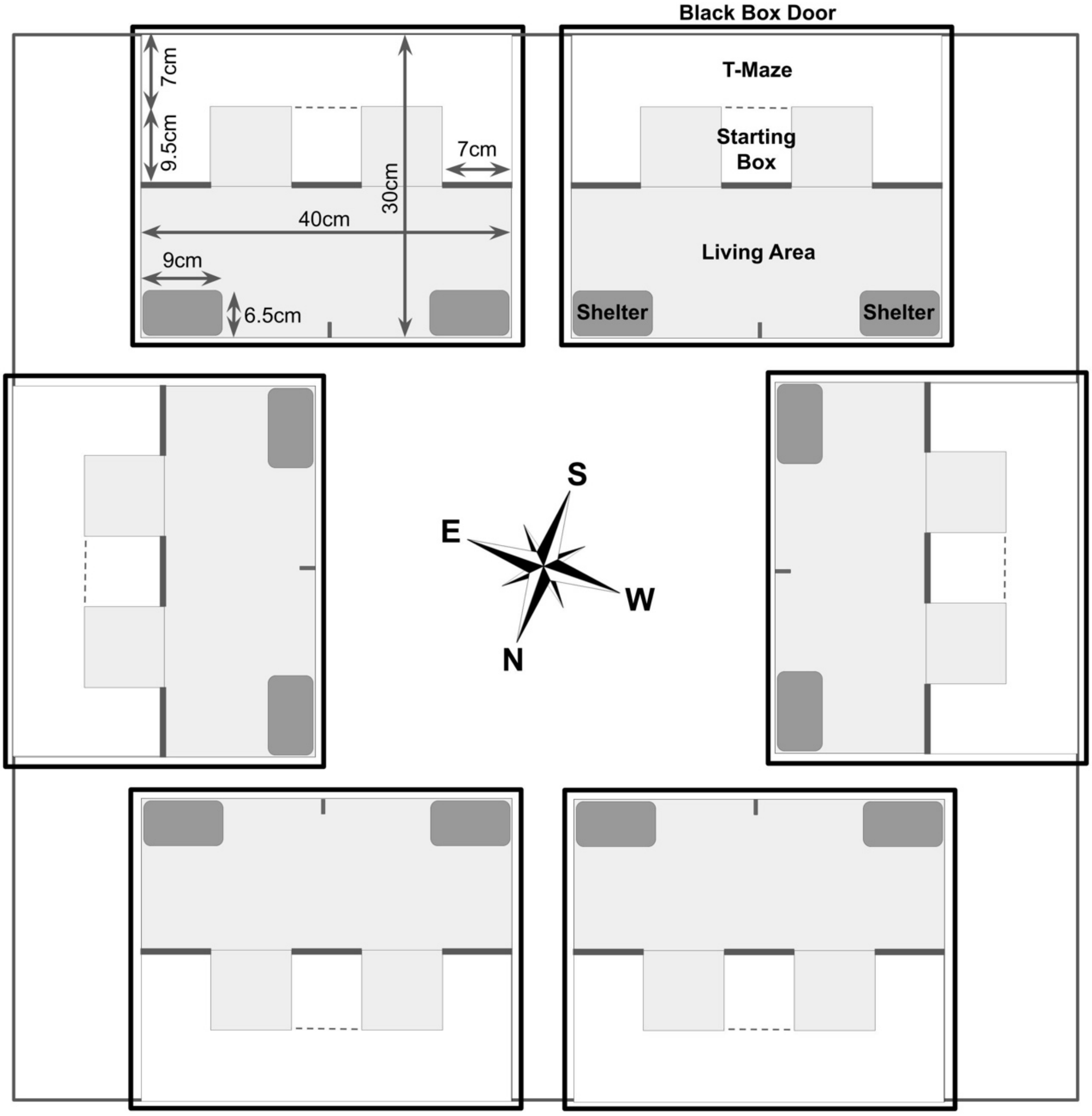


Figure 16 – Top view of the cognitive test setup: six arenas (see Figure 15 for details) on a backlight table (Noldus Technology), each inside a black box (thick black rectangles surrounding each arena), of which the access door for the experimenter was facing outward the backlight table. Note the different orientation of the arenas, randomizing potential geomagnetic cues usable for navigation.

Variables of Interest

The number of trials necessary for each subject to reach the 9/10 training learning criterion (trials to training criterion, TTC) was recorded as a proxy for the individual learning efficiency. The proportion of learners vs. non-learners in each group was recorded as a group-level indicator of learning capacity. Proportions of motor responses vs. beaconing strategies chosen during probes were used to assess navigational strategies prioritized. All of these variables were compared between treatments, morphs, boldness scores, and all of their interactions.

3.3.3 Measuring Underlying Brain Gene Expression

I also investigated the neural mechanisms involved in the development and evolution of spatial cognition and personality, in response to the structural complexity of the environment.

For this, I used individuals from Cohort B that (1) had previously been screened for boldness personality trait the OFT experiment (section 3.3.1) and (2) consecutively had their spatial cognitive abilities characterized by completing the cognitive experiment (section 3.3.2). Twenty minutes after the subject finished its last probe trial, its brain was dissected out and total mRNA was extracted from three different brain regions essential to spatial cognition and boldness: the telencephalon, the cerebellum and the optic tectum. I used quantitative PCR (qPCR, also called real-time PCR or real-time quantitative PCR) to assess the relative expression of 18 genes, related to neural plasticity and its epigenetics (*bdnf*, two paralogs of *c-fos*, *egr1*, *neurod1*, *neurod1-like*, *pcna*, *reelin*, *crebbp*, and five sequences related to the catalytic subunits of *pp1*), to stress regulation (*mr*, *gr*), and to monoamine metabolism (*dbh*, *mao*). The development, optimization, validation and detailed description of the primers and methods used are available in Paper IV and its supplements.

3.3.4 Sex Determination

While being dissected for brains, fin clip samples were also collected on each subject, which were used for sex determination by PCR amplification of the male-specific *SdY* gene on extracted DNA (detailed method in Paper IV).

3.4 Statistical Analyses

All statistical procedures were performed with R v. 4.3.0 software (R Core Team, 2018) unless specified otherwise.

3.4.1 Personality Profiles Comparisons

In Paper I, I addressed hypotheses **H1**, **H2** and **H3** focussing on the impact of feeding modalities as the environmental cue for personality development and evolution. Mean boldness score and the group-level proxy of boldness were compared between morphs to investigate the genetic influence on the development of personality, and between treatments and batches to assess boldness plasticity in response to feeding modalities. Boldness score variances and repeatability were compared between morphs to explore differences in canalization of boldness.

In Paper II, I addressed hypotheses **H1**, **H2** and **H3**, this time focussing on structural complexity as the environmental cue for personality development and evolution. In this Paper we additionally broke down personality traits into several components (mean, within- and among-individual variabilities, repeatability, and plasticity), and specifically examined the development and evolution of each of those components of personality. Similar to Paper I, mean boldness scores were compared between morphs to investigate the genetic influence on the development of personality. I also assessed the plasticity of each boldness component in response to structural complexity, which would translate into boldness components varying between treatments, either overall (environment effect) or within morphs (genotype-by-environment effect). All components of boldness were compared between morphs to investigate whether their development and evolution could be independent from each other.

Statistical comparisons of boldness scores are provided in detail in the R script accompanying Paper I (Dellinger et al., 2023) and in Paper II: they consisted in linear mixed models using the brms package v. 2.19.0 (Bürkner, 2017) in the Bayesian language Stan (Carpenter et al., 2017). We adapted the statistical approach described by Royauté & Dochtermann (2021) to extract among- and within-individual variances in boldness scores from these models, which were used to calculate repeatability of boldness scores. The proportion of non-responding fish was compared between morphs, treatments, and morph-by-treatment batches, with χ^2-tests calculated manually in Excel v. 16.78.

3.4.2 Linking Cognition, Personality and Neural Mechanisms

In Paper IV, I addressed hypotheses **H1**, **H2**, **H3** and **H4**, focussing on the impact of structural complexity as the environmental cue for the development and evolution of spatial cognition, and how it relates to personality traits. I also addressed hypothesis **H5**, assessing the neural mechanisms involved in the development and evolution of spatial cognition and personality, in response to the structural complexity of the environment. Spatial cognitive abilities investigated were spatial learning speed, spatial learning capacity and navigation strategy prioritised when navigational cues are set in conflict.

To detect the presence of a cognition-personality syndrome, I tested for relationships between boldness personality trait and the different spatial learning abilities. I compared spatial cognitive abilities, as well as their relationship with boldness, between morphs to assess the eco-evolutionary and genetic influence on the development of spatial cognition and on cognition-personality syndromes. They were also compared between treatments and batches to assess the developmental plasticity of spatial cognition and its syndrome with boldness in response to early environmental structural complexity. For that, I used χ^2-tests (manually calculated with Microsoft Excel v. 16.85 (Microsoft Corporation, 2018)), two sample t-tests or Wilcoxon rank sum tests, or a linear model again with the brms package v. 2.19.0 (Bürkner, 2017) in Stan Bayesian language (Carpenter et al., 2017) where appropriate.

To unravel neural mechanisms underlying differential development across eco-evolutionary factors of cognitive abilities, personality, and their interactions, I firstly fitted a specific Bayesian brms linear model predicting the influence of spatial learning speed, boldness, and all combinations of their interactions with morphs and treatments, on the relative expression (RE) of each gene of interest within each brain region (cerebellum, optic tectum and telencephalon). Secondly, for spatial learning capacity, I used two sample t-tests or Wilcoxon rank sum tests comparing learners and non-learners in terms of mean gene RE within each brain region.

4 General Results

This thesis shows that the development of mean boldness personality trait and spatial cognitive abilities are predominantly influenced by the genetic background, *i.e.*, morph-specific and very little influenced by the early environment in which the animals developed (low developmental plasticity). Indeed, in Paper I, the benthic feeding treatment led to bolder individuals overall compared to the pelagic feeding treatment, but the environmental effect was rather weak and the degree of developmental plasticity depended on the morph, and structural complexity did affect neither mean boldness in Paper II, nor directly the cognitive abilities in Paper IV. Notably, even though the Arctic charr showed the capacity to use both motor response and beaconing to solve the maze, neither the morph nor the structural complexity treatment had an influence on the navigation strategy prioritized by the fish when navigation cues were set in conflict, as the motor response was the strategy used in vast majority. However, Paper II shows that despite boldness repeatability not being overall affected by environmental complexity either, a morph-by-treatment interaction effect was found, in which boldness tended to be more consistent (*i.e.*, more repeatable and less variable within individuals) within the treatment mimicking the structural complexity of a given morph's natural habitat, hinting that personality emergence might be favoured for individuals experiencing conditions to which they have been adapted. Also, the structurally enriched treatment was shown to engender higher among-individual variance in boldness than the plain treatment, but this effect was also morph-specific.

Paper I also showed that the evolutionary history had an impact on boldness personality trait: clues of boldness canalization were found through a tendency to greater boldness repeatability and a reduction in boldness plasticity along the evolutionary gradient, as well as lower boldness variances in morphs harbouring the longest evolutionary history in their natural habitat. However, these patterns do not follow the predictions of the "plasticity-first hypothesis" (Levis & Pfennig, 2016). The results are more suggestive of an initial decanalization and increased plasticity of boldness upon ancestral colonisation, followed by independent evolution of the different components of personality profiles, depending on the specific conditions met in the new colonized environment.

These findings were confirmed in Paper II, showing that personality components evolve and develop independently from each other: mean boldness increases with divergence from the ancestor, is predominantly genotype-dependent as mentioned earlier and suspectedly highly heritable, while boldness repeatability, that tended to increase with divergence, might rather depend on the ecological niches exploited by each morph. On the contrary, variability-related components of personality (within- and among-individual variances), including their plasticity in response to environmental complexity, were rather dependent on genotype-by-environment effects and are seemingly evolving and developing jointly.

Paper III is a methodological short communication presenting the specificities of the cognitive test, that proved to be efficient in measuring spatial cognitive abilities in juvenile Arctic charr. The experimental approach was designed to avoid all biases one can face during cognitive testing (*e.g.*, differences between subjects in behavioural lateralization or in motivation to perform the task), and took particular care to avoid stress in the animals at all stages of the test. This approach can easily be adapted to all kinds of cognitive experiments carried out on virtually any fish species.

The cognitive test described in Paper III was used in paper IV to explore the eco-evo-devo of spatial cognitive abilities, its interaction with boldness personality trait, and their underlying neural mechanisms. The results show the existence of a cognition-personality syndrome in the Arctic charr, and so, at different scales. At the individual scale, relationships between boldness and spatial cognitive abilities were complex and depended on the morph, the treatment, the morph-by-treatment interaction, and the cognitive ability considered. On a global scale, it seems that mean boldness increases (Paper I, Paper II) and spatial learning speed decreases (Paper IV) with the degree of divergence from the common ancestor. I also found that the syndrome showed developmental plasticity in response to structural complexity, and that this plasticity also decreased with divergence. These findings are, to my knowledge, the first empirical support to the hypothesis that cognition and personality could be co-evolving (Carere & Locurto, 2011).

Results regarding brain gene expression in Paper IV highlighted that the genes coding for dopamine beta-hydroxylase (*dbh*) that converts dopamine into noradrenaline, as well as for the subunits of the memory suppressor serine/threonine protein phosphatase (*pp1*) were particularly implicated in spatial cognition, boldness personality trait, and their syndrome in this species. But again, our results regarding underlying neural mechanisms showed just as much complexity as the cognition-personality individual relationship itself, as the expression of each gene relative to spatial learning or to boldness differed depending all at once on the morph, the treatment, their interaction, and the brain region considered.

5 General Conclusions and Perspectives

This doctoral project uncovered the remarkable complexity of biological mechanisms shaping spatial cognition and personality. It partly unveiled the roles of genetic influence, environmental responsiveness (developmental plasticity in response to both feeding modalities and structural complexity) and evolutionary history in shaping boldness personality trait, spatial cognition, their syndrome, and their underlying neural processes.

I showed that spatial learning and mean boldness were dependent principally on the genetic background (morph-dependent), little influenced by environmental inputs (low developmental plasticity). Given that mean levels of boldness appear to be highly heritable, and considering the syndrome found globally between boldness and spatial learning showing potential co-evolution between the two traits (mean boldness increasing and learning speed decreasing with divergence from the common ancestor), it is likely that spatial learning is also a heritable trait. The pioneering empirical support found herein for the co-evolution between cognition and personality should encourage further research to confirm or infirm the latter inference, notably by including family crossing designs and heritability calculations. This surely represents a challenge given the sample size needed for such designs along with the amount of time needed to train individual fish.

In the introduction (section 1.6.3), I presented how the evolution of behaviour is inherently multidimensional, and that there are inevitable interdependences among all traits of the organism and its ecological conditions. This thesis illustrates this very well, as spatial cognition, personality profiles, cognition-personality syndromes, and their underlying neural mechanisms were essentially dependent all at once on the morph, the treatment, the morph-by-treatment interaction, the spatial cognitive ability examined, and the scale (global or individual) considered, implying extremely intertwined eco-evo-devo interactions at play. This underscores that generalising theories modelling traits evolution like the plasticity-first hypothesis (Levis & Pfennig, 2016), or modelling cognition-personality syndromes like the speed-accuracy trade-off (Sih & Del Giudice, 2012), are too restrictive for such complex phenotypes. The present thesis rather supports a model in which the development of personality traits (including their multiple components), cognitive abilities, cognition-personality syndromes, and their plastic potential, is a "case-by-case" phenomenon. This allows each trait to develop and evolve independently from the others, each shaped by fine-scale and intricate influences of evolutionary, ecological, and developmental factors according to specific local demands for a given genotype in a given ecosystem (*e.g.*, Lande, 2015).

The findings I present here additionally suggest strong evolutionary implications for species adaptability and sympatric diversification, as change in behaviour is indeed the first adaptive response of organisms facing environmental changes (Bateson, 2004; Bogert, 1949; Duckworth, 2009; Levins & Lewotin, 1985; Mayr, 1963). Personality is sometimes viewed as constraining behaviour to consistency by definition (Duckworth, 2010), which might sometimes hamper the attainment of optimal behaviours (Sih et al., 2003). Plus, as exposed in the introduction (section 1.6.2), personality and spatial cognition co-evolving in a syndrome could also slow evolution down by imposing trade-offs where both traits are then sub-optimally expressed (Dochtermann & Dingemanse, 2013; Wolf & Weissing, 2012). This could lead to seemingly maladaptive phenotypes. However, I showed that personality mean, variabilities, repeatability, and plasticity evolve and develop independently from each other, and that the cognition-personality syndrome described here was shown to be plastic, which grants the ability to come up with different combinations of those traits, providing a wide diversity of fine-tuned and optimized behavioural phenotypes to adapt more accurately to subtleties of a broad array of environmental demands (Lande, 2015). Moreover, I also introduced the fact that as much behavioural syndromes can be a brake to evolution, as much they can also accelerate it (section 1.6.1) by fostering rapid and integrated shifts in a whole set of phenotypic traits following environmental shifts, rather than waiting for the right mutation to happen independently for each trait (Wolf & Weissing, 2012). As a matter of fact, abrupt environmental changes (in the present case, forced switch from anadromous migration to lake residency) impose drastic behavioural shifts to cope with new environmental features. Developmental plasticity in personality, cognitive abilities and their syndrome, grants higher adaptability via the potential to quickly reach new adaptive peaks (Rice & Pfennig, 2007; Snell-Rood, 2013), by rapid formation of environmentally induced new consistent behavioural phenotypes at the population level (Baldwin, 1896; Bateson, 2004; Robinson & Dukas, 1999). In oligotrophic systems like artic freshwaters, lowering intra-specific competition by exploiting different niches in the new environment optimizes resource availability and the systems' productivity. Personality and cognitive shifts favouring the use of different resources can hence parallelly create niche shifts as well (Kerr, 2007; Stamps & Groothuis, 2010; Wolf & Weissing, 2010). Differential selective pressures in newly colonized niches can sort out these environmentally induced personalities and cognitive abilities in potentially opposite directions (Pfennig et al., 2010). Eventually, the best-fitted personality-cognition patterns selected for in respective niches might become fixed over time. Ultimately, divergence between individuals in the separate behaviourally constructed niches could occur (Duckworth, 2009). As behavioural traits are often genetically correlated with morphological or physiological traits (*e.g.*, Kern et al., 2016; Muñoz, 2014), selection acting on personality and cognitive traits could also apply indirect selection on the other correlated traits (Huey et al., 2003; Levins & Lewotin, 1985; Wolf & Weissing, 2012), eventually leading to polymorphic or polyphenic species (Dall et al., 2012), as described through resource polymorphism (Levis & Pfennig, 2019; Skúlason & Smith, 1995; Smith & Skúlason, 1996)). Ultimately, given sufficient subsequent reproductive barriers forming between the obtained morphs, this could potentially lead to speciation (Dukas, 2004; Ingley, 2018).

To date, it is still very unclear whether personality, cognitive abilities, and their interplay, could be a cause or a by-product of species diversification. A key challenge to test this scheme would be to define whether populations displaying more plasticity in those traits evolve more rapidly under new selective pressures compared to poorly plastic populations. This would undoubtedly provide outstanding new insights into how such a diversity of behavioural traits can appear and be maintained within and between populations over the course of evolution.

I would like to raise readers' awareness of the fact that these interpretations should be taken with caution. Firstly, this fully laboratory-based design focuses on only two environmental aspects (feeding modality and structural complexity), which is still far from mimicking all potential selective and developmental cues available in nature. Secondly, the expression of boldness in the lab might not necessarily fully reflect natural behaviours (Beukeboom, 2023). Finally, associative learning demonstrated in these laboratory conditions captures only a small fraction of learning abilities in this species. The full ecological relevance of my findings would be better uncovered by experiments conducted directly in natural habitats. This comes of course with its own challenges, like the lack of control over the past experience of each subject, confounding factors of differences in nutritional and health status, etc (Morand-Ferron et al., 2016). Moreover, we found only rare occasions where a neural marker served as a common denominator for personality and cognitive patterns highlighted in Paper IV. It is also possible that other neural markers are more directly related to cognition, personality and their syndrome, or that these traits are governed by multiple genes simultaneously, that were maybe not tested here. These first results regarding the neural mechanisms behind cognition-personality syndromes were nevertheless promising, and greatly encourage further research on that matter. I would suggest the combined use of broad transcriptomic screenings like RNA-Sequencing to get a thorough assessment of all genes involved in these complex phenotypes, alongside immunohistochemistry techniques to pinpoint exact cerebral subregions involved.

Altogether, this doctoral work supports the idea that animals’ behaviour cannot be decoupled from the environment they occupy, as together they form an inseparable set of instruments essential for a harmonious orchestra. Behaviours can result from individuals adapting to their surroundings, and can also initiate changes in their environment through niche construction, or both simultaneously, creating an endless evolutionary arms race dynamic. On an even broader scale, those multidimensional behaviours-driven changes in the niche influence not only the evolution of their conductors, but also influences the evolution of the entire ecosystem they are part of. Again, we can quote here the example of beavers building dams for themselves, but influencing in the meantime the whole ecosystem’s biodiversity and other species’ migration patterns. This even includes humans in the picture by providing essential hydrologic ecosystem services enabling urbanization in zones that would otherwise not be suitable for people to dwell (Bailey et al., 2019). Opposite to this macro-systemic impact of animals’ behaviours, another interesting ecosystemic level that behaviours can influence is the microscopic level of animals’ inner worlds: indeed, through the brain-gut-microbiome axis recently put to light, gut microbial communities interact with the central nervous system of their host, mutually influencing each other. On the one hand, the gut microbiome influences the host’s behaviour, mood and cognition while on the other hand, the host’s behaviour and mental states influence gastrointestinal microbial diversity and assemblages, hence microbial communities’ evolution (see Cryan & Dinan, 2012; Dellinger, 2019; Foster et al., 2017; Martin et al., 2018 for reviews). The role of the gastrointestinal flora, notably parasitism, in the evolution of personality and behavioural syndromes started to be investigated (Barber & Dingemanse, 2010), but this field of research is still in its infancy, therefore offering exciting perspectives for further investigations.

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Data and Accompanying R Script

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Variation in personality shaped by evolutionary history, genotype, and developmental plasticity in response to feeding modalities in the Arctic charr

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Personality; Plasticity; Polymorphism; Arctic charr; Development; Canalization

Abstract

Animal personality has been shown to be influenced by both genetic and environmental factors and shaped by natural selection. Currently, little is known about mechanisms influencing the development of personality traits. This study examines the extent to which personality development is genetically influenced and/or environmentally responsive (plastic). We also investigated the role of evolutionary history, assessing whether personality traits could be canalized along a genetic and ecological divergence gradient. We tested the plastic potential of boldness in juveniles of five Icelandic Arctic charr morphs (*Salvelinus alpinus*), including two pairs of sympatric morphs, displaying various degrees of genetic and ecological divergence from the ancestral anadromous charr, split between treatments mimicking benthic vs. pelagic feeding modalities. We show that differences in mean boldness are mostly affected by genetics. While the benthic treatment led to bolder individuals overall, the environmental effect was rather weak, suggesting that boldness lies under strong genetic influence with reduced plastic potential. Finally, we found hints of differences by morphs in boldness canalization through reduced variance and plasticity, and higher consistency in boldness within morphs. These findings provide new insights on how behavioural development may impact adaptive diversification.

Introduction

Animal personality is defined as among-individual behavioural differences that are consistent across time and/or contexts (Réale et al., 2007; Sih et al., 2004). Variation in personality traits within populations has been shown to be subjected to natural selection due to ecological conditions (Dingemanse & Réale, 2005), with heritability estimated between 0.32 (Réale et al., 2007) and 0.52 (Dochtermann et al., 2015) in various species. Personality can also be plastic, as individuals' behaviour can respond to environmental factors, while remaining consistent among individuals (*e.g.,* the most active individual being less active around predators, but still being the most active relative to other individuals (Sih et al., 2003)). Such behavioural plasticity may influence biological diversification and speciation (Pfennig et al., 2010). Indeed, phylogenetic clades with greater behavioural plasticity display higher species richness (Nicolakakis et al., 2003; Sol et al., 2005). Several theoretical models suggest how different personality traits appear and coexist among individuals within and between populations (reviewed in Dingemanse & Wolf (2010); Wolf & Weissing (2010)): personality differences could arise from individual differences in life-history strategies (Réale et al., 2010), from individual differences in state that can be either directly due to heterogeneous environmental factors or indirectly due to differences in parental contributions (Reddon, 2012), or even from stochastic chaos dynamics (Bierbach et al., 2017; Fisher et al., 2018). Those personality differences could be further maintained if they lead to similar fitness payoffs or through frequency-dependent selection (Dall et al., 2004). Stamps & Groothuis (2010) posited that variations in personality could be at the origin of differential niche-picking or niche construction between individuals (see also Wolf & Weissing (2010)), leading to separation of personalities into sympatric distinct niches for which they are more fitted, which we hypothesize promotes sympatric divergence between personalities within species.

Comparative study of personality variations between species, or between populations occupying different niches within a species, can allow identification of ecological factors driving the evolution of personality. Nevertheless, some pitfalls may hamper the use of this approach: intrinsic interspecies differences in behaviour, and geographical confounding effect as ecological conditions differ between sites. In this project, we aimed at partly by-passing those issues by using different morphs of the same species, the Arctic charr (*Salvelinus alpinus*), a fish of the Salmonid family found in sympatry in Icelandic freshwater lakes and rivers. In order to simultaneously disentangle genetic and environmental effects on the development of personality, these morphs were raised in a common garden design where a key environmental variable was manipulated: benthic vs. pelagic modality of food distribution.

In many freshwater fishes, utilization of benthic vs. pelagic habitats has driven ecological specialization and evolutionary divergence within species, leading to the repeated evolution of coexisting sympatric ecomorphs within lakes (Cooper et al., 2010; Parsons et al., 2020; Skúlason et al., 2019). This high evolvability is believed to be due to a high plastic potential, notably in morphology of feeding apparatus structures linked with different diets (Adams et al., 2003). Such plastic changes are suspected to be initiated by behavioural differences (Snorrason & Skúlason, 2012). As behaviour is the most immediate adaptive mechanism available for animals against environmental change (Bateson, 2004; Bogert, 1949; Duckworth, 2009; Levins & Lewotin, 1985; Mayr, 1963), Wilson and McLaughlin (2010) posited that studying populations exhibiting behavioural divergence, yet low morphological divergence, is relevant to understand the initial stages of resource polymorphism (Skúlason & Smith, 1995).

In Iceland, Arctic charr morphs tend to be either benthic, *i.e.*, feeding on the bottom with stocky dark-brown bodies and subterminal lower jaw seemingly adapted to benthic prey exploitation, or pelagic, *i.e.*, feeding in the water column with fusiform silvery bodies and pointy snouts with terminal mouth seemingly adapted to plankton filtration or even to piscivory (Gíslason et al., 1999; Malmquist et al., 1992; Snorrason et al., 1994). The morphs studied here (Fig.S1) display various degrees of genetic, ecological and morphological divergence in the wild, due to partial reproductive isolation and differential niche-use in lakes (Gíslason et al., 1999; Guðbrandsson et al., 2019; Skúlason et al., 1989). The Large Benthic (LB, benthic) and the Planktivorous (PL, pelagic) sympatric morphs from Lake Þingvallavatn are an extreme example of such divergence, with obvious morphological differences, distinct specialized diets and spatio-temporally separated reproduction (Jonsson et al., 1988; Malmquist et al., 1992; Sandlund et al., 1987, 1992; Skúlason et al., 1989; Snorrason et al., 1994). The Brown (VB) and the Silver (VS) sympatric morphs from Lake Vatnshlíðarvatn display more subtle morphological and ecological differences: while VB are closer to the previous description of a benthic morph and VS are closer to the description of a pelagic morph, morphological divergence is not as pronounced as between LB and PL (Brachmann et al., 2021).

These morphs are thought to descend from the same ancestral population (Brunner et al., 2001; A. J. Wilson et al., 2004), this ancestor likely represented contemporarily by extant anadromous populations retaining higher genetic diversity (Brachmann et al., 2021). Ancestral Arctic charr were landlocked in lakes formed at different geological times after the last glaciation. Although the specific events during colonization and early divergence are unknown, it is assumed that the time since separation from the ancestral population translates into a gradient of evolutionary divergence from the common ancestor between Arctic charr populations throughout Iceland (Brachmann et al., 2021, 2022). Although broader sampling and more genetic data is needed to estimate the origin and history of each population, we use the monomorphic anadromous population in Fljótaá River (AN) to represent the putative ancestral form, sympatric VS and VB morphs from Lake Vatnshlíðarvatn as moderately diverged from the ancestor (landlocked 6,000 to 8,000 years ago (Jónsson & Skúlason, 2000)), and sympatric LB and PL morphs from Lake Þingvallavatn as highly diverged forms (landlocked approximately 10,000 years ago (Saemundsson, 1992)). Consistently, genetic analyses show strong genetic divergence between Lake Þingvallavatn sympatric morphs (LB and PL, in advanced divergence state), but weaker genetic separation between Lake Vatnshlíðarvatn sympatric morphs (VB and VS, in early state of divergence) (Brachmann et al., 2021; Gíslason et al., 1999; Guðbrandsson et al., 2019; Kapralova et al., 2011; Steele et al., n.d.).

Developmental plasticity is the property of a given genotype to produce different phenotypes depending on the environmental conditions under which development takes place (Lafuente & Beldade, 2019), observed as differences in means by conditions (reaction norms). Canalization refers to the capacity of organisms to produce standard phenotypes despite genetic and environmental perturbations, reducing variance around reaction norms (Loison, 2019; Stearns, 1989). Diet treatment where the same food items are either provided as floating (pelagic) or on the bottom (benthic) has been shown to elicit plastic response in growth, body shape and craniofacial/skeletal features in the Arctic charr morphs studied here (Adams et al., 2003; Küttner et al., 2013; Parsons et al., 2010, 2011). The data suggest differences in morphological plasticity, where morphs from Lake Þingvallavatn (LB and PL, more diverged) were less plastic over ontogeny with a more canalized development than morphs from Lake Vatnshlíðarvatn (VS and VB, moderately diverged), and where LB and VB morphs were more morphologically canalized than their respective sympatric counterparts (Parsons et al., 2010, 2011). The "plasticity-first hypothesis" states that ancestral phenotypic plasticity induced by environmental perturbations leads to developmental reorganization and uncovers cryptic genetic variation, producing novel trait variants on which selection can act, and that undergo phenotypic accommodation and ultimately genetic accommodation (Levis & Pfennig, 2016). In this sense, genetic fixation of new behavioural accommodations acquired through ancestral behavioural plasticity and decanalization when the environment changes or when invading a new environment, *i.e.,* Baldwin effect (Baldwin, 1896; Bateson, 2004; Robinson & Dukas, 1999), could allow individuals to survive until genetic assimilation (Waddington, 1953). The costs of maintaining behavioural plasticity could then be reduced over evolutionary time, when the optimal behavioural trait value for the new conditions has been reached, by genetically fixing and canalizing this phenotype in the population. To our knowledge, whether this mechanism could be happening concerning personality traits has not been addressed.

In the present study, we examined what roles genotype, plasticity and canalization play in the development of personality in the Arctic charr. We focused on a widely studied aspect of animal personality, boldness – the individual propensity to take risks (Réale et al., 2007) – that has recently been reported in the Arctic charr (Benhaïm et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022). We hypothesized that (a) differences in ecological backgrounds lead to the evolution of different personality profiles between morphs (genotype), (b) the early environment influences the developmental trajectories of personality traits (developmental plasticity of personality) within morphs, (c) the degree of evolutionary divergence influences the degree of developmental plasticity of personality and the magnitude of personality range between morphs, potentially via canalization.

To address this, juvenile full sibs of AN, VS, VB, LB and PL morphs were reared in common garden in benthic vs. pelagic feeding modality treatments. We tested for differences in boldness repeatability, averages, and variances across morphs and treatments to characterize personality profiles. If personality development is under genetic influence (a), we expected mean boldness to be morph-specific, or at least population-specific (between lakes/rivers). If personality development responds to environmental conditions, *i.e.*, is plastic (b), we expected differences in mean boldness between treatments, either overall or within morphs. If a longer time since divergence leads to canalization of personality traits (c), we expected reduced plasticity, lower variance and higher consistency in boldness in more diverged morphs. Note that these predictions are not mutually exclusive. To our knowledge, this study is the first empirical attempt to simultaneously assess the role of evolutionary history, genetic and environmental influences, and in particular the influence of feeding modalities, in shaping personality during early life.

Material and Methods

Biological Material

This study utilized offspring of wild-caught Arctic charr morphs: AN from Fljótaá River; VS and VB sympatric morphs from Lake Vatnshlíðarvatn; LB and PL sympatric morphs from Lake Þingvallavatn. For each morph, three male and three female wild adults were captured with gill nets or electrofishing during spawning season: AN on the 22/10/2018 (65.999641°, -19.004670°); VS on the 17/09/2018 (65.510907°, -19.666710°); VB on the 03/09/2018 (65.516883°, -19.612980°); LB on the 09/08/2018 (64.234195°, -21.047049°); PL from 07/10/2018 to 14/10/2018 (64.190976°, -21.093420°). Each fish was stripped of gametes and pure crosses were made on site, creating three families within each morph. Eggs from each family were kept separated and raised in common garden. Incubation conditions were complete darkness at 4.3 ± 0.14°C, pH 7.3 ± 0.15, dissolved O_2 saturation 101.4 ± 1.8% and eggs were checked daily.

Housing Conditions

One week before anticipated hatching date, families were transferred to separate 20L cylindrical tanks (water renewal kept above 200% per hour (40-50L/h) to maintain oxygen saturation; 12h/12h light/dark photoperiod at 80 Lux). Fish were fed ground commercial aquaculture start food (INICO 0.4mm) three times a day. Before first feeding, the water temperature was maintained at 4.0 ± 0.3°C matching Þingvallavatn spawning beds temperatures (Adalsteinsson et al., 1992), then progressively increased to 7.6 ± 1.1°C between 50 and 90 degree days (daily temperature x age in days) after first feeding to match more typical groundwater temperatures (Adalsteinsson et al., 1992) and to increase growth rates during feeding treatment. At 1226.3 ± 20.7 degree days after hatching, individuals within families were individually tagged with Visual Implants Elastomer (VIE, Northwest

Marine Technology, 2008), and half was randomly assigned to a benthic feeding treatment, the other half to a pelagic feeding treatment (see next paragraph). Hence, we had 10 different batches (5 Morphs x 2 Treatments). Specificities regarding number of tank replicates, fish density and families' distribution over replicate tanks are detailed in Table S1.

Feeding Treatments

Feeding treatments, following Parsons and colleagues (2010, 2011), started 16 days after tagging and lasted for 120 days. Fish from both treatments received the same food quantity (4% of overall biomass) and quality: from day 1 to day 30, they received a 60:40 percent mixture respectively of INICO commercial aquaculture pellets (to ensure complete nutrition) and bloodworms (to expose individuals to a prey item foraged by juveniles in the wild), then a 50:50 percent mixture from day 31 to day 120. Pellets were distributed manually five times a day and bloodworms once a day. Pellet granulometry was 0.8 mm from day 1 to day 60, and 1.1 mm from day 61 to day 120, to adapt to fish growth. Only the distribution modality differed between treatments, mimicking prey shape and accessibility in natural habitats. In the benthic treatment, food was accessible exclusively at the bottom of the tank: whole pellets were distributed through a funnel and whole bloodworms were distributed in a food trap placed at the bottom to encourage scrapping of food from the substrate like benthic-feeders would in the wild. In the pelagic treatment, ground pellets and chopped bloodworms were spread evenly at the water surface and distributed homogeneously through the water column with water flow. A mesh placed 2 to 3 cm above the bottom of pelagic tanks prevented benthic feeding, forcing the fish to catch or filter drifting items like pelagic-feeders would.

Open Field Test

After the completion of the feeding experiment on day 120, and throughout the behavioural testing, fish were maintained on pellets only, using the same distribution modality as the feeding experiment. Bloodworms were no longer provided because behavioural testing would take entire days, and provisioning bloodworms only once a day as during the feeding experiment, could bias behaviour between fish that had access to it prior to being tested and fish that had not. After three days of adjustment to this diet, at day 124, 18 individually tagged fish per family per batch, equally distributed across replicate tanks (AN, LB: 6 individuals per replicate; PL, VB, VS: 9 individuals per replicate to compensate for lesser replicate tanks per family, see Table S1 for details), were chosen to be submitted twice to an Open Field Test (OFT) with shelter, with a 7-day interval between each replication, to assess boldness personality trait (18 individuals x 3 families = 54 fish per batch, *i.e.* 108 fish per morph, *i.e.*, N=540, Table S1). These fish were semi-randomly chosen in order to cover a size gradient to account for possible influence of body size on behaviour (Benhaïm et al., 2003) as notable size variations existed between and within replicate tanks, morphs and families. Individuals from each family within each tank were divided in three size classes, small, medium and large, corresponding respectively to the 1st, 2nd and 3rd quantiles of the body size distribution of family within tank. Two fish per size class were picked at random for each family and tank. For technical reasons, the test started on day 131 instead of day 124 for the AN. All morphs started OFT trials at 276 to 292 days old from hatching, well before maturity. The OFT arena (Philip et al., 2022) consisted of an opaque white rectangle tank (39.7 x 29.5 x 25 cm) filled with 6 cm water depth at home tanks' temperature, with a shelter in a corner (14 x 6 x 6 cm) provided with a removable door. The set-up was placed on a white LED backlight (Noldus Information Technology) set at 70 Lux to increase contrast. A Basler Ace acA1920-150um camera placed 110 cm above the arena recorded each test at 30 frames per second. At the beginning of the test, the fish was placed in the closed shelter. After a 5-minute acclimation, the shelter door was opened, and the fish was free to explore the arena for 20 minutes. Once the 20 minutes were completed, the fish was gently netted, anaesthetized in a 300 ppm phenoxyethanol solution, weighed, and fork length and total length were measured. Water in the arena was replaced between each trial.

Boldness Score Assessment

The fish were tracked from the obtained videos with Ethovision XT version 14 (Noldus Information Technology). The arena was divided into four virtual zones: the shelter zone covering the shelter area, overlapping with an entry zone extended 3 cm from the shelter door; the border zone corresponding to a buffer of 3.5 cm along the edges of the arena; the centre zone being the remaining part of the arena and corresponding to the riskiest area since thigmotaxis (tendency to remain close to the enclosure walls) is commonly associated with shyness (Dahlbom et al., 2014; Simon et al., 1994). The fish barycentre was used to calculate the latency to first exit the shelter (s), frequency of visits in each zone, cumulative time spent in each zone (duration, s), mean velocity (corrected by body length, s^{-1}), absolute angular velocity ($deg.s^{-1}$) and the total distance travelled (cm). If an individual did not exit the shelter (head and trunk not visible) after 20 minutes in either replication, it was considered not to have responded to the test and was excluded from the subsequent Principal Component Analysis (PCA) (justification in Fig.S2). Eventually, a total of N=463 fish were included in the PCA analyses (details in Table S1).

We can consider that the first OFT replication is the most accurate assessment of boldness, as all individuals are completely naive to the test at that point (Benhaïm et al., 2023; Brown, 2001). Hence, we reduced all behavioural variables recorded during the first OFT replication into a primary axis of behavioural variation with a PCA, removing variables showing high correlation with others on the factor map. We retained total distance travelled, absolute angular velocity, entry frequency, and shelter, centre and entry durations. The first axis of this PCA (PC1) explained 58.0% of the variance in the data, to which the variables with strongest contributions were total distance travelled, shelter duration and entry frequency (respective PC1 loadings: 0.941, -0.935 and 0.839; See Fig.S2 and accompanying R script (Dellinger et al., 2023)). Consequently, PC1 represents a gradient from shyest (negative values) to boldest individuals (positive values), as high PC1 coordinates indicate that the fish explored the arena instead of spending time in the shelter and re-entered the entry zone numerous times. We then used this PCA as a template applied to the whole dataset. Each individual was hence assigned a boldness score for each replication, corresponding to its coordinate on PC1, the boldness scores at the second OFT replication being predicted based on the PCA parameters calculated with the first replication, consequently taking repeated measures into account while conserving the multiple-behaviours complexity of risk-taking.

Statistical Analyses

As time spent in the shelter was a major component of shyness in the PCA, we considered the proportion of non-responding fish – technically spending the maximum time in the shelter – as a complementary group-level indicator of boldness. The proportion of non-responding individuals was compared between morphs, treatments and batches with χ^2 tests.

All other statistics were performed with R v.4.3.0 software (64) and are provided in the R script accompanying this article (63). Mean boldness score and proportion of non-responding individuals were compared between morphs to investigate the genetic influence on the development of personality, and between treatments and batches to investigate boldness plasticity. Boldness score repeatability (*i.e.*, consistency) and variances were compared between morphs to assess differences in canalization of boldness. We partitioned the total variance in boldness into among- and within-individual variances for each morph. Among-individual variance (*Vamong*) represents the group-level variance in boldness, *i.e.*, how much individuals behaved differently from each other across replications. Within-individual variance (*Vwithin*) represents the variance in boldness at the data level, *i.e.*, how much an individual behaved differently from itself across replications.

Mean Boldness Scores

We fitted a linear mixed model predicting z-scaled boldness score in the Bayesian language Stan (Carpenter et al., 2017) using the brms package v.2.19.0 (Bürkner, 2017). The model (referred to as model.0, details in accompanying R script (Dellinger et al., 2023)), included morph, treatment, their interaction, z-scaled total length of the fish and OFT replication as fixed predictors. Random factors included individual ID, family, tank, time-category (as OFT tests were run over entire days, we attributed a time-category to each trial according to running slots to control for potential differences in diel activity) and date (as one OFT replication would last for six days per morph, to account for possible stress accumulation over days). Diagnostics of model.0 were validated by inspecting potential scale reduction factors, effective sample sizes, tails heaviness and lightness, trace plots, densities, autocorrelation plots, and trace rank plots for each model parameter. We also verified the robustness of the results by running model.0 again using several different seeds.

We calculated marginal (R2m), conditional (R2c) and inclusive (IR2) R2 values for model.0, describing the amount of variance in boldness scores explained by the fixed predictors, the whole model, and each predictor respectively (method adapted from Hertel et al. (2023), details in accompanying R script (Dellinger et al., 2023)). We applied the functions emmeans and contrast (package emmeans v.1.7.8 (Lenth, 2023)) under Tukey linear hypothesis to model.0, to perform pairwise post hoc comparisons of boldness scores between morphs and between treatments within morphs.

Boldness Repeatability, among- and within-individual variances

Boldness repeatability (R) evaluates the proportion of the total variance that is due to among-individual variance in boldness (Dochtermann et al., 2015), corresponding to the behavioural consistency at the group level over replications. In other words:

$$R = Vamong / Total\ variance = Vamong / (Vamong + Vwithin)$$

On the one hand, we extracted the posterior samples corresponding to the standard deviations for the ID random predictor and the residuals of model.0, squared them to obtain *Vamong* and *Vwithin* respectively, which we used to calculate R. On the other hand, we calculated those three variables for each morph and compared them statistically by following the method proposed by Royauté and Dochtermann (2021), fitting a multi-level structure brms model (model.morph), with boldness score as the response variable, the morph as fixed predictor, random effects for both the intercept and the morph-related effect, which were nested within the individual IDs, allowing capture of *Vamong* per morph, and finally a sigma term accounting for potential variations in the residual standard deviation among morphs, capturing *Vwithin* per morph (details in accompanying R script (Dellinger et al., 2023)). Diagnostics of model.morph were produced as described in the previous paragraph.

Still using Royauté and Dochtermann's guidelines (2021), we examined pairwise differences in the posterior distributions of *Vamong*, *Vwithin* and R between morphs (Δ*Vamong*, Δ*Vwithin* and ΔR respectively). All estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD), indicating the proportion of samples that are of the estimate's sign, *i.e.*, roughly the probability that the estimate is different from zero. We also calculated the effect sizes for each pairwise comparison to estimate the amplitudes of Δ*Vamong*, Δ*Vwithin* and ΔR between morphs, by calculating the probability of superiority between the posterior distributions of each parameter for each pair of morphs (A statistic (Ruscio, 2008), package RProbSup v.3.0 (Ruscio, 2020)). Finally, we tested whether model.morph detected the correct pattern of differences in variance components among morphs (Royauté & Dochtermann, 2021), by comparing models similar to model.morph, allowing (m1) neither *Vamong* nor *Vwithin*, (m2) *Vamong* only, (m3) *Vwithin* only or (m4) both *Vamong* and *Vwithin*, to vary by morph (details in accompanying R script (Dellinger et al., 2023)).

Note: as a side investigation, we also compared *Vamong*, *Vwithin* and R between treatments within morphs to test the genotype-by-environment influence on the emergence of personality (see accompanying R script (Dellinger et al., 2023)). Results are presented and discussed in Table S2.

Note: Bayesian statistics do not use frequentist p-values. The significance of an estimate is obtained when its CI or its highest posterior density interval (HPDI) does not overlap zero. We considered a tendency for an estimate when the CI or HPDI overlapped zero no further than 0.09 units with a $PD \geq 0.92$. Regarding the A statistic, we considered a large effect when $A \in [0.00;0.10] \cap [0.90;1.00]$, a small effect when $A \in [0.11;0.20] \cap [0.80;0.89]$, and a negligible effect otherwise.

Results

Mean Boldness Score

The morph had significant impact on boldness score, the post hoc test highlighting gradual increase in mean boldness along the divergence gradient (with AN as a reference: AN = VS < VB < LB < PL) with a tendency for the PL to have a higher mean boldness than LB and VB morphs (Table 1, Fig.1A). The treatment itself had no significant impact on boldness score, but the morph-by-treatment interaction had a slight effect within morphs (Table 1), VS-Benthic fish being significantly bolder compared to VS-Pelagic fish, with a similar tendency within PL (Table 1, Fig.1B). Larger fish were also significantly bolder (Table 1). Model.0 explained 62.0% of the variance in the data (R2c), with 30.9% explained by the fixed effects (R2m). Further examination of R2 values (Table 1) indicated that the morph explained the vast majority of the variance (26.8%) while the morph-by-treatment interaction and the total length had a minute impact (0.7% and 0.5% respectively). Variation in boldness scores was similar between families within a morph (visual inspection, see Fig.S3).

Proportions of Non-Responding Individuals

The proportions of non-responding fish (Table 2) depended on the morph (χ^2 (4, N = 536) = 21.27, $p < 0.001$), treatment (χ^2 (1, N = 536) = 4.73, $p < 0.05$) and batch (χ^2 (9, N = 536) = 28.18, $p < 0.001$), and broadly followed the same pattern as mean boldness scores: the more diverged the morph, the bolder, with the fewest non-responding fish (except VB and LB standing out of this pattern). With 6.5% more fish coming out compared to the pelagic treatment, fish from the benthic treatment appeared significantly bolder overall. This was also the case within morphs, with the AN showing the largest proportion difference between treatments, but more marginally for the VB in which proportions were almost equal between treatments.

Boldness Repeatability, Vamong and Vwithin

The boldness score was repeatable over the whole data set – confirming (along with Benhaïm et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022) that boldness can be considered a personality trait in the Arctic charr (Réale et al., 2007) – as well as within each morph. A notable exception was the AN (Table 3). Interestingly, repeatability tended to increase along the divergence gradient, with the AN and PL morphs at the two extremes of the gradient showing a significant difference (AN < (VS = VB = LB) < PL, Table 3). The A statistics revealed notable differences in repeatability in pairwise comparisons involving AN and PL morphs (Table 3). Also, AN had generally lower *Vamong* compared to the other morphs, and when considering *Vwithin*, AN and PL morphs had similar values and were significantly less variable than VS, VB and LB morphs, which were also similar to each other (*i.e.*, the general *Vwithin* pattern (AN = PL) < (VS = VB = LB), Table 3, Fig.1A). The A statistics revealed large differences in pairwise comparisons of *Vamong* involving AN, and the only negligible difference in *Vwithin* was between LB and VS morphs (Table 3). Out of the four models compared, m4 was best supported, indicating that both *Vamong* and *Vwithin* varied by morph (Royauté & Dochtermann, 2021).

Discussion

Boldness Under Strong Genetic Influence

The results show that the development of boldness in this species is predominantly influenced by genetic background and less so by the environment (feeding modality) (see also (Benhaïm et al., 2023; Philip et al., 2022)). We and previous studies found no differences in mean boldness score between sympatric morphs (*e.g.*, VS-VB, LB-PL, SB-PL, (Benhaïm et al., 2023; Horta-Lacueva et al., 2021)). This suggests that genetic differences between closely related sympatric morphs are too subtle to engender detectable differences in boldness. However, PL and LB sympatric morphs, which are also more genetically, ecologically and morphologically diverged from each other than VB from VS, tended to differ in mean boldness score (Table 1, Fig.1A). This indicates genetic influence on boldness may be more easily discovered in more diverged populations between lakes and rivers and suggests that differences in average boldness could widen as sympatric morphs further diverge from each other.

An increase in boldness with the morph's degree of divergence from the common ancestor was observed (roughly, AN < VS < VB < LB < PL, Table 1, Fig.1A). Similar ranking of boldness by morph was seen in independently reared offspring (same morphs sampled three years prior in (Benhaïm et al., 2023); one to two years later in Dellinger et al. (Dellinger et al., n.d.)). Such consistency within morphs across years further suggests that boldness patterns are heritable, with stable selective pressures maintaining them in the wild. The boldness gradient among the five morphs also appears to be consistent over social rearing contexts, as individuals raised in isolation (Benhaïm et al., 2023) responded similarly to our individuals raised in high-density groups. This corroborates previous studies showing no effect of direct early-life social exposure on mean personality trait (Bierbach et al., 2017), or on repeatability of behaviour (Edenbrow & Croft, 2013), unlike the highly influential effect of social contexts on personality usually described in other taxa (Webster & Ward, 2011). All these observations constitute even more clues in favour of a strong and heritable genetic effect on Arctic charr boldness development.

Boldness Plasticity

The treatment engendered a plastic response in boldness, with significantly fewer non-responding fish in the benthic treatment compared to the pelagic treatment (Table 2). Although we did not detect this relationship between boldness score and benthic treatment in the fish that responded to the OFT, such a pattern can be perceived (Fig.1B) as all morphs except the VB show lower boldness scores in pelagic treatment (significant for VS, tendency for PL), the morph-by-treatment interaction weakly but significantly influencing boldness scores (Table 1). If non-responding individuals could have been attributed a (mostly likely low) boldness score (discussed in Fig.S2), one can imagine stronger boldness scores differences between treatments within morphs, hence the detection of higher plasticity levels. This would be especially apparent within the AN, showing a rather flat boldness score reaction norm, but the largest difference in proportions of non-responding fish, between treatments. These elements considered parallelly indicate that the development of boldness is plastic, and that the degree of plasticity differs by genotypes. Higher boldness in benthic treatment may be due to competitive access to localized food. Agonistic behaviours towards conspecifics are well-known in the Arctic charr, with dominance typically established through a high degree of aggressiveness by larger fish monopolizing access to food (Adams et al., 1995). A positive correlation between boldness and dominance has already been demonstrated in salmonids (Sundström et al., 2004) which parallels our results showing a significant positive effect of size on boldness. Hence, interindividual competition in our benthic treatment may have fuelled higher levels of boldness to fight for first access to the resource. Competition was probably much lower in the pelagic treatment where food was equally accessible by all through the water column. In this case, further work is necessary to assess whether these observed behavioural patterns relate to developmental plasticity (long-term effect) or temporal plasticity (or flexibility) in boldness (Stamps, 2016).

Two explanations could be mentioned for the overall low plasticity level we found. Firstly, the treatment used may not elicit strong plastic changes in this species, or was not administered long enough. Exposure to the same feeding modalities only elicited minimal plastic response in morphological traits among these morphs (Parsons et al., 2011). Additionally, environmental enrichment administered from hatching had no detectable effect on boldness in an Arctic charr aquaculture strain (Philip et al., 2022). This suggests that weak plastic response may not necessarily reflect insensibility to a particular treatment, but rather a lack of environmental responsiveness of boldness in the species. Secondly, environmental canalization might have evolved rapidly and only small amounts of remaining plasticity are detectable, like Parsons and colleagues (2011) suggested about Arctic charr morphology.

Potential Effects of Evolutionary History on Variation in Personality

If a longer time since divergence leads to canalization of personality traits, we expected reduced plasticity, overall lower boldness variance and higher boldness consistency in more diverged morphs. Following our predictions, boldness consistency showed a tendency to increase with divergence degree. A higher sample size and/or more numerous OFT replications would most likely have turned this tendency into significant increase (Dingemanse & Dochtermann, 2013), given the notable effect sizes detected between morphs in terms of repeatability (Table 3). The ancestral proxy AN showed an extremely low repeatability, which can be explained by this morph's general behaviour in the OFT: consistent with AN's low mean boldness score, almost all AN individuals actually spent most of their time hiding in the shelter (median of 89% of the time, for a dataset median of 56%). Therefore, AN's low boldness repeatability was not due to individuals not behaving consistently over replications, but rather because they all behaved the same (Réale et al., 2007). AN also had significantly lower boldness variance components. A current extremely canalized state of boldness in the AN, as it is the morph with the longest adaptation time to its habitat, would explain the apparent lack of boldness variability in this morph, despite their higher genetic diversity (Brachmann et al., 2021). Nevertheless, AN morph still showed substantial plastic potential, as it had the largest difference in percentage of non-responding fish between treatments. The "plasticity-first hypothesis" (Levis & Pfennig, 2016) suggests plasticity in ancestral populations provides opportunity for adaptive divergence when colonizing a new environment, describing ancestral plasticity as a key for rapid evolution, predicted to be reduced after acclimation to the new habitat. Our data does not support this model (Levis & Pfennig, 2016), as 1) even though boldness plasticity differed to some extent between morphs, submitting our ancestral proxy to "derived" conditions (treatments) 2) did not trigger the expression of boldness levels found in diverged morphs and 3) did not uncover ancestral cryptic genetic variation as boldness variance components in the AN were among the lowest. However, it is worth noting that in another study in which no differential treatments were applied to the same morphs (Benhaïm et al., 2023), the AN was 1) the only morph showing temporal plasticity in boldness over multiple OFT replications, with a boldness score increasing and ultimately reaching both 2) a boldness mean equivalent to the most diverged morphs and 3) boldness variance equivalent to the most variable morph.

The data may fit better with a scenario where mean, plasticity and variances of a trait can evolve independently (*e.g.*, Lande, 2015). In this view, the trait is canalized in the ancestral environment, and a transient boost in plasticity and its potential further maintenance after drastic environmental change is contingent on multiple factors, such as the difference in mean, variance and predictability of the new environmental conditions for instance. Independent evolution of boldness mean, plasticity, variances and consistency could explain discrepancies in boldness components observed here between sympatric morphs.

The trend for increasing boldness repeatability with divergence across morphs – that is, more pronounced personalities in more diverged morphs – suggests an initial decanalization of boldness upon ancestral colonization, followed by progressive recanalization of this trait over time (Bell et al., 2009). This is consistent with higher boldness variability found in more recently diverged VS and VB, possibly representing a state of decanalization or weak canalization (Parsons et al., 2010, 2011). Also, the fact that AN and VS had the highest plasticity, respectively in proportions of non-responding fish (Table 2) and in boldness score reaction norm (Fig.1B, Table 1) between treatments, also supports a possible boost in boldness plasticity in colonizing ancestors. Indeed, the recently diverged VS has retained numerous ancestral features: body and head morphology, colourations, migration (Jónsson & Skúlason, 2000), resource use (Brachmann et al., 2021) and even mean boldness (as seen here and in Benhaïm et al., 2023), hence probably also its seemingly high remnant plasticity. The loss of plasticity in VB could be attributed to ecological divergence among these sympatric morphs. VB specialize on one prey-type and spend their whole life cycle within the lake, while generalist VS alternate prey according to seasonal abundance and migrate to adjacent streams to spawn (Brachmann et al., 2021; Jónsson & Skúlason, 2000). VS's generalist lifestyle might promote plasticity persistence to be able to juggle with those seasonal changes. Highly diverged LB and PL both showed high consistency and somewhat equal levels of plasticity, but differed in boldness within-individual variance. Low variability and high consistency in boldness in the PL is likely attributed to narrow specialization in a "simple" open-water environment where they shoal in small groups (Sandlund et al., 1992). This probably favours predictable and consistent behaviours for better coordination necessary for shoal formation, hence a high degree of behavioural canalization, also allowed by their longer divergence time. PL residual degree of boldness plasticity (tendency, Table 1) might be maintained to allow for behavioural adjustments to a pelagic lifestyle after ontogenetic shift from spawning grounds. In contrast, its sympatric counterpart LB dwells solitarily in a highly complex habitat amidst lava rocks and crevasses (Snorrason & Skúlason, 2012) probably equally favouring the use of multiple behavioural sub-niches (multiple behavioural optima), leading to an array of coexisting consistent boldness profiles: bold individuals actively defending territories, intermediate fish searching for food in unoccupied spots, and shy individuals hiding from predators/conspecifics between crevasses. The higher within-individual variance in boldness may additionally grant LB individuals the flexibility to exploit those different behavioural sub-niches when needed (*e.g.*, a shy fish sometimes risking exiting its crevasse to find food). Consequently, despite the common evolutionary history of Þingvallavatn morphs, major ecological differences between them could explain the hampering of boldness canalization towards a single boldness profile in the LB, as opposed to the highly canalized PL. In both sympatric pairs, the more specialized morph tended to be bolder, consistent with specialist lifestyle favouring plasticity loss and fixation of inflexible behavioural routine often associated with higher boldness (Coppens et al., 2010).

Interestingly, boldness seems to follow a canalization fashion similar to morphological canalization among these morphs (Parsons et al., 2011). This hints towards potential genetic correlation between these morphological and behavioural traits, hence selection acting on personality traits could lead to correlated changes in morphology (Huey et al., 2003; Levins & Lewotin, 1985; Wolf & Weissing, 2012). However, LB was found to be more morphologically canalized than PL (Parsons et al., 2011) when we found the contrary for boldness, suggesting that evolution of canalization can be trait-specific. All in all, these interpretations should be taken with caution, as this fully lab-based design focuses on only one aspect of foraging, still far from mimicking all potential selective and developmental cues available in nature, and as expression of boldness in the lab might not necessarily fully reflect natural behaviours. An experimental design including more families would be beneficial to confirm our conclusions.

Conclusion and Implication for Sympatric Diversification and Adaptability

This study contributed to partly unveil the roles of genetic influence, environmental responsiveness (developmental plasticity) and evolutionary history in shaping boldness as a personality trait. We showed that the level of boldness was dependent mostly on genetic influence (morph-dependent and heritable), little influenced by environmental inputs (low plasticity). We also found clues of boldness canalization by showing a tendency to greater boldness consistency and a reduction in plasticity along the evolutionary gradient, and lower boldness variance in morphs harbouring the longest evolutionary history in their habitat. These findings suggest strong evolutionary implications for species diversification, as change in behaviour is indeed the first adaptive response of organisms facing environmental changes (Bateson, 2004; Bogert, 1949; Duckworth, 2009; Levins & Lewotin, 1985; Mayr, 1963). Radical environmental changes (in this case, from anadromous migration to lake residency) impose behavioural shifts to cope with new environmental features. Developmental plasticity in personality grants higher adaptability via the potential to quickly reach new adaptive peaks (Rice & Pfennig, 2007; Snell-Rood, 2013), by rapid formation of environmentally-induced new consistent behavioural phenotypes at the population level (Baldwin, 1896; Bateson, 2004; Robinson & Dukas, 1999). In low productivity systems, lowering intra-specific competition by exploiting different resources in the new environment optimizes resource availability. Hence, personality shifts favouring the use of different resources, parallelly create niche shifts as well (Stamps & Groothuis, 2010; Wolf & Weissing, 2010). Differential selective pressures in newly colonized niches can sort out these environmentally-induced personalities in potentially opposite directions (Pfennig et al., 2010). Eventually, the best fitted personality patterns selected for in respective niches might become fixed over time. Ultimately, divergence between individuals in the separate behaviourally-constructed niches could occur (Duckworth, 2009). As behavioural traits are often genetically correlated with morphological or physiological traits, selection acting on personality traits could also apply indirect selection on the other correlated traits (Huey et al., 2003; Levins & Lewotin, 1985; Wolf & Weissing, 2012), eventually leading to polymorphic or polyphenic species (resource polymorphism (Levis & Pfennig, 2019; Skúlason & Smith, 1995; Smith & Skúlason, 1996)). To date, it is still very unclear whether the emergence of personality could be a cause or a by-product of species diversification. A key challenge to test this scheme would be to define whether more plastic populations in terms of personality evolve more rapidly under new selective pressures than populations with low personality plasticity. This would for sure provide outstanding new insights on how such a diversity of personality traits can appear and be maintained within and between populations over the course of evolution.

Ethics

Fishing in lakes and rivers was done with permission obtained from the landowners, and ethics' committee approval was not needed for regular or scientific fishing in Iceland at this time accord- ing to the Icelandic Animal Protection Act (Act 15/1994, last updated with Act 11/2014). Sampling was performed by Hólar University Aquaculture Research Station (HU-ARC) personnel, and the fish were kept under an operational license to this station, which includes clauses of best practices for animal care and experiments. All pro- cedures were designed to minimize stress in the tested animals, in line with the European standard animal care protocols, and respect the Icelandic Animal Protection Act. Optimal dosage of anaesthesia with phenoxyethanol was adjusted to the reaction of the individual, following the recommendations of the laboratory facility.

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Paper I - References

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Paper I - Tables and Figures

*Table 1 – **Left**: output of model.0, Bayesian brms linear mixed model predicting z-scaled boldness score including the morph (AN from Fjlótaá River taken as reference, sympatric VS and VB from Vatnshlíðarvatn, and sympatric LB and PL from Þingvallavatn), treatment (Benthic or Pelagic, Benthic being taken as reference) and their interaction (AN morph and Benthic treatments taken as references), z-scaled total length of the fish and OFT replication (first replication taken as reference) as fixed predictors, and individual ID, family, tank, date and time-categories as random predictors. **Middle**: variance explained (IR2) by each predictor of model.0. **Right**: results of the Tukey post hoc pairwise comparisons of boldness scores between morphs, and between treatments within morphs, carried out on model.0, with significant estimates highlighted in bold black font, while tendencies are highlighted in bold grey font. Estimate: median of posterior distribution for each variable or contrast; SE: Standard Error; CI: Credible Interval; HPDI: 95% Highest Posterior Density Intervals.*

Model.0				Variance explained			Post Hoc			
Predictor	**Estimate**	**SE**	**CI**	**Predictor**	**IR2**	**HPDI**	**Comparison**	**Pair**	**Estimate**	**HPDI**
Intercept	-0.81	0.31	[-1.43 ; -0.18]							
VS	0.67	0.41	[-0.14 ; 1.47]	Morph	0.268	[0.10 ; 0.43]	Between morphs	VS - AN	0.49	[-0.31 ; 1,26]
VB	0.82	0.41	[0.00 ; 1.63]					VB - AN	**0.89**	[0.12 ; 1.68]
LB	0.97	0.41	[0.17 ; 1.77]					LB - AN	**0.92**	[0.13 ; 1.69]
PL	1.72	0.41	[0.91 ; 2.52]					PL - AN	**1.63**	[0.85 ; 2.43]
Treatment pelagic	-0.04	0.15	[-0.34 ; 0.25]	Treatment	0.008	[0.00 ; 0.02]		VB - VS	0.39	[-0.39 ; 1.15]
Total length	0.12	0.04	[0.05 ; 0.19]	Total Length	0.005	[0.00 ; 0.02]		LB - VS	0.43	[-0.32 ; 1.22]
OFT Replication 2	-0.01	0.08	[-0.17 ; 0.14]	OFT Replication	0.001	[0.00 ; 0.00]		PL - VS	**1.15**	[0.37 ; 1.92]
VS Pelagic	-0.35	0.21	[-0.75 ; 0.06]	Morph-by-Treatment interaction	0.007	[0.00 ; 0.06]		LB - VB	0.03	[-0.76 ; 0.80]
VB Pelagic	0.13	0.22	[-0.30 ; 0.56]					PL - VB	**0.75**	[-0.04 ; 1.52]
LB Pelagic	-0.10	0.21	[-0.51 ; 0.30]					PL - LB	**0.72**	[-0.05 ; 1.49]
PL Pelagic	-0.18	0.21	[-0.58 ; 0.23]							
ID	0.30	0.05	[0.20 ; 0.39]	ID	0.094	[0.04 ; 0.15]	Between treatments within morphs (Benthic - Pelagic)	AN	0.04	[-0.25 ; 0.33]
Family	0.43	0.12	[0.26 ; 0.73]	Family	0.167	[0.05 ; 0.44]		VS	**0.39**	[0.11 ; 0.67]
Tank	0.08	0.05	[0.00 ; 0.20]	Tank	0.006	[0.00 ; 0.03]		VB	-0.09	[-0.39 ; 0.23]
Date	0.19	0.04	[0.12 ; 0.28]	Date	0.035	[0.01 ; 0.07]		LB	0.15	[-0.16 ; 0.43]
Time-categories	0.20	0.15	[0.06 ; 0.59]	Time-categories	0.035	[0.01 ; 0.07]		PL	**0.22**	[-0.06 ; 0.51]
Sigma	0.67	0.02	[0.63 ; 0.72]							

Table 2 – Proportion of non-responding individuals, i.e., fish that did not exit the OFT shelter after 20 minutes, per morph, per treatment and per batch (N=73 out of 540 fish tested).

		Morph *All treatments considered*	AN	VS	VB	LB	PL
			19.4 %	*13.0 %*	*19.6 %*	*15.1 %*	*0.9 %*
Treatment	*All morphs considered*						
Benthic	*10.4 %*		13.0 %	9.3 %	19.2 %	10.9 %	0.0 %
Pelagic	*16.9 %*		25.9 %	16.7 %	20.0 %	19.6 %	1.9 %

Table 3 – Estimates per morph (AN from Fjlótaá River, sympatric VS and VB from Vatnshlíðarvatn, and sympatric LB and PL from Þingvallavatn), pairwise differences in the posterior distributions between morphs (Δ) and their effect sizes, of boldness score repeatability (R), among-individual variance (Vamong) and within-individual variance (Vwithin). Significant Δ estimates and large effect sizes are highlighted in bold black font, while tendencies for Δ and small effect sizes are highlighted in bold grey font. ***Estimates & Δ****: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ, indicating the proportion of samples that are of the estimate's sign, i.e., roughly the probability that the estimate is different from zero.* ***Effect size****: probability of superiority (A) with its standard error (SE) and 95% confidence interval (CI), between the posterior distributions of each variable for each pair of morphs. The A statistic is a probability-based measure of effect size, indicating the probability that a randomly chosen member of group 1 scores higher on the response variable than a randomly chosen member of group 2, i.e., in our case if A < 0.5, morph 2 outscores morph 1 and vice versa.*

Variable	Estimates				Pairwise comparison	Δ				Effect size			
	Morph	Estimate	Lower CI	Upper CI		Estimate	Lower CI	Upper CI	PD	A	SE	Lower CI	Upper CI
R	AN	0.03	0.00	0.18	VS - AN	**0.21**	-0.03	0.41	0.95	**0.95**	0.00	0.95	0.96
					VB - AN	**0.18**	-0.06	0.40	0.92	**0.92**	0.00	0.92	0.93
	VS	0.25	0.03	0.44	LB - AN	**0.24**	0.00	0.45	0.98	**0.98**	0.00	0.97	0.98
					PL - AN	**0.40**	0.19	0.56	1.00	**1.00**	0.00	1.00	1.00
	VB	0.22	0.01	0.42	VB - VS	-0.03	-0.32	0.26	0.58	0.42	0.01	0.41	0.43
					LB - VS	0.03	-0.25	0.31	0.58	0.58	0.01	0.57	0.59
	LB	0.28	0.08	0.47	PL - VS	**0.19**	-0.06	0.44	0.93	**0.93**	0.00	0.92	0.93
					LB - VB	0.06	-0.22	0.34	0.65	0.66	0.01	0.65	0.67
	PL	0.44	0.27	0.58	PL - VB	**0.22**	-0.04	0.47	0.94	**0.95**	0.00	0.94	0.95
					PL - LB	0.16	-0.09	0.40	0.89	**0.89**	0.00	0.89	0.90
Vamong	AN	0.03	0.00	0.19	VS - AN	**0.60**	0.03	1.25	0.98	**0.98**	0.00	0.98	0.99
					VB - AN	**0.66**	0.00	1.51	0.98	**0.98**	0.00	0.97	0.98
	VS	0.65	0.08	1.27	LB - AN	**0.72**	0.15	1.40	0.99	**1.00**	0.00	0.99	1.00
					PL - AN	**0.52**	0.23	0.83	1.00	**1.00**	0.00	1.00	1.00
	VB	0.71	0.04	1.55	VB - VS	0.06	-0.86	1.05	0.55	0.55	0.01	0.54	0.56
					LB - VS	0.12	-0.74	0.99	0.61	0.61	0.01	0.59	0.62
	LB	0.76	0.21	1.44	PL - VS	-0.09	-0.76	0.53	0.61	0.40	0.01	0.39	0.41
					LB - VB	0.05	-0.93	1.03	0.54	0.55	0.01	0.53	0.56
	PL	0.56	0.32	0.87	PL - VB	-0.14	-1.01	0.58	0.64	0.36	0.01	0.34	0.37
					PL - LB	-0.20	-0.92	0.43	0.73	0.27	0.01	0.26	0.29
Vwithin	AN	0.91	0.73	1.14	VS - AN	**1.00**	0.48	1.77	1.00	**1.00**	0.00	1.00	1.00
					VB - AN	**1.57**	0.88	2.54	1.00	**1.00**	0.00	1.00	1.00
	VS	1.91	1.44	2.67	LB - AN	**1.02**	0.49	1.76	1.00	**1.00**	0.00	1.00	1.00
					PL - AN	**-0.21**	-0.48	0.08	0.92	**0.08**	0.00	0.07	0.09
	VB	2.49	1.83	3.43	VB - VS	0.57	-0.40	1.62	0.88	**0.87**	0.00	0.87	0.88
					LB - VS	0.01	-0.87	0.88	0.51	0.52	0.01	0.51	0.53
	LB	1.94	1.45	2.64	PL - VS	**-1.20**	-1.97	-0.69	1.00	**0.00**	0.00	0.00	0.00
					LB - VB	-0.54	-1.60	0.41	0.86	**0.13**	0.00	0.12	0.14
	PL	0.71	0.54	0.94	PL - VB	**-1.77**	-2.71	-1.06	1.00	**0.00**	0.00	0.00	0.00
					PL - LB	**-1.23**	-1.94	-0.69	1.00	**0.00**	0.00	0.00	0.00

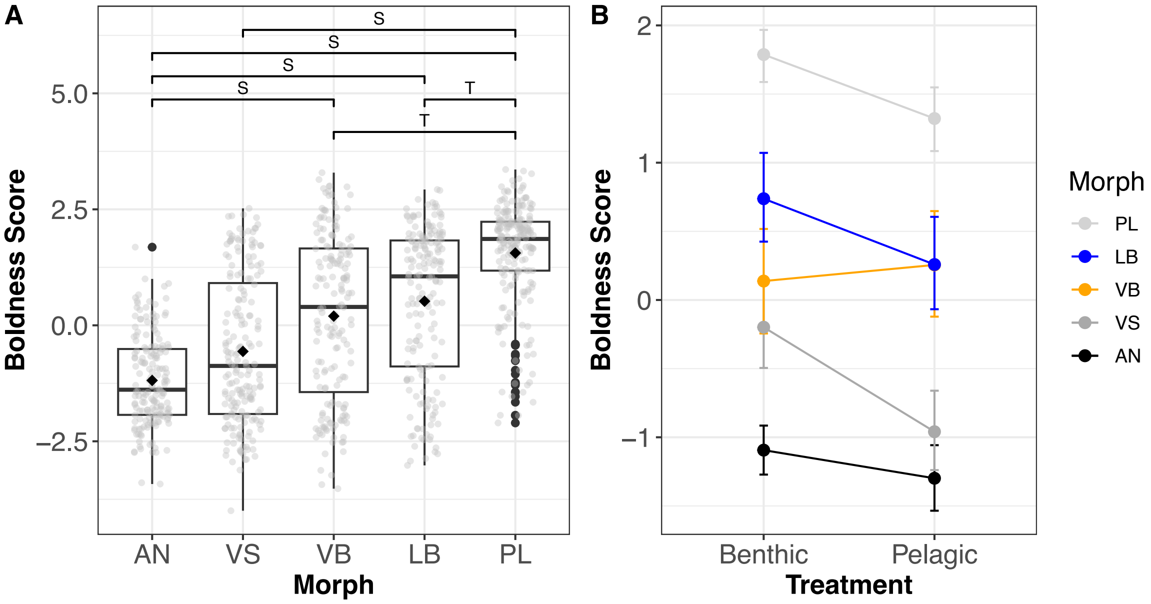


Figure 1 – Boldness scores across the five morphs of Arctic charr, from the least to the most diverged: AN from Fljótaá River, sympatric VS and VB from Vatnshlíðarvatn, and sympatric LB and PL from Þingvallavatn. ***(A)*** *Boxplots of boldness scores for each morph: the rhombus represents the mean, grey dots are scattered individual values, the central line indicates the median, ends of the boxes denote upper and lower quartiles, whiskers cover 95% of values. Significance levels from Tukey post hoc pairwise comparisons of boldness scores between morphs, carried out on model.0: S – significant; T – tendency (see also Table 1).* ***(B)*** *Reaction norms of boldness scores across treatments (Benthic and Pelagic) for each morph: the dot represents the mean, vertical bars indicate one standard error, the slope of the line across treatments indicates the direction and amplitude of boldness plasticity. The benthic fish were significantly bolder than the pelagic fish within the VS, and tended to be within the PL (see also Table 1).*

Paper I – Supplements

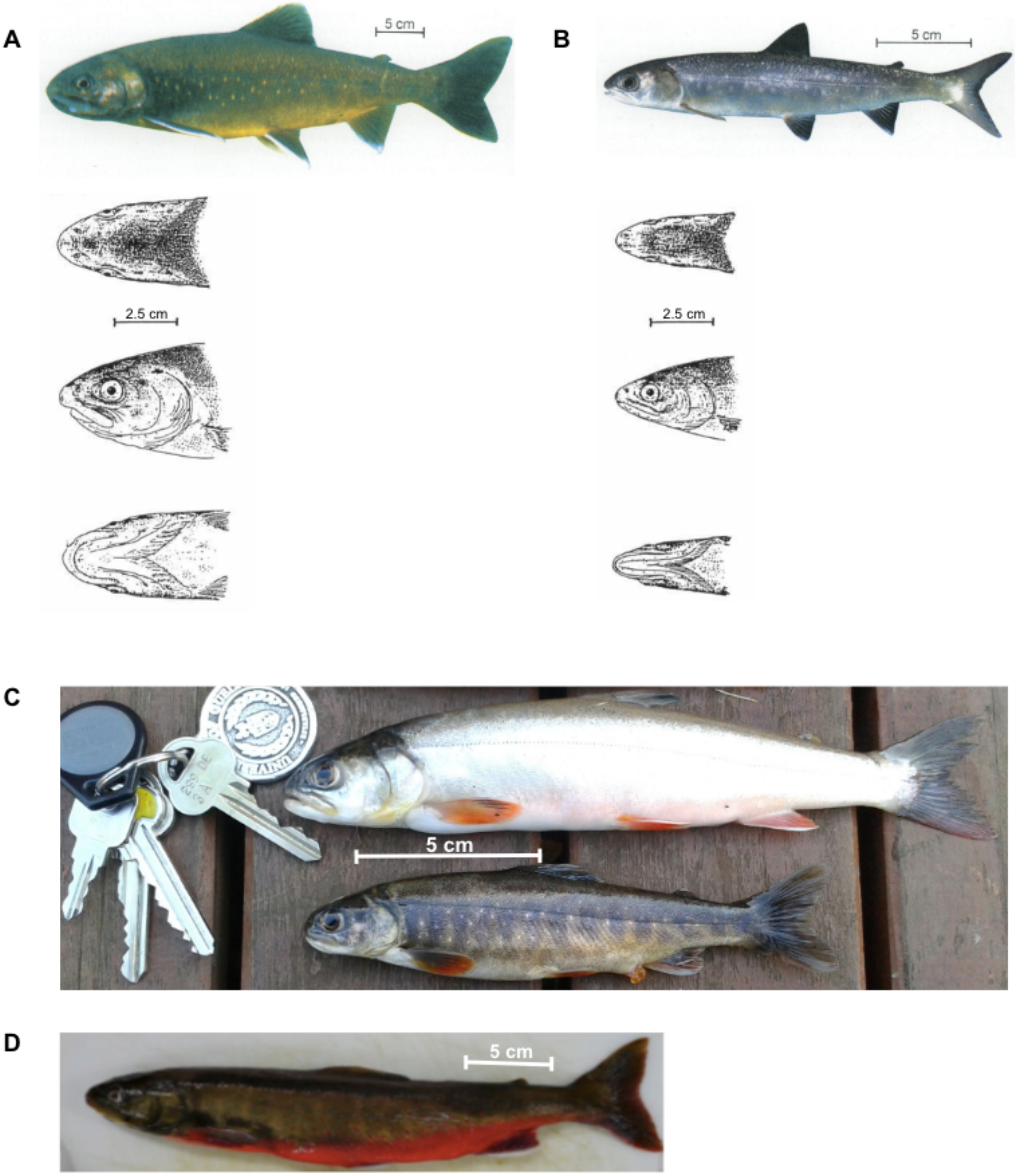
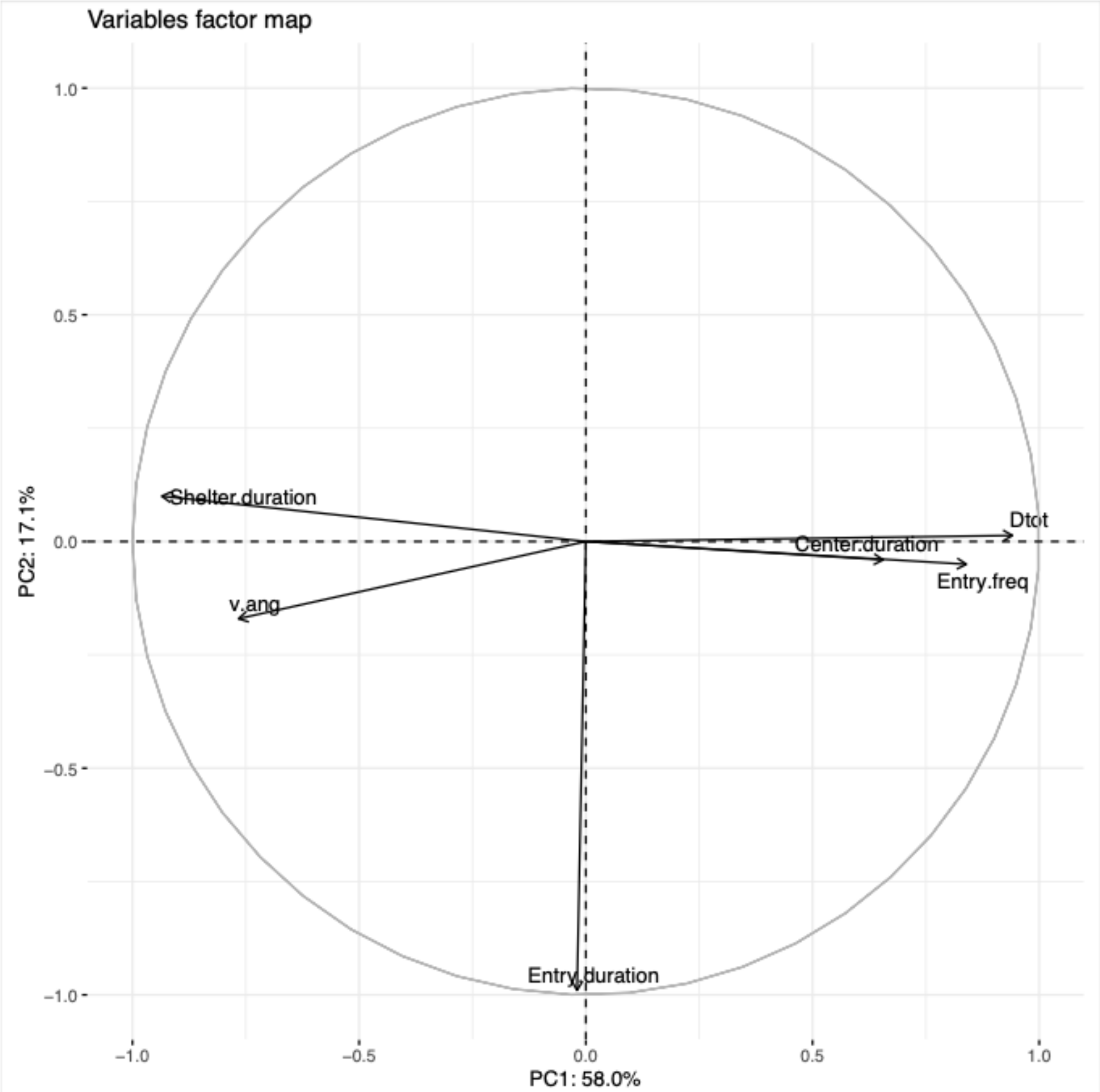


Figure S1 – The five Arctic charr morphs studied (species: Salvelinus alpinus). Note the important differences in coloration, body depth, head and mouth morphology and in size (see scales in cm). ***(A)*** *Large Benthic (LB) and* ***(B)*** *Planktivorous (PL) sympatric morphs from Þingvallavatn: top rows – photograph of each morph in left lateral view (Modified* *from: Sandlund OT, Gunnarsson K, Jónasson PM, Jonsson B, Magnússon KP, Malmquist HJ, et al. The arctic charr Salvelinus alpinus in Thingvallavatn. Oikos. 1992;64(1/2):305–51. https://doi.org/10.2307/3545056); Second, third and fourth rows – drawings of the head and feeding apparatus for each morph in dorsal, left lateral and ventral views respectively, illustrating the typical differences in feeding apparatus morphology respectively between benthic (A) vs. pelagic (B) morphs (Modified from: Snorrason SS, Skúlason S, Jonsson B, Malmquist HJ, Jónasson PM, Sandlund OT, et al. Trophic specialization in Arctic charr Salvelinus alpinus (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. Biological Journal of the Linnean Society. 1994;52(1):1–18. https://doi.org/10.1111/j.1095-8312.1994.tb00975.x).* ***(C)*** *Photograph of the Silver (VS, top row) and the Brown (VB, bottom row) sympatric morphs from Vatnshlíðarvatn in left lateral view (Photograph courtesy: Skúli Skúlason).* ***(D)*** *Photograph of the anadromous morph (AN) from Fjlótaá River in left lateral view (Photograph courtesy: Samantha V. Beck).*

A



B

Dimension	Contributions						Correlations						Eigenvalues	Variance proportion	Cumulative variance
	Dtot	Shelter. duration	Entry. freq	v.ang	Center. duration	Entry. duration	Dtot	Shelter. duration	Entry. freq	v.ang	Center. duration	Entry. duration			
PC1	25.423	25.125	20.230	16.857	12.353	0.011	0.941	-0.935	0.839	-0.766	0.656	-0.019	3.482	0.580	0.580
PC2	0.017	0.977	0.246	2.834	0.160	95.765	0.013	0.100	-0.050	-0.170	-0.040	-0.990	1.023	0.171	0.751
PC3	1.729	7.526	18.358	13.005	58.362	1.021	-0.125	-0.260	-0.406	0.342	0.723	-0.096	0.897	0.150	0.901
PC4	12.456	1.388	13.119	67.056	3.459	2.522	0.223	-0.074	0.229	0.517	-0.117	-0.100	0.398	0.066	0.967
PC5	21.922	15.010	45.771	0.219	17.007	0.069	0.190	-0.157	-0.274	-0.019	-0.167	0.011	0.165	0.027	0.994
PC6	38.453	49.973	2.275	0.028	8.659	0.612	-0.115	-0.132	0.028	0.003	-0.055	-0.015	0.035	0.006	1.000

Figure S2 – Principal Component Analysis (PCA) on the first OFT replication, used to obtain individual boldness scores (see Chapter I of the R script accompanying the main text: Dellinger M, Steele SE, Sprockel E, Philip J, Pálsson A, Benhaïm D. Variation in personality shaped by evolutionary history, genotype, and developmental plasticity in response to feeding modalities in the Arctic charr. Dryad Repository. 2023. https://doi.org/10.5061/dryad.4f4qrfjk2). Shelter.duration, Entry.duration and Center.duration (s): cumulative time spent respectively in the shelter, entry and centre zones; Dtot (cm): total distance travelled; Entry.freq: frequency of visits in the entry zone; v.ang (deg.s^{-1}): absolute angular velocity. ***(A)*** *PCA Factor map on PC1 and PC2* ***(B)*** *Contributions and correlations per variable, eigenvalue, variance proportion and cumulative variance, for each PC dimension.* ***Comment:*** *non-responding fish (N = 73 out of 540 fish tested, see Table 2 in the main text and Table S1 for details) were not included in* *the PCA analyses because attributing them all with the same arbitrary boldness score, hence considering them all equally shy, is nonsensical in the context of testing for among-individual differences in behaviour. Indeed, if the test could last an infinite amount of time, each individual would eventually exit the shelter, behave differently in the arena, and consequently have their own boldness score. A single boldness score attributed to all non-responding fish would just fallaciously skew and bias the overall boldness score distribution, potentially in a tremendous way depending on the score arbitrarily chosen, and create a ceiling effect. Instead, proportions of non-responding fish were compared between groups using* χ^2 *tests (see results in the main text and in Table 2).*

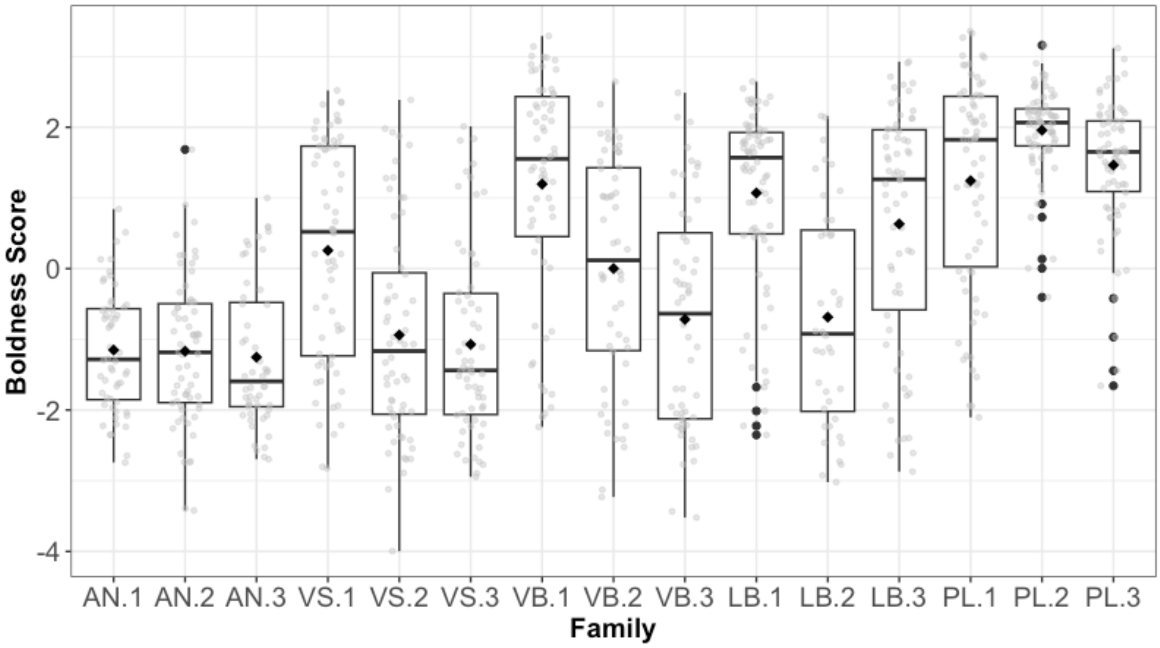
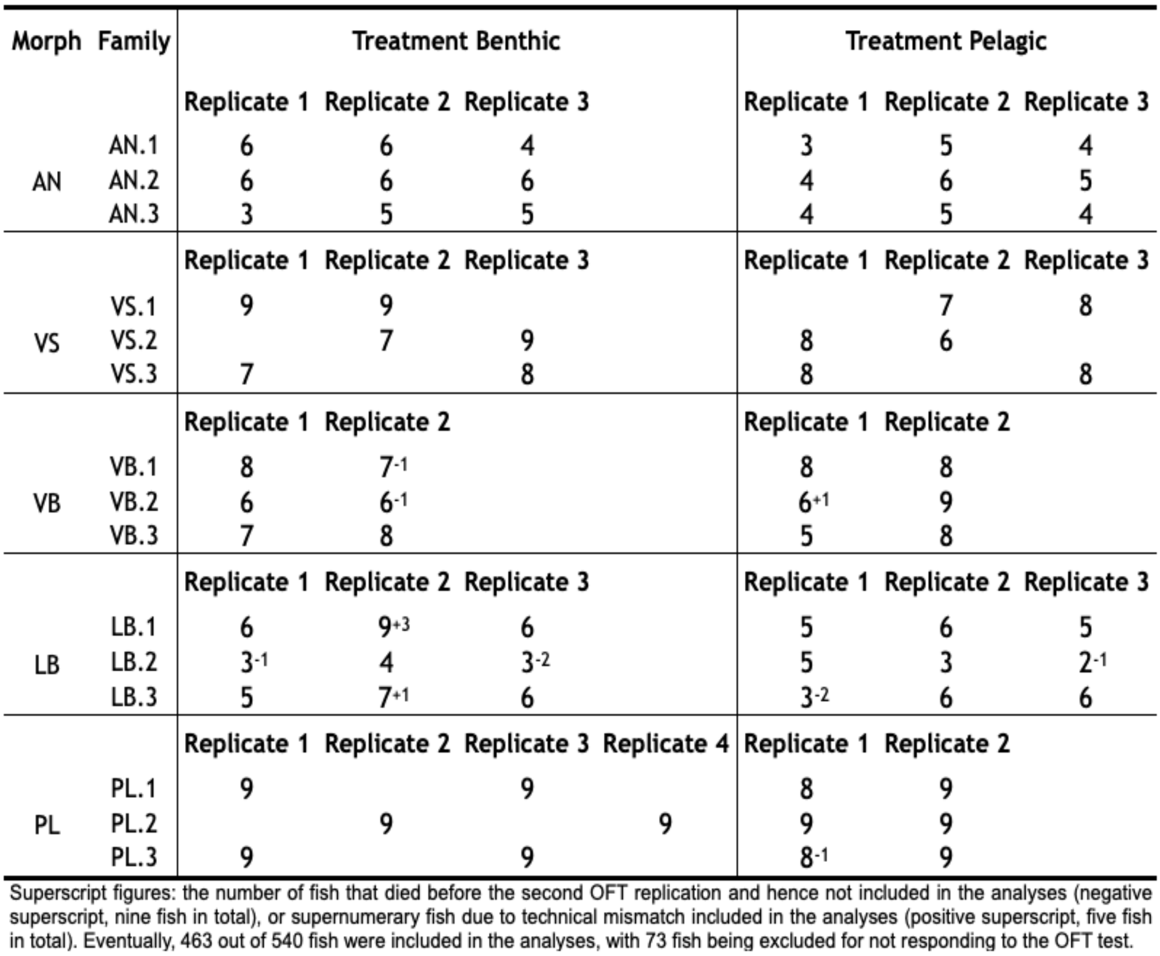


Figure S3 – Boxplots of boldness scores across the three families within each morph, from the least to the most diverged morph: AN from Fjlótaá River, sympatric VS and VB from Vatnshlíðarvatn, and sympatric LB and PL from Þingvallavatn. The rhombus represents the mean, grey dots are scattered individual values, the central line indicates the median, ends of the boxes denote upper and lower quartiles, whiskers cover 95% of values. Note that variation in boldness scores between families within a morph were similar, with just one oddity in PL, where family PL.1 displayed more variability than PL.2 and PL.3.

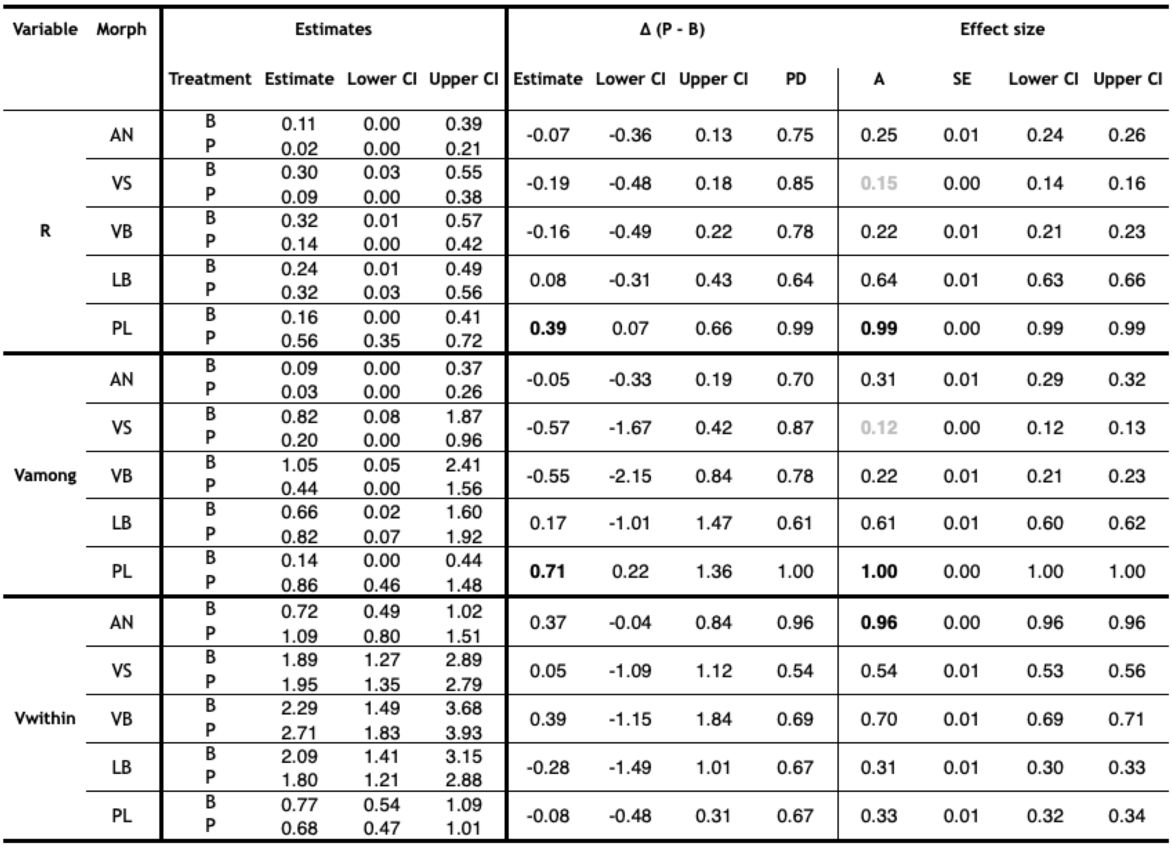
Table S1 – Sample size distribution between treatments, tank replicates and families for each morph, indicating the number of fish included in the analyses. Each replicate tank contained a total density of 136 fish, but only experimental fish are listed here. Fish from AN and LB morphs were distributed over three replicate tanks per treatment and per morph. VB crossings only provided enough individuals to be distributed over two replicate tanks per treatment. Variation in the experimental design/distribution concerning VS and PL morphs was due to the rearing of additional families not included in this study (that is, VS and PL families not represented in all replicate tanks, and discrepancies in the number of replicate tanks in PL). Families were however equally distributed over each replicate tank. For each family, 18 individuals were tested per treatment and per morph (6 or 9 fish per tank depending on the families' distribution and number of tank replicates). Discrepancies between theoretical and actual sample size for each subgroup are either due to individuals excluded from analyses for not responding to the OFT, or other reasons detailed in the footnote.



Morph	Family	Treatment Benthic				Treatment Pelagic		
		Replicate 1	Replicate 2	Replicate 3		Replicate 1	Replicate 2	Replicate 3
AN	AN.1	6	6	4		3	5	4
	AN.2	6	6	6		4	6	5
	AN.3	3	5	5		4	5	4
		Replicate 1	Replicate 2	Replicate 3		Replicate 1	Replicate 2	Replicate 3
VS	VS.1	9	9				7	8
	VS.2		7	9		8	6	
	VS.3	7		8		8		8
		Replicate 1	Replicate 2			Replicate 1	Replicate 2	
VB	VB.1	8	7^{-1}			8	8	
	VB.2	6	6^{-1}			6^{+1}	9	
	VB.3	7	8			5	8	
		Replicate 1	Replicate 2	Replicate 3		Replicate 1	Replicate 2	Replicate 3
LB	LB.1	6	9^{+3}	6		5	6	5
	LB.2	3^{-1}	4	3^{-2}		5	3	2^{-1}
	LB.3	5	7^{+1}	6		3^{-2}	6	6
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	
PL	PL.1	9		9		8	9	
	PL.2		9		9	9	9	
	PL.3	9		9		8^{-1}	9	

Superscript figures: the number of fish that died before the second OFT replication and hence not included in the analyses (negative superscript, nine fish in total), or supernumerary fish due to technical mismatch included in the analyses (positive superscript, five fish in total). Eventually, 463 out of 540 fish were included in the analyses, with 73 fish being excluded for not responding to the OFT test.

Table S2 – Estimates per treatment (B: Benthic and P: Pelagic) within morphs (AN from Fjlótaá River, sympatric VS and VB from Vatnshlíðarvatn, and sympatric LB and PL from Þingvallavatn), pairwise differences in the posterior distributions between treatments within morphs (Δ) and their effect sizes, of among-individual variance, within-individual variance and repeatability of boldness (Vamong, Vwithin and R respectively). Results were obtained from morph-specific multi-level structure Bayesian brms linear mixed models for boldness score, including the treatment as fixed predictor, random effects for both the intercept and the treatment-related effect nested within the individual IDs, and a sigma term accounting for potential variations in the residual standard deviation among treatments. See "Supplemental Chapter" in the R script accompanying the main text (Dellinger M, Steele SE, Sprockel E, Philip J, Pálsson A, Benhaïm D. Variation in personality shaped by evolutionary history, genotype, and developmental plasticity in response to feeding modalities in the Arctic charr. Dryad Repository. 2023. https://doi.org/10.5061/dryad.4f4qrfjk2). Significant Δ estimates and large effect sizes are highlighted in bold black font, while tendencies for Δ and small effect sizes are highlighted in bold grey font. ***Estimates & Δ****: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ, indicating the proportion of samples that are of the estimate's sign, i.e., roughly the probability that the estimate is different from zero.* ***Effect size****: probability of superiority (A) with its standard error (SE) and 95% confidence interval (CI), between the posterior distributions of each variable for each pair of morphs. The A statistic is a probability-based measure of effect size, indicating the probability that a randomly chosen member of group 1 scores higher on the response variable than a randomly chosen member of group 2, i.e., in our case if A < 0.5, benthic fish outscore pelagic fish and vice versa.* ***Commentary****: We did not have sufficient power for these tests to show significant results except for very large differences, and a higher sample size and/or more numerous OFT replications would most likely turn these tendencies to significance, as suggested in Dingemanse NJ, Dochtermann NA. Quantifying individual variation in behaviour: Mixed-effect modelling approaches. Journal of Animal Ecology. 2013;82(1):39–54. https://doi.org/10.1111/1365-2656.12013 and in Royauté R, Dochtermann NA. Comparing ecological and evolutionary variability within datasets. Behavioral Ecology and Sociobiology. 2021;75:127. https://doi.org/10.1007/s00265-021-03068-3. But the results still show an interesting pattern: the morphs seemed to respond differently to the treatments in terms of boldness consistency (lower repeatability in the pelagic treatment for the sympatric morphs from Vatnshlíðarvatn, the contrary for the PL from Þingvallavatn, and no difference for its sympatric counterpart LB and the AN) suggesting that the environment in interaction with the genotype could be an important driver for the emergence and development of consistent behaviours among-individuals, i.e., personality. It is worth noting that boldness repeatability seems higher when the treatment mimics the proper ecology of the morph: higher in the benthic treatment for morphs feeding close to the bottom in shallow environments like the VS and VB; equal between treatments for the benthic-feeder LB that however lives in a deep lake in which pelagic lifestyle is accessible, and the AN morph switching between shallow rivers and deeper seas; higher in the pelagic treatment for the PL feeding in deep open waters. This would suggest that the expression of behavioural consistency could be favoured when the environment meets the conditions for which the genotype has been adapted to, whereas the lack of behavioural consistency would highlight a situation of maladaptiveness. Notably, the potential behavioural maladaptiveness to benthic conditions expressed during juvenile stage in the PL could be hypothesized to be the origin of the ontogenetic shift from benthic spawning grounds to the pelagos in this morph* *(personality profiles engendering differential niche picking). However, this statement should be taken with caution, as our fully lab-based design focuses on only one aspect of foraging, still far from mimicking all the potential selective and developmental cues available in nature, and as expression of boldness in the lab might not necessarily fully reflect natural behaviours. Further research is hence needed to confirm or infirm the hypothesis that personality emerges as a result of an interaction between genes and environment in the Arctic charr, potentially in an adaptive way.*



Variable	Morph	Estimates				Δ (P - B)				Effect size			
		Treatment	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI	PD	A	SE	Lower CI	Upper CI
R	AN	B P	0.11 0.02	0.00 0.00	0.39 0.21	-0.07	-0.36	0.13	0.75	0.25	0.01	0.24	0.26
	VS	B P	0.30 0.09	0.03 0.00	0.55 0.38	-0.19	-0.48	0.18	0.85	0.15	0.00	0.14	0.16
	VB	B P	0.32 0.14	0.01 0.00	0.57 0.42	-0.16	-0.49	0.22	0.78	0.22	0.01	0.21	0.23
	LB	B P	0.24 0.32	0.01 0.03	0.49 0.56	0.08	-0.31	0.43	0.64	0.64	0.01	0.63	0.66
	PL	B P	0.16 0.56	0.00 0.35	0.41 0.72	**0.39**	0.07	0.66	0.99	**0.99**	0.00	0.99	0.99
Vamong	AN	B P	0.09 0.03	0.00 0.00	0.37 0.26	-0.05	-0.33	0.19	0.70	0.31	0.01	0.29	0.32
	VS	B P	0.82 0.20	0.08 0.00	1.87 0.96	-0.57	-1.67	0.42	0.87	0.12	0.00	0.12	0.13
	VB	B P	1.05 0.44	0.05 0.00	2.41 1.56	-0.55	-2.15	0.84	0.78	0.22	0.01	0.21	0.23
	LB	B P	0.66 0.82	0.02 0.07	1.60 1.92	0.17	-1.01	1.47	0.61	0.61	0.01	0.60	0.62
	PL	B P	0.14 0.86	0.00 0.46	0.44 1.48	**0.71**	0.22	1.36	1.00	**1.00**	0.00	1.00	1.00
Vwithin	AN	B P	0.72 1.09	0.49 0.80	1.02 1.51	0.37	-0.04	0.84	0.96	**0.96**	0.00	0.96	0.96
	VS	B P	1.89 1.95	1.27 1.35	2.89 2.79	0.05	-1.09	1.12	0.54	0.54	0.01	0.53	0.56
	VB	B P	2.29 2.71	1.49 1.83	3.68 3.93	0.39	-1.15	1.84	0.69	0.70	0.01	0.69	0.71
	LB	B P	2.09 1.80	1.41 1.21	3.15 2.88	-0.28	-1.49	1.01	0.67	0.31	0.01	0.30	0.33
	PL	B P	0.77 0.68	0.54 0.47	1.09 1.01	-0.08	-0.48	0.31	0.67	0.33	0.01	0.32	0.34

Paper II

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Do animal personality components independently evolve and develop in response to environmental complexity?

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Keywords

Animal personality; Arctic charr; Environmental complexity; Development; Enrichment; Evolution; Personality components; Plasticity; Polymorphism; Variability

Abstract

Widespread existence of consistent differences in behaviour among individuals even within species and populations, *i.e.*, animal personality, has been established since the last decades in a wide array of taxa. However, little is known about personality traits' ontogeny and evolution. This study aimed at exploring eco-evolutionary mechanisms driving the emergence and development of animal personality. Focusing on boldness as a personality trait, we assessed how personality components (mean, among- and within-individual variabilities, repeatability, and plasticity in response to environmental complexity) develop at early age. Investigating development of all personality components, rather than averages only, offers a more exhaustive and comprehensive picture of how personality emerges, develops, and evolves. We compared personality components between juveniles of five morphs of Arctic charr (*Salvelinus alpinus*) ranging along gradients of ecological and genetic divergence from a common ancestor, raised from hatching in plain vs. structurally complex treatments. On the one hand, we show that some of these personality components evolve and develop independently from the others: mean boldness, which increases with divergence from the ancestor, was predominantly genotype-dependent and suspectedly a highly heritable trait with strong and stable selective pressures acting on it in the wild, while boldness repeatability might rather depend on the ecology of each morph. These two components were not affected by environmental complexity. On the other hand, variability-related components of personality, including their plasticity in response to environmental complexity, were rather dependent on genotype-by-environment effects and seemingly evolve and develop jointly. Boldness tended to be more consistent within the treatment mimicking the structural complexity of a given morph's natural habitat, hinting that personality emergence might be favoured for individuals experiencing conditions to which they have been adapted. These findings suggest mechanisms by which personality components could be implicated in adaptability to environmental changes or even sympatric diversification and biodiversity.

Introduction

The growing interest in animal personality – consistent among-individual behavioural differences across time and/or contexts (Réale et al., 2007; Sih et al., 2004) – focuses on its eco-evolutionary causes and consequences. Variations in personality may drive differential niche-picking or niche construction (Stamps & Groothuis, 2010; Wolf & Weissing, 2010), which could promote intraspecific sympatric divergence between personalities, leading to polymorphism (Dall et al., 2012), or even ultimately to speciation (Ingley, 2018). However, the emergence and development of animal personality traits among individuals within and between populations remain poorly understood, although elucidating the ontogeny of personality is crucial to understanding its proximate and ultimate implications (Stamps & Groothuis, 2010).

Therefore, increasing scientific efforts aim to understand how early experiences and environmental stimuli shape animal personality over ontogeny (personality developmental plasticity, reviewed in Stamps, 2016). Developmental plasticity of personality is defined as the variation in personality across individuals from a same genotype, at a same age and time, as a function of the conditions in which these individuals developed (Stamps & Groothuis, 2010). Environmental complexity is potentially a key factor shaping personality development. Indeed, habitat complexity in the wild, or enrichment of the rearing conditions have been shown to influence various behaviours and cognitive abilities in animals (de Azevedo et al., 2023; Mikheev et al., 2010; Shumway, 2008; White & Brown, 2014; Zentall, 2021). Notable studies showed contrasting effects of environmental complexity and enrichment on personality traits, *e.g.*, environmental complexity increasing (Liedtke et al., 2015) or decreasing (Xu et al., 2021) boldness and exploration, or having no effect on boldness but increasing territoriality (Church & Grant, 2018). Further empirical research is needed to clarify these inconsistencies. Furthermore, these studies examined the impact of environmental complexity solely on average personality traits, neglecting valuable insights into the plasticity of other personality components.

Personality traits in groups of individuals can be broken down into several components: mean, among- and within-individual variabilities, repeatability, and plasticity. The mean component is the average behaviour over the group and across behavioural measurements. The among-individual variance component (*Vamong*) represents the group-level variance in behaviour, *i.e.*, how much individuals behave differently from each other, within and across behavioural measurements. The within-individual variance component (*Vwithin*) represents the variance in behaviour at the individual level, *i.e.*, how much an individual behaves differently from itself across repeated behavioural measurements. To compare relevant magnitudes of personality variation between groups, as variances may scale with the mean, it is advised to mean-standardize variances (Dochtermann & Royauté, 2019), using Houle's I (Houle, 1992) as a scaled measure of among- and within-individual variability (henceforth *Iamong* and *Iwithin* respectively). The repeatability component (R) represents how pronounced personality traits are at the group level, measured as the proportion of the total variance that is due to among-individual variance (Dochtermann et al., 2015). In other words:

$$R = Vamong / Total\ variance = Vamong / (Vamong + Vwithin).$$

The plasticity component of personality could itself be broken down into several subtypes of plasticity (Stamps, 2016), but we focus here on developmental plasticity as already defined. Different groups of individuals can display different combinations of values for each component of a given personality trait, forming what we coin personality profiles.

Investigating the development of all personality components, in other words the ontogeny of personality profiles, offers a more exhaustive and comprehensive picture of how personality emerges, develops, and evolves, than when focussing on the mean component only. *Iamong* may be influenced within a group by environmental complexity (Akhund-Zade et al., 2019; Lee & Berejikian, 2008), as subtle variation in microenvironmental experiences between individuals within a same complex environment (*e.g.*, minute differences in food intake, social interactions, visual stimuli, etc) could lead to individual variations in state (*e.g.*, energy reserves, metabolic rate, hormonal content, neural circuitry, etc) (Willmore et al., 2007) possibly encouraging the expression of an array of different consistent behaviours among individuals (Freund et al., 2013, 2015; Körholz et al., 2018; Zocher et al., 2020). This behavioural divergence can ultimately be transcribed into increased variability around the mean of a personality trait among individuals at a later age ('fanning-out' reaction norms over time, (Sih et al., 2015; Stamps & Biro, 2016)). In contrast, homogeneous stimuli in a plain environment might favour progressive convergence of personalities among individuals in the group ('fanning-in' reaction norms over time, (Sih et al., 2015; Stamps & Biro, 2016)). Moreover, *Iamong* differences in response to environmental complexity have been shown to depend on the genotype and on genotype-by-environment interactions, implying a genotype-dependent plasticity in behavioural variability (Akhund-Zade et al., 2019) with potentially important eco-evolutionary implications (Pfennig et al., 2010). Finally, in the case of *Iwithin* and repeatability and how they may be influenced by environmental complexity, we suggested in a previous study that the expression of more consistent behaviours could be favoured when individuals experience environmental conditions they are adapted to (Dellinger et al., 2023). This would translate into more stable behaviours within individuals (lower *Iwithin*) and/or more pronounced personality across individuals (higher repeatability). Notably, repeatability component as defined earlier is conceptually dependent on variability components as it is calculated from among- and within-individual variances. However, as repeatability is the resultant of both variances' ratio, the direction taken by the repeatability's development and evolution can still be different than the direction taken by variance components (*e.g.*, repeatability could still be increasing even though both variance components would decrease, or remain stable even though both variance components change). In this sense, we will henceforth consider that development and evolution of the repeatability component have the potential to be considered independent from other personality components.

The present study investigates the development and evolution of personality components in response to environmental complexity. The Arctic charr (*Salvelinus alpinus*), a polymorphic Salmonid found in sympatry in Icelandic freshwaters, constitutes an ideal study system for its morphs' various degrees of genetic, ecological, morphological, and behavioural divergence in the wild (Brachmann et al., 2021; Gíslason et al., 1999; Guðbrandsson et al., 2019; Jónsson & Skúlason, 2000; Sandlund et al., 1987; Skúlason et al., 1993; Snorrason et al., 1994). Habitat complexity is of major interest in this species system, as sympatric morphs tend to specialize in benthic *versus* pelagic habitats, naturally varying in structural complexity within lakes. The emergence of coexisting sympatric morphs within lakes could results from such differential ecological specialization (Cooper et al., 2010; Parsons et al., 2020; Skúlason et al., 2019). The Arctic charr's high evolvability is thought to stem from a significant plastic potential, with plastic changes presumably triggered by behavioural differences (Snorrason & Skúlason, 2012; Wilson & McLaughlin, 2010). Additionally, all Arctic charr populations in Iceland are suggested to descend from the same ancestral anadromous population (Brunner et al., 2001; Wilson et al., 2004), which became landlocked in newly formed lakes at different geological times after the last glaciation. This putatively created a gradient of evolutionary divergence from the common ancestor among morphs based on time since separation.

In this study (see also Benhaïm et al., 2023 and Dellinger et al., 2023), we compared morphs that have diverged in varying degrees from the ancestral type. The monomorphic anadromous population from Fljótaá River (AN) retains high genetic diversity and is assumed to be representative of the ancestral form (Brachmann et al., 2021, 2022; Dellinger et al., 2023). We assume that the sympatric Silver (VS) and Brown (VB) morphs from Lake Vatnshlíðarvatn, which was landlocked 6000 to 8000 years ago (Jónsson & Skúlason, 2000), are moderately diverged from the ancestor. Finally, the sympatric Large Benthic (LB) and Planktivorous (PL) morphs from Lake Þingvallavatn, which was landlocked approximately 10 000 years ago (Saemundsson, 1992), are assumed to be the most highly diverged from the ancestor. Additionally, VS and VB morphs are genetically closely related while the LB and PL morphs are genetically differentiated (Brachmann et al., 2021; Gíslason et al., 1999; Kapralova et al., 2011; Steele et al., in prep.). As morphs form specific genetic clusters both within and among lakes and rivers, we henceforth consider morphs as separate genotypes (not pertaining to the inter-individual genetic variability within a morph). Comparisons between these morphs provide the opportunity for inferring how personality components evolved, and whether they evolve jointly or independently from each other.

The different niches exploited by sympatric Arctic charr morphs within the lakes studied here can vary spatiotemporally in their availability of structurally complex features. LB and PL sympatric morphs from the large and deep Lake Þingvallavatn are an extreme example of polymorphic divergence, in which morphological differences are obvious and diet differences remain distinct throughout the year. Both morphs reproduce at the bottom of rocky shores of the lake, though spatiotemporally distinctively from one another. LB are benthic and reside within structurally complex lava rock beds, crevasses, and algae at the bottom throughout their whole life cycle, while pelagic PL undergo a habitat shift and mature in open waters offering scarce to no structurally complex features (Jonsson et al., 1988; Malmquist et al., 1992; Sandlund et al., 1987, 1992; Skúlason, Snorrason, et al., 1989; Snorrason et al., 1994). In Lake Vatnshlíðarvatn, while VB is typically described as a benthic morph and VS as a pelagic morph, those sympatric morphs display more subtle morphological and ecological divergence, not as pronounced as between LB and PL (Brachmann et al., 2021, 2022). Vatnshlíðarvatn being a very small and shallow lake, both VB and VS sympatric morphs evolve in a similar habitat. But VS migrate to the winding gravel-bed tributary streams to spawn, hence probably experiencing relatively higher structural complexity than the VB spending its whole life cycle within the simple muddy-bottom lake (Jónsson & Skúlason, 2000).

We specifically focused on one of the most studied aspects of animal personality, boldness – the individual propensity to take risks (Wilson et al., 1994) – that has recently been described as a personality trait in the Arctic charr (Benhaïm et al., 2023; Dellinger et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022). Juveniles of AN, VS, VB, PL, and LB morphs were lab-reared in common gardens manipulating environmental complexity, with a plain treatment (bare tanks, offering little stimuli similar to low structural complexity environments in the wild) vs. a structurally enriched treatment (featuring volcanic rocks and plants, found in environments of higher structural complexity in the wild). To characterize personality profiles (*i.e.*, group-specific combinations of personality components: mean, among- and within-individual variabilities, repeatability, and plasticity in response to environmental complexity), we tested for differences in all boldness components across morphs and treatments.

The first question tested with this design (H1, Table 1) was whether environmental complexity experienced at young age influences the development of personality components (developmental plasticity of personality), which would translate into boldness components varying between treatments, either overall (environment effect) or within morphs (genotype-by-environment effect). We had specific predictions for each personality component. (i) The mean component: if environmental complexity has a uniform effect at the group level on personality development, we expected differences in mean boldness between treatments. (ii) The among-individual variability component: if an enriched/complex environment provides a variety of microenvironments experienced differently and eliciting different personality responses for each individual, we would expect a higher boldness variability among individuals raised in the enriched treatment when compared to the plain one. (iii) The within-individual variability and repeatability components: if behaviours are more consistently expressed when an individual's current environmental conditions match those to which it has been adapted, this would translate into boldness being more repeatable, and/or less variable within individuals in the enriched treatment than in the plain one in every morph (de Azevedo et al., 2023). We might also expect a morph-by-treatment interaction effect, resulting in boldness being more repeatable, and/or less variable within individuals, in the enriched treatment for morphs whose natural habitats are structurally more complex: at a lower extent for the VS migrating through streams of relative complexity but residing in an otherwise structurally simple lake; at an intermediary extent for the AN migrating along a quite complex and extended river bed; and at the greatest extent for the LB living in highly-complex lava rock beds and crevasses – as opposed to morphs living in lower-complexity environments that would display more consistent boldness in the plain treatment: at a lower extent for the VB native and resident of a relatively low-complexity lake, and at greater extent for the pelagic morph PL living in overly homogeneous open waters.

The second question of this study (H2, Table 1) was how the evolutionary history of individuals could impact the development of personality profiles and their response to environmental complexity. The "plasticity-first hypothesis" (Levis & Pfennig, 2016) proposes that the key for rapid evolution and adaptive divergence in a novel environment is ancestral plasticity, which should then be reduced with acclimation to the new habitat. This is accompanied by canalization (production of standard phenotypes despite genetic and environmental perturbations, characterized by a reduction of variance around the mean (Loison, 2019)) of the phenotype most fitted to the new fitness optimum in the subsequently diverged populations. In this view, personality components values should evolve as a package and be correlated to the divergence degree: plasticity and variabilities should decrease, and repeatability should increase, when divergence from the ancestral population increases (gradients of personality components along the gradient of divergence). The results of Dellinger et al., 2023 suggested that, instead of evolving jointly as a block, personality components (mean, *Vamong*, *Vwithin*, repeatability and plasticity) rather evolve independently from each other. If this is the case, we expected morph-specific personality profiles, of which personality components do not show gradient-like values correlated to the divergence degree across morphs.

Material and Methods

Subjects and Housing Conditions

We used offspring of five wild-caught Arctic charr morphs as described in Dellinger et al. (2023) and Benhaïm et al. (2023): AN from Fljótaá River (ancestral proxy); VS and VB sympatric morphs from Lake Vatnshlíðarvatn (moderately diverged); LB and PL sympatric morphs from Lake Þingvallavatn (highly diverged). For each morph, eggs and sperm from wild adult fish (6 to 12 females and 6 males) were collected and pooled together in order to enhance genetic variability. The number of females sampled was adjusted according to the targeted morph to ensure a consistent number of eggs collected across all morphs, as size variation between morphs implied variation in clutch size among gravid females. Adults from LB morph were sampled on the 31/07/2019 (6 females, 64.13930°, -21.03110°); AN on the 14/10/2019 (6 females, 65.99964°, -19.00467°); VB on the 08/09/2020 (12 females, 65.51688°, -19.61298°); VS on the 18/09/2020 (11 females, 65.51091°, -19.66671°); PL on the 16/10/2020 (11 females, 64.19098°, -21.09342°). Eggs from each morph were kept separate, raised in common garden and checked daily, incubated in complete darkness at 4°C and dissolved O_2 saturation always above 100%. At the end of the incubation period of the PL morph's eggs, when a fraction already started hatching, a technical issue raised the water temperature for a few hours which accelerated the hatching of the remaining eggs. However, the total incubation time for this clutch (106 days) remained similar to the usual mean incubation time for this morph in these conditions (107 ± 3 days (Beck, 2019)).

After 100% of the eggs had hatched within a morph, fish were distributed over six 20-L cylindrical tanks in a flow-through system, at a density of approximately 275 individuals per tank (to meet the welfare requirements of this species (Jobling et al., 1993)), between two treatments modulating the structural complexity of the rearing environment (Philip et al., 2022): plain treatment (bare tank) vs. enriched treatment (with one plastic plant and five volcanic rocks at the bottom of each tank, mimicking natural features of the Arctic charr environment and providing different colours and textures, and structural heterogeneity both horizontally and vertically to the tank). Hence, we obtained 10 different batches (5 Morphs x 2 Treatments), with three replicates tanks per batch. Once the majority of the fish reached a mass of 1g and a total length (from the tip of the snout to the tip of the tail) of 5cm (320 to 327 days post hatching), 55 fish per tank chosen randomly were intraperitoneally marked with a PIT tag (Oregon RFID FDX-B skinny tag: 8 x 1.4 mm), providing individual identification. On that day, the density in the experimental tanks was reduced to 85 fish per tank.

For the VS morph, there were initially five tanks (three enriched and two plain) because the sampled females did not provide enough eggs to create six tanks. Then, one enriched tank was lost due to technical issues (at 200 days post hatching), which reduced the VS morph to four tanks (two enriched and two plain). However, at tagging and density-reduction, we used the extra fish to create two pseudo-replicates (one enriched and one plain), recovering the six tanks for the VS as planned. For the VB morph, there were initially six tanks, but a disease outbreak drastically reduced the density in one plain tank, hence excluded from the experiment. Moreover, further mortality made it impossible to create a pseudo-replicate to replace the plain tank missing. The experiment for the VB morph was therefore performed on five tanks only (three enriched and two plain). Fish were fed commercial aquaculture pellets (BioMar INICO) three times a day. To adapt to fish growth and oxygen consumption, the granulometry of the pellets and the water renewal rate were progressively increased over the rearing period (respectively: 0.4, 0.8 and 1.1 mm according to manufacturer's instructions, at a total amount of 1% overall biomass; 400, 800 and 1000 mL/min, *i.e.*, water renewal of 120, 240 and 300% per hour to maintain oxygen saturation between 90-100%). The photoperiod was 12h/12h light/dark at 300 Lux, and water temperature was maintained between 3.3 and 7.3 °C to match natural conditions thus expecting low growth.

Open Field Test

After a recovery period of 15 to 18 days following the tagging procedure (334 to 341 days post hatching), 52 fish per tank (N=1508 in total, see Table S1 for detail) were chosen at random to be subjected twice to an Open Field Test (OFT) with shelter, with a 7-day interval between each replication, to assess among-individual consistency in boldness *i.e.*, personality (Dellinger et al., 2023). The OFT arena (Philip et al., 2022) consisted of an opaque white rectangle tank (39.7 x 29.5 x 25 cm) filled with 6 cm water depth at home tanks' temperature, with a shelter in a corner (14 x 6 x 6 cm) provided with a removable door. The set-up was composed of 4 adjacent arenas, allowing to test 4 fish in a single round, and 13 rounds a day were necessary to test the 52 fish of a given tank for a given morph. The set up was placed on a white LED backlight (Noldus Information Technology) set at 70 Lux to increase contrast. A Basler Ace acA1920-150um camera placed 110 cm above the arenas recorded each round at 30 frames per second. At the beginning of the test, the fish were placed in the closed shelter where they acclimated for 5 minutes. The shelter doors were then opened, and the fish were free to explore the arena for 20 minutes. Once the 20 minutes were completed, the fish were gently netted, anaesthetized in a 200 ppm 2-phenoxyethanol solution, weighed, and fork length (from the tip of its snout to the fork of the tail) and total length were measured. They were then left to recover from anaesthesia for 20 minutes and transferred back in their home tank. Water temperature in each arena was recorded before the start and after the completion of each trial, and water in the arenas was replaced between each trial.

Boldness Score Assessment

The fish were tracked using Ethovision XT version 15 (Noldus Information Technology) based on the recorded videos. Arenas were divided into four virtual zones: the border zone along the edges of the arena (to control for size differences between morphs, the width of this zone was morph-dependent: half of the total length of the largest fish tested of the morph + 1 cm, *i.e.*, between 4.6 and 5.2 cm); the shelter zone covering the shelter area, overlapping with an entry zone with a width equal to half of the border zone width, extended from the shelter door; the centre zone being the remaining part of the arena and corresponding to the riskiest area since thigmotaxis (tendency to remain close to the enclosure walls) is commonly associated with shyness (Dahlbom et al., 2014; Simon et al., 1994). The fish barycentre was used to calculate the latency to first exit the shelter (s), the frequency of visits in each zone, cumulative time spent in each zone (duration, s), the total distance travelled (cm), the mean velocity (body length cm/s) and the absolute angular velocity (deg/s). To calculate boldness scores, we used the same principal component analysis (PCA) method as described in Dellinger et al. (2023), that takes repeated measures into account while conserving the multiple-behaviours complexity of risk-taking. To respect the objective of testing for among-individual differences in behaviour, and to avoid skewed data and ceiling effect, this method excludes individuals not exiting the shelter (with their head and trunk not visible) within 20 minutes in either replication, considered non-responsive to the test (N=270 non-responding fish excluded, Table S2). A total of N=1185 fish were included in the PCA analyses (see details in Table S1). Briefly, all behavioural variables recorded during the first OFT replication were reduced into a primary axis of behavioural variation with a PCA, removing variables showing high correlation with others on the factor map (border frequency and mean velocity). The first axis of this PCA (PC1) explained 56.2% of the variance in the data, to which the variables with strongest contributions were total distance travelled, centre frequency, border and shelter durations (respective PC1 loadings: 0.944, 0.894, 0.881 and -0.869; see Fig. S1 and accompanying R script). Consequently, PC1 represents a gradient from shyest (negative values) to boldest individuals (positive values), as high PC1 coordinates indicate that the fish explored the arena instead of hiding in the shelter. We then used this PCA as a template applied to the whole dataset: each individual received a boldness score for each OFT replication, corresponding to its predicted coordinate on PC1 with the aforementioned PCA parameters (see accompanying R script).

Statistical Analyses

All statistics analyses (Table 1), following those of Dellinger et al. (2023), were performed with R v.4.3.0 software (R Core Team, 2018) and are provided in the R script accompanying this article. Models described were linear mixed models fitted in Stan Bayesian language (Carpenter et al., 2017) using the brms package v.2.19.0 (Bürkner, 2017).

Mean Boldness Scores

A first model (henceforth model.0, Table 1, details in accompanying R script) predicted z-scaled boldness score, with morph, treatment, their interaction, OFT replication and z-scaled total length of the fish (accounting for potential influence of body size on behaviour (Benhaïm et al., 2003)) as fixed predictors. Random predictors included individual ID, tank, round (to control for potential differences in diel activity) and date (to account for possible stress accumulation over days, as one OFT replication lasted for five to six days per morph). We validated model.0's diagnostics by inspecting effective sample sizes, potential scale reduction factors, tails weights, densities, trace plots, trace rank plots, and autocorrelation plots, for each model parameter. We also verified the results robustness by fitting model.0 again with several different seeds.

We calculated conditional (R2c), marginal (R2m), and inclusive (IR^2) R squared values for model.0, respectively describing the amount of variance in boldness scores explained by the whole model, the fixed predictors together, and each single predictor (method adapted from Hertel et al. (2023), details in accompanying R script). We applied the functions emmeans and contrast (package emmeans v.1.7.8 (Lenth, 2023)) under Tukey linear hypothesis to model.0, to perform pairwise post hoc comparisons of boldness scores between morphs and between treatments within morphs.

Boldness among- and within-individual variabilities, and repeatability between morphs

We calculated *Vamong*, *Vwithin* and R for each morph and compared them statistically by following the method proposed by Royauté & Dochtermann (2021), fitting a multi-level structure brms model (model.morph, Table 1), with boldness score as the response variable, the morph as fixed predictor, random effects for both the intercept and the morph-related effect, which were nested within the individual IDs, allowing to capture *Vamong* per morph, and finally a sigma term accounting for potential variations in the residual standard deviation among morphs, capturing *Vwithin* per morph (details in accompanying R script). As variances might scale with the mean, and to assess relevant magnitudes of personality variability between groups (Dochtermann & Royauté, 2019), we standardized *Vamong* and *Vwithin* for each morph by the square of the morph's mean, as described in Houle (1992) (details in accompanying R script). This led to two new variables, closely related to coefficients of variations, that we used as scaled measure of among- and within-individual variability: *Iamong* and *Iwithin* respectively. Diagnostics of model.morph were produced as per model.0.

Adapting Royauté & Dochtermann's guidelines (2021), we examined pairwise differences in the posterior distributions of *Iamong*, *Iwithin* and R between morphs (Δ*Iamong*, Δ*Iwithin* and ΔR respectively). We also calculated the effect sizes for each pairwise comparison to estimate the amplitudes of Δ*Iamong*, Δ*Iwithin* and ΔR between morphs, by calculating the probability of superiority between the posterior distributions of each parameter for each pair of morphs (A statistic (Ruscio, 2008), package RProbSup v.3.0 (Ruscio, 2020)). The A statistic is a probability-based measure of effect size, indicating the probability that a randomly chosen member of group 1 scores higher on the response variable than a randomly chosen member of group 2. In our case if $A < 0.5$, morph 2 outscores morph 1 and *vice versa*.

Boldness among- and within-individual variabilities, and repeatability within morphs between treatments

To compare *Iamong*, *Iwithin* and R between treatments within morphs, we fitted one model per morph (models.treatment, Table 1) similar to model.morph, but this time with the treatment as fixed predictor, random effects for the intercept and the treatment-related effect both nested within the individual IDs, and a sigma term accounting for potential variations in the residual standard deviation between treatments. For each model, diagnostics, calculation of Δ*Iamong*, Δ*Iwithin*, ΔR and their effect sizes, and model comparisons were carried out as described above (all details in accompanying R script).

Note: All estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) if applicable, indicating the proportion of samples that are of the estimate's sign, *i.e.*, roughly the probability that the estimate is different from zero. Bayesian statistics do not use frequentist p-values. The significance of an estimate is obtained when its CI or its highest posterior density interval (HPDI) does not overlap zero. We considered a tendency for an estimate when the CI or HPDI overlapped zero no further than 0.09 units with a $PD \geq 0.92$. Regarding the A statistic, we considered a large effect when $A \in [0.00;0.10] \cap [0.90;1.00]$, a small effect when $A \in [0.11;0.20] \cap [0.80;0.89]$, and a negligible effect otherwise.

Note: we also calculated estimates of raw unstandardized *Vamong*, *Vwithin* and of pairwise differences in the posterior distributions of *Vamong*, *Vwithin* (Δ*Vamong*, Δ*Vwithin*) and their effect sizes between morphs as well as between treatments within morphs. We tested whether model.morph and models.treatment detected the correct pattern of differences in variance components among groups (Royauté & Dochtermann, 2021), by comparing WAIC criteria of models allowing neither *Vamong* nor *Vwithin*, *Vamong* only, *Vwithin* only or both *Vamong* and *Vwithin*, to vary by morph. All details and results are presented in the accompanying R script and in Tables S3 and S4.

Ethical Note

Fishing in lakes and rivers was done with permission obtained from the landowners. All procedures were approved through an animal care permit (MAST – #2019-01.-09, Ref.190564), were designed to minimize stress in the tested animals, in line with the European standard animal care protocols, and respect the Icelandic Animal Protection Act (Act 15/1994, last updated with Act 11/2014). Particularly, handling procedures always involved optimal dosage of anaesthesia with 2-phenoxyethanol adjusted to the reaction of the individual, following the clauses of best practices for animal care and experiments of Hólar University Aquaculture Research Station (HU-ARC) operational licence. The Arctic charr performs better at high stocking density as they switch from territorial agonistic behaviour to shoaling. Therefore, the fish were held in densities (5 to 14 fish/L) that might seem high, but that did not exceed the welfare recommendations for aquaculture (up to 250 fish/L, Jobling et al., 1993). Their growth was followed up monthly and food rations were adjusted accordingly, strictly following instructions from BioMar INICO manufacturer, an aquaculture feed developed specifically for Arctic charr, providing optimal nutrition.

Results

Mean Component of Boldness

Boldness score was significantly impacted by the morph, mean boldness score increasing along the degree of divergence (roughly, AN = VS < VB ≤ PL < LB, respective mean boldness score ± standard error (SE) : -0.73 ± 0.08, -1.14 ± 0.06, -0.18 ± 0.08, 0.33 ± 0.10, 1.72 ± 0.11, Table 2, Fig. 1a), whereas neither the treatment nor the morph-by-treatment interaction showed an effect (Table 2, Fig. 1b). Mean boldness score was not influenced by size, but the fish on average displayed significantly shyer behaviour on the second OFT replication compared to the first (estimated decrease in boldness score ± SE of 0.27 ± 0.07, Table 2). Model.0 explained 61.0% of the variance in the data (R2c), with 25.4% explained by the fixed effects (R2m). The quasi totality of the variance explained by the fixed predictors was attributable to the morph (IR^2 = 20.7%), the other fixed predictors showing negligible impact (Table 2). Random factors variation was attributable mostly to the individual ID (IR^2 = 28.2%, Table 2), consistent with the concept of personality.

Among- and Within-Individual Variabilities, and Repeatability Components of Boldness

Comparisons Between Morphs

Table 3 shows that all morphs differed significantly in terms of within-individual variability (*Iwithin*: VS < LB < AN < PL < VB, respective estimates: 0.87, 1.35, 3.82, 26.64, 48.87), and except for the LB-AN pair showing no significant difference, among-individual variability followed a similar fashion across morphs (*Iamong*: VS < LB = AN < PL < VB, respective estimates: 0.36, 0.90, 1.25, 13.52, 39.33), with important effect sizes in all cases (almost all A statistics of 0 or 1). The boldness score was repeatable within each morph (Table 3). The morphs considered benthic, VB and LB, tended to show higher repeatabilities than the three other morphs (VB significantly more repeatable than AN, and a tendency for VB and LB to be more repeatable than VS and AN respectively, see ΔR estimates in Table 3), also hinted by the A statistics showing large differences only in comparisons involving LB and VB morphs against others (Table 3). AN seemingly had the lowest repeatability, which was not significantly different from VS but tended to be lower than PL (small difference in effect on R between AN and PL, Table 3). Hence, repeatability pattern could be summarized as AN = VS ≤ PL < (VB = LB) (respective R estimates: 0.25, 0.29, 0.34, 0.44, 0.40, Table 3), showing signs of a general increase with divergence from the ancestral proxy.

Comparisons Between Treatments Within Morphs

The boldness score was repeatable within each batch (Tables 4). The results highlight differences in plasticity of variability in boldness among morphs. That is, compared to the plain treatment, VB and PL in the enriched treatment were ostensibly more variable, both among and within individuals (for VB and PL respectively: Δ*Iamong* estimates -610.88 and -2658.19; Δ*Iwithin* estimates -679.32 and -4416.20, Table 4), and effect sizes for ΔR revealed a tendency for them to also be more repeatable (ΔR estimates for VB and PL both inferior to -0.10, with A statistics inferior to 0.20, Table 4). Conversely, boldness scores were significantly less variable within AN individuals from the enriched treatment than from the plain treatment (Δ*Iwithin* estimate 2.08, Table 4), with effect sizes indicating a similar tendency for VS and LB (respective A statistics for *Iwithin*: 0.90 and 0.85, Table 4). Differences in plasticity of variability in boldness among morphs also manifest through a morph-by-treatment interaction effect, as magnitudes of both Δ*Iamong* and Δ*Iwithin* between treatments were higher within PL than within VB, themselves higher than within AN in the case of Δ*Iwithin* (Table 4). Plasticity in variability of boldness pattern could be summarized as (VS = LB) < AN < VB < PL.

Discussion

Non-responsiveness in the OFT

About 18% of the fish tested in the OFT were excluded from the PCA analyses for not responding (see results and discussion on statisticalstatistical comparisons of the proportions of excluded fish per group excluded fish per groupth results on boldness scores in Table S2). This percentage is not unusual in fish tested with OFT (*e.g.*, Ferrari et al., 2016). However, more participation in the behavioural test would capture a more accurate estimation of among-individual variation in behaviour. This can be obtained by increasing the open field duration over 20 minutes, which can be logistically challenging with an important number of individuals to test repeatedly.

(H1.i) No Plastic Response to Environmental Complexity in Mean Personality

The structural complexity treatment did not have a significant impact on mean boldness scores, consistent with a previous study showing that the same environmental enrichment treatment did not influence mean boldness scores in an aquaculture strain of Arctic charr (Philip et al., 2022). However, we previously demonstrated that mean boldness responded plastically to feeding modalities (Dellinger et al., 2023), indicating that mean boldness is a developmentally plastic trait in this species. Consequently, the current lack of differences in mean boldness between plain- and enriched-raised fish suggests a probable failure of this specific complexity treatment to engender a mean plastic response in boldness in the Arctic charr. This failure could have several sources (Stamps & Groothuis, 2010): (1) the treatment itself is not a developmental cue for mean boldness (either because structural complexity in general is not, or because the specific enrichment structures provided here do not equate the natural structural complexity relevant to elicit developmental plasticity in the wild, where other factors such as predation avoidance strategies are at stake); (2) the treatment was applied outside of sensitive developmental windows during which plastic responses to environmental complexity in boldness are triggered (the notable developmental stage covered by this experimental design was the switch from endogenous to first exogenous feeding, but maybe the Arctic charr would be more sensitive to environmental complexity at later stages, *e.g.*, sexual maturation); plastic developmental processes were indeed triggered by this early environmental complexity exposure (*i.e.*, exposure during an actual sensitive developmental window) but the resulting behavioural phenotypes (3) would only manifest in later life stages not monitored in this study (for instance, influencing boldness in mating context) or (4) manifested for a given period of time at a younger age and then normalized prior to OFT testing. Monitoring the ontogeny of personality in future research, over additional strategic developmental time points and longer periods covering the lifespan, appears necessary (Stamps & Groothuis, 2010).

(H1.ii) Genotype-by-Environment Plastic Responses to Environmental Complexity in Among-Individual Variability in Personality

We hypothesized that a structurally complex treatment providing a variety of microenvironments could elicit different behavioural responses among individuals, resulting in a higher variability in boldness among individuals compared to the plain treatment (Freund et al., 2013, 2015; Körholz et al., 2018; Zocher et al., 2020). Here, the results hint at such a pattern, but it seems the effect might be morph-specific, as *Iamong* was significantly higher in the enriched treatment only for VB and PL morphs. Interestingly, VB and PL are both the more specialized morphs from their respective sympatric pairs, and exploit niches with lower structural complexity in the wild compared to their sympatric counterparts (VB being sedentary in a small, simple, muddy-bottom lake; PL living in the water column of a deep lake). We might hypothesize that these two morphs are particularly prone to behavioural shifts when exposed to the enriched treatment, due to the unusualness of structural diversity in their natural habitat.

Körholz and colleagues (2018) found no differences in among-individual variance in locomotion in an open field between mice reared in structurally enriched cages and mice reared in plain, control conditions. They however showed that mice raised in enriched conditions exhibited higher among-individual variance compared to those raised in plain conditions, both in territory coverage and interaction time with novel objects (see also Zocher et al., 2020). In the steelhead trout, fish reared in tanks stably enriched with rocks and plants had lower among-individual variation in latency to exit the shelter than fish from plain tanks (Lee & Berejikian, 2008). All in all, studies focusing on the effects of environmental complexity/enrichment on behavioural among-individual variances specifically, not just averages, are very scarce, and provide contrasting results. Our results partly align with those of Akhund-Zade and colleagues (2019), who showed that these effects depend on the genotype, the enrichment type proposed to the animals, genotype-by-enrichment interactions, and the specific behaviour tested. Further empirical, variance-focused research is needed to elucidate the mechanisms behind those intricate dependencies to better understand evolutionary outcomes of consistent environmentally-induced among-individual variation in behaviour, *i.e.*, environmentally-induced variation in personality.

(H1.iii) Genotype-by-Environment Plastic Responses to Environmental Complexity in Personality Consistency

We predicted that boldness would be more repeatable and/or less variable within individuals either (1) in the enriched treatment overall, as an enriched treatment is more representative of natural conditions than a bare plain treatment, or (2) in the treatment most reflecting the structural complexity experienced by each morph in the wild (i.e., in the enriched treatment for AN, VS and LB morphs living in naturally more complex niches, vs. in the plain treatment for VB and PL morphs living in more homogeneous habitats), the extent of the response depending on the degree of structural complexity in the natural habitat of each morph (higher extent for PL and LB respectively living in environments at the two extremes of structural complexity; intermediary extent for the AN migrating through quite complex river beds; lower extent for VB and VS morphs living in a low-complexity lake).

On the one hand, the responses in terms of repeatability seem to follow our first prediction, boldness seeming generally more repeatable in the enriched treatment (all ΔR estimates being negative, Table 4). This was, nevertheless, only a non-significant pattern, with a potential tendency for VB and PL weakly supported by small effect sizes observed between treatments in terms of repeatability. On the other hand, within-individual variability in personality responses to structural complexity partially follows our second prediction: *Iwithin* was generally lower in the enriched treatment within AN, VS and LB morphs, and lower in the plain treatment within VB and PL morphs. The extent of responses for VB and PL morphs also corresponded to our predictions, as *Iwithin* in the enriched treatment for the PL was more than 6 times higher than for the VB. However, the extent of responses for AN, VS and LB morphs differed from predicted, AN showing greater plasticity than VS and LB.

All in all, the results show a morph-by-treatment interaction in how boldness consistency responds to structural complexity. This generally corresponds to our prediction that behavior is expressed more consistently when animals are reared in environments that resemble their natural habitats, *i.e.*, personality emergence being favored when the current environmental conditions experienced by individuals match the conditions to which they have been adapted. These patterns resonate with the concept of nature-based welfare of farmed animals (Huntingford et al., 2006), in which welfare conditions are met when animals are able to express their natural behaviours. The potentially lower behavioural consistency in a given treatment would highlight a situation of maladaptiveness and lower welfare, where fish have impaired capacities to express stable behaviours. This could explain how separation of personality profiles between niches can be maintained: impairment manifesting in certain types of environments might prevent individuals to switch to a niche not allowing the expression of stable behaviours for their given personality profile. It is interesting to note that just as for Δ*Iamong*, major differences in Δ*Iwithin* and ΔR concern VB and PL morphs, more ecologically specialized and exploiting niches of lower structural complexity than their sympatric counterparts, reinforcing the hypothesis of a potentially higher behavioural sensitivity to unusually complex conditions for those two morphs.

(H2) Effects of Evolutionary History on Personality Profiles

Boldness Development Under Strong Genetic Influence

All boldness repeatability estimates being significant is a first clue in favour of heritability of this personality trait (Dochtermann et al., 2015). Consistent with previous experiments on these Arctic charr morphs (Benhaïm et al., 2023; Horta-Lacueva et al., 2021; Philip et al., 2022)), the present results confirm the dominant influence of genetic background over environmental factors on the development of boldness in this species. They also reproduce the increasing mean boldness along the morph's divergence gradient (roughly, AN = VS < VB ≤ PL < LB, Tables 1, Fig. 1a). This suggests a genetic origin over maternal effects, as this pattern was already reported in the offspring of the same five morphs independently sampled and reared in different years (four to five years prior in Benhaïm et al., 2023; one to two years prior in Dellinger et al., 2023), and despite different social rearing contexts (Benhaïm et al., 2023), ages and feeding modalities (Dellinger et al., 2023)). This remarkable within- and between-morph consistency over the years strongly evinces high heritability of these mean boldness patterns, suggesting their maintenance in the wild through stable and morph-specific selective pressures.

The current results demonstrate significant within-lake differences in mean boldness scores between sympatric morphs of Arctic charr for the first time (Table 2, Fig. 1a), where previous studies on Arctic charr morphs only identified non-significant tendencies (Dellinger et al., 2023; Horta-Lacueva et al., 2021) or differences in boldness limited to more distantly related populations across lakes and rivers (Benhaïm et al., 2023; Dellinger et al., 2023). In the present study, the sample size used was markedly higher (around 235 fish per morph) compared to the previous studies (32 fish per morph in Benhaïm et al., 2023; 15 to 37 fish per morph in Horta-Lacueva et al., 2021; around 100 fish per morph in Dellinger et al., 2023). The (subtle) genetic differences between closely related sympatric morphs required a large sample size and power to accurately detect the fine resulting behavioural differences.

Independence of Personality Components Development and Evolution

Our previous study (Dellinger et al., 2023) suggested that personality components (mean, variances, repeatability and plasticity) might not coevolve as a block, but rather evolve independently from each other. After a drastic environmental change (in this case, forced lake residency for anadromous fish), the evolution of each personality component could independently be driven by specific combinations of factors, including the plasticity cost, the difference in mean, variance and predictability of the new environment, the magnitude of difference in phenotypic optima between ancestral and derived environments, and the amount of time since the change occurred (Lande, 2015). If this is the case, we expected different developmental outcomes for each component within and between morphs. Specifically, we did not expect the boldness components to exhibit gradient-like values along the degree of divergence across morphs.

We showed that mean personality component might evolve according to the divergence degree (boldness mean increasing with the divergence degree: AN = VS < VB ≤ PL < LB), while the other components seem independent from divergence. Repeatability — while showing signs of a general increase with divergence from the ancestral proxy as found in Dellinger et al. (2023) — appeared to rather depend on the "type" of morph considered: interestingly, non-migratory "benthic" morphs VB and LB, considered the most derived from the ancestral form due to pedomorphism (Skúlason, Noakes, et al., 1989), tended to have the highest repeatabilities. The accentuation of juvenile traits in these morphs might also lead to accentuation of personalities. Additionaly, resident benthic morphs may establish more stable hierarchies resulting in more pronounced personalities (*e.g.*, bold fish actively defending bottom territories vs. shy fish acting more submissively). Mechanisms underlying this phenomenon are yet uncertain and deserve further investigations. Among- and within-individual variabilities, unrelated to the divergence gradient among morphs, appear to be linked to each other (roughly VS < LB < AN < PL < VB for both *Iamong* and *Iwithin*), hence seemingly evolving co-dependently within a genotype. Plasticity in response to structural complexity was found only in both variability components of personality, but not in terms of mean boldness, and was not related to the divergence degree. Morphs displayed different degrees of plasticity in variability components ((VS = LB) < AN < VB < PL), which also follow approximately the patterns of variability estimates per morph.

Our results therefore do not support the "plasticity-first hypothesis" (Levis & Pfennig, 2016). It appears that mean personality and repeatability of personality develop and evolve independently from each other and from from variability-related components. Notably, variability-related components do not decrease with divergence and may co-evolve to some extent. In the present study, we used mean standardized variances (Houle's I (Houle, 1992)) as measures of variability, which explains discrepancies with among- and within-individual variances estimates found in our previous work (Dellinger et al., 2023). However, even the unstandardized raw variances estimates found in this study (Tables S3 and S4) differ from those of the previous study, where fish were subjected to feeding modality treatments instead of structural complexity. This suggests that the development of among- and within-individual variabilities in boldness is extremely flexible and influenced significantly by genotype-by-environment interactions. Particularly, we observed significant differences in boldness plasticity among morphs in terms of both among- and within-individual variability. Akhund-Zade et al. (2019) demonstrated that among-individual variability in behaviour is a plastic trait and that different complexity enrichment conditions did not always change behavioural among-individual variance in the same direction depending on the genotype. Our results further demonstrate that within-individual variability of behaviour is also a plastic and genotype-by-environment dependent trait, and that variability-related components of personality develop and plausibly evolve jointly, but independently from the other personality components. The mechanisms leading to the coevolution of those traits remain unclear. We hypothesize that having all aspects of personality variability and its plasticity covarying together may be a less costly way to grant behavioural flexibility to accommodate to changing/unstable environments at the population level. As noted, the more ecologically specialized morphs are also the most variable and plastic. This is surprising, as ecological specialization is usually associated with reduced flexibility and plasticity (*e.g.*, Svanbäck & Schluter, 2012). Maintaining such high degree of behavioural flexibility in specialists' populations could be seen as an evolutionary backup strategy. In case of environmental changes, specialists might require extensive behavioural shifts to adapt to new conditions, hence requiring latent flexibility. In contrast, generalists already exploiting broad niches would require little to no behavioural shift, hence little to no flexibility to accommodate. This further emphasizes the importance of plasticity research to not only consider traits means, but also expand the understanding to plasticity in variability as well (Akhund-Zade et al., 2019), for an exhaustive insight into how biodiversity emerges.

Conclusion and Implication for Adaptability and Biodiversity

This study sheds light on the roles of genetics, developmental plasticity and evolutionary history in shaping animal personality. Mean boldness showed no plastic response to environmental complexity, being primarily genotype-dependent and seemingly highly heritable, with an increasing pattern along the divergence gradient most likely under strong and stable selective pressure in the wild. Personality emergence seems favoured when individuals experience environmental conditions they are adapted to. Personality components related to variability might evolve and develop jointly, while the others do so independently. We stress the importance of genotype-by-environment interactions for these components' development. The results suggest potential mechanisms underlying personality-driven adaptability to sudden environmental changes and its implication for sympatric diversification. Coping with drastic environmental changes (from anadromy to lake residency in our case) imposes behavioural shifts (Duckworth, 2009). Personality is sometimes viewed as constraining behaviour (Duckworth, 2010) potentially hampering the achievement of optimal behaviours (Sih et al., 2003). But personality mean, variabilities, repeatability, and plasticity evolving and developing independently from each other allow for a wide diversity of combinations of these components, to adapt more accurately to the various subtleties of environmental demands (Lande, 2015). In oligotrophic ecosystems like Arctic freshwaters, reducing intra-specific competition by exploiting different niches is a key strategy to maximize resources availability for a species. This ability to modulate each component to optimally adapt personality profiles for consistent exploitation of different resources facilitates these niche shifts and separation between personality profiles (Stamps & Groothuis, 2010; Wolf & Weissing, 2010). After fine-tuning personality profiles, the allegedly impaired capacity to express stable behaviours in unsuitable environments might reinforce and maintain niche separation between personality profiles, allowing differential selective pressures to drive sympatric diversification and polymorphism (Dall et al., 2012), or even speciation (Ingley, 2018). To date, the relative importance of each personality component in species adaptability and diversification is unknown. Further research should explore the ontogeny of their different combinations and their subsequent consequences on fitness. This would provide outstanding appreciation of the link between biodiversity and the observable diversity in animal personality traits.

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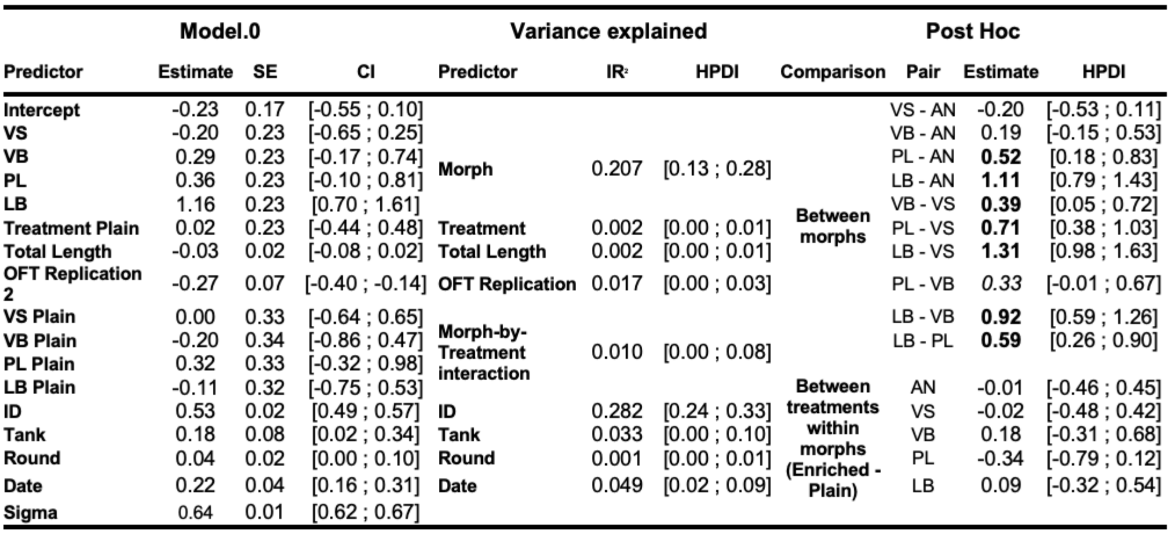
Paper II - Tables and Figures

Table 1 – Summary of the different models used, and the corresponding hypotheses explored.

Model structure	Calculation allowed	Hypotheses explored
Model.0		
scale(Boldness Score) ~ Morph*Treatment + scale(Total length) + OFT Replication + (1|ID) + (1|Tank) + (1|Round) + (1|Date)	Mean component by morph	**H2** – Components evolution: mean
	Mean component by treatment	**H1.i** – Developmental plasticity: environment effect
	Mean component by batch	**H1.i** – Developmental plasticity: genotype-by-environment effect **H2** – Components evolution: plasticity
Model.morph		
BoldnessScore ~ Morph + (0+Morph||ID), sigma ~ 0+Morph	*Iamong*, *Iwithin* and R components, by morph	**H2** – Components evolution: *Iamong*, *Iwithin*, R
Models.treatment (one per morph)		
BoldnessScore ~ Treatment + (0+Treatment||ID), sigma ~ 0+Treatment	*Iamong* component by treatment within morph	**H1.ii** – Developmental plasticity: fanning-in and fanning-out effect
	Iwithin and R components, by treatment within morph	**H1.iii** – Developmental plasticity: consistent behaviours favoured in adapted conditions

Personality component: mean, among-individual variability (*Iamong*) and within-individual variability (*Iwithin*), repeatability (R), and plasticity. The hypotheses are labelled as per the introduction.

Table 2 – Results of model.0, variance explained by each predictor, and post hoc comparisons regarding boldness scores.



Model.0				Variance explained			Post Hoc			
Predictor	Estimate	SE	CI	Predictor	IR^2	HPDI	Comparison	Pair	Estimate	HPDI
Intercept	-0.23	0.17	[-0.55 ; 0.10]				Between morphs	VS - AN	-0.20	[-0.53 ; 0.11]
VS	-0.20	0.23	[-0.65 ; 0.25]					VB - AN	0.19	[-0.15 ; 0.53]
VB	0.29	0.23	[-0.17 ; 0.74]	Morph	0.207	[0.13 ; 0.28]		PL - AN	**0.52**	[0.18 ; 0.83]
PL	0.36	0.23	[-0.10 ; 0.81]					LB - AN	**1.11**	[0.79 ; 1.43]
LB	1.16	0.23	[0.70 ; 1.61]					VB - VS	**0.39**	[0.05 ; 0.72]
Treatment Plain	0.02	0.23	[-0.44 ; 0.48]	Treatment	0.002	[0.00 ; 0.01]		PL - VS	**0.71**	[0.38 ; 1.03]
Total Length	-0.03	0.02	[-0.08 ; 0.02]	Total Length	0.002	[0.00 ; 0.01]		LB - VS	**1.31**	[0.98 ; 1.63]
OFT Replication 2	-0.27	0.07	[-0.40 ; -0.14]	OFT Replication	0.017	[0.00 ; 0.03]		PL - VB	*0.33*	[-0.01 ; 0.67]
VS Plain	0.00	0.33	[-0.64 ; 0.65]	Morph-by-Treatment interaction	0.010	[0.00 ; 0.08]		LB - VB	**0.92**	[0.59 ; 1.26]
VB Plain	-0.20	0.34	[-0.86 ; 0.47]					LB - PL	**0.59**	[0.26 ; 0.90]
PL Plain	0.32	0.33	[-0.32 ; 0.98]							
LB Plain	-0.11	0.32	[-0.75 ; 0.53]				Between treatments within morphs (Enriched - Plain)	AN	-0.01	[-0.46 ; 0.45]
ID	0.53	0.02	[0.49 ; 0.57]	ID	0.282	[0.24 ; 0.33]		VS	-0.02	[-0.48 ; 0.42]
Tank	0.18	0.08	[0.02 ; 0.34]	Tank	0.033	[0.00 ; 0.10]		VB	0.18	[-0.31 ; 0.68]
Round	0.04	0.02	[0.00 ; 0.10]	Round	0.001	[0.00 ; 0.01]		PL	-0.34	[-0.79 ; 0.12]
Date	0.22	0.04	[0.16 ; 0.31]	Date	0.049	[0.02 ; 0.09]		LB	0.09	[-0.32 ; 0.54]
Sigma	0.64	0.01	[0.62 ; 0.67]							

Model.0: output of model.0. AN morph taken as reference for the morph factor, enriched treatment taken as reference for the treatment factor, AN morph and enriched treatments taken as references for the morph-by-treatment interaction factor, first OFT replication taken as reference for the OFT replication factor. **Variance explained**: variance in boldness scores explained (IR^2) by each predictor of model.0. **Post Hoc**: results of the Tukey post hoc pairwise comparisons of boldness scores between morphs, and between treatments within morphs, carried out on model.0, with significant estimates highlighted in bold font, while tendencies are highlighted in italics. Estimate: median of posterior distribution for each variable or contrast; SE: Standard Error; CI: Credible Interval; HPDI: 95% Highest Posterior Density Intervals.

Table 3 – Estimates per morph, pairwise differences in the posterior distributions between morphs (Δ) and their effect sizes, of boldness score repeatability (R), among-individual variability (Iamong) and within-individual variability (Iwithin).

Variable		**Estimates**		**Pairwise comparison**		**Δ**		**Effect size**	
	Morph	**Estimate**	**CI**		**Estimate**	**CI**	**PD**	**A**	**CI**
R									
	AN	0.25	[0.12 ; 0.36]	VS - AN	0.05	[-0.12 ; 0.21]	0.69	0.70	[0.69 ; 0.71]
				VB - AN	**0.20**	[0.05 ; 0.36]	0.99	**0.99**	[0.99 ; 0.99]
	VS	0.29	[0.17 ; 0.41]	PL - AN	0.09	[-0.08 ; 0.26]	0.86	*0.86*	[0.85 ; 0.86]
				LB - AN	*0.16*	[0.00 ; 0.31]	0.97	**0.97**	[0.97 ; 0.98]
	VB	0.44	[0.33 ; 0.55]	VB - VS	*0.16*	[-0.01 ; 0.31]	0.97	**0.97**	[0.97 ; 0.97]
				PL - VS	0.04	[-0.12 ; 0.22]	0.71	0.71	[0.69 ; 0.72]
	PL	0.34	[0.22 ; 0.45]	LB - VS	0.11	[-0.05 ; 0.27]	0.91	**0.91**	[0.91 ; 0.92]
				PL - VB	-0.11	[-0.27 ; 0.05]	0.91	**0.09**	[0.08 ; 0.09]
	LB	0.40	[0.29 ; 0.50]	LB - VB	-0.05	[-0.20 ; 0.10]	0.73	0.27	[0.26 ; 0.29]
				LB - PL	0.06	[-0.09 ; 0.22]	0.79	0.79	[0.78 ; 0.80]
Iamong									
	AN	1.25	[0.57 ; 1.93]	VS - AN	**-0.88**	[-1.59 ; -0.20]	0.99	**0.01**	[0.01 ; 0.01]
				VB - AN	**38.05**	[25.98 ; 52.39]	1.00	**1.00**	[1.00 ; 1.00]
	VS	0.36	[0.20 ; 0.54]	PL - AN	**12.28**	[7.12 ; 18.43]	1.00	**1.00**	[1.00 ; 1.00]
				LB - AN	-0.34	[-1.08 ; 0.40]	0.82	*0.18*	[0.17 ; 0.19]
	VB	39.33	[27.44 ; 53.63]	VB - VS	**38.96**	[27.10 ; 53.24]	1.00	**1.00**	[1.00 ; 1.00]
				PL - VS	**13.17**	[8.00 ; 19.20]	1.00	**1.00**	[1.00 ; 1.00]
	PL	13.52	[8.36 ; 19.56]	LB - VS	**0.54**	[0.22 ; 0.90]	1.00	**1.00**	[1.00 ; 1.00]
				PL - VB	**-25.72**	[-41.23 ; -12.42]	1.00	**0.00**	[0.00 ; 0.00]
	LB	0.90	[0.62 ; 1.22]	LB - VB	**-38.40**	[-52.73 ; -26.49]	1.00	**0.00**	[0.00 ; 0.00]
				LB - PL	**-12.61**	[-18.68 ; -7.43]	1.00	**0.00**	[0.00 ; 0.00]
Iwithin									
	AN	3.82	[3.20 ; 4.60]	VS - AN	**-2.94**	[-3.75 ; -2.30]	1.00	**0.00**	[0.00 ; 0.00]
				VB - AN	**45.05**	[36.82 ; 55.51]	1.00	**1.00**	[1.00 ; 1.00]
	VS	0.87	[0.73 ; 1.06]	PL - AN	**22.81**	[18.36 ; 28.39]	1.00	**1.00**	[1.00 ; 1.00]
				LB - AN	**-2.47**	[-3.26 ; -1.78]	1.00	**0.00**	[0.00 ; 0.00]
	VB	48.87	[40.65 ; 59.46]	VB - VS	**48.02**	[39.74 ; 58.51]	1.00	**1.00**	[1.00 ; 1.00]
				PL - VS	**25.75**	[21.38 ; 31.34]	1.00	**1.00**	[1.00 ; 1.00]
	PL	26.64	[22.33 ; 32.19]	LB - VS	**0.46**	[0.20 ; 0.79]	1.00	**1.00**	[1.00 ; 1.00]
				PL - VB	**-22.24**	[-33.88 ; -12.29]	1.00	**0.00**	[0.00 ; 0.00]
	LB	1.35	[1.14 ; 1.62]	LB - VB	**-47.52**	[-58.05 ; -39.25]	1.00	**0.00**	[0.00 ; 0.00]
				LB - PL	**-25.26**	[-30.81 ; -20.91]	1.00	**0.00**	[0.00 ; 0.00]

Significant Δ estimates and large effect sizes are highlighted in bold font, while tendencies for Δ and small effect sizes are highlighted in italics. **Estimates & Δ**: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ. **Effect size**: probability of superiority (A) with its 95% confidence interval (CI), between the posterior distributions of each variable for each pair of morphs.

Table 4 – Estimates per batch, pairwise differences (Δ) in the posterior distributions between treatments (E: Enriched vs. P: Plain) within morphs and their effect sizes, of boldness score repeatability (R), among-individual variance (Iamong) and within-individual variance (Iwithin).

Variable	**Morph**	**Estimates**			**Δ (P - E)**			**Effect size**	
		Treatment	**Estimate**	**CI**	**Estimate**	**CI**	**PD**	**A**	**CI**
R									
	AN	E	0.26	[0.06 ; 0.41]	-0.03	[-0.28 ; 0.22]	0.61	0.39	[0.38 ; 0.40]
		P	0.22	[0.03 ; 0.39]					
	VS	E	0.33	[0.16 ; 0.47]	-0.08	[-0.32 ; 0.16]	0.74	0.26	[0.25 ; 0.27]
		P	0.24	[0.06 ; 0.43]					
	VB	E	0.47	[0.34 ; 0.59]	-0.10	[-0.37 ; 0.13]	0.80	*0.20*	[0.19 ; 0.21]
		P	0.38	[0.14 ; 0.55]					
	PL	E	0.38	[0.21 ; 0.52]	-0.13	[-0.36 ; 0.11]	0.85	*0.15*	[0.14 ; 0.15]
		P	0.25	[0.05 ; 0.42]					
	LB	E	0.41	[0.26 ; 0.54]	-0.02	[-0.22 ; 0.18]	0.58	0.43	[0.41 ; 0.44]
		P	0.39	[0.24 ; 0.52]					
Iamong									
	AN	E	1.02	[0.23 ; 1.85]	0.40	[-1.04 ; 1.98]	0.70	0.70	[0.69 ; 0.71]
		P	1.43	[0.21 ; 2.83]					
	VS	E	0.38	[0.18 ; 0.64]	-0.06	[-0.40 ; 0.33]	0.68	0.38	[0.37 ; 0.39]
		P	0.32	[0.07 ; 0.64]					
	VB	E	616.61	[410.98 ; 883.98]	**-610.88**	[-877.44 ; -403.73]	1.00	**0.00**	[0.00 ; 0.00]
		P	5.80	[2.02 ; 10.36]					
	PL	E	2660.12	[1388.03 ; 4191.14]	**-2658.19**	[-4189.87 ; -1384.21]	1.00	**0.00**	[0.00 ; 0.00]
		P	2.14	[0.46 ; 4.02]					
	LB	E	0.86	[0.50 ; 1.31]	0.09	[-0.51 ; 0.71]	0.61	0.62	[0.60 ; 0.63]
		P	0.96	[0.55 ; 1.45]					
Iwithin									
	AN	E	2.99	[2.35 ; 3.97]	**2.08**	[0.60 ; 3.75]	1.00	**1.00**	[1.00 ; 1.00]
		P	5.07	[3.92 ; 6.63]					
	VS	E	0.79	[0.62 ; 1.02]	0.21	[-0.12 ; 0.59]	0.90	**0.90**	[0.89 ; 0.90]
		P	1.01	[0.76 ; 1.35]					
	VB	E	689.05	[548.13 ; 876.98]	**-679.32**	[-869.99 ; -537.97]	1.00	**0.00**	[0.00 ; 0.00]
		P	9.70	[7.15 ; 13.92]					
	PL	E	4422.29	[3470.40 ; 5769.55]	**-4416.20**	[-5762.17 ; -3462.41]	1.00	**0.00**	[0.00 ; 0.00]
		P	6.47	[5.01 ; 8.55]					
	LB	E	1.24	[0.98 ; 1.59]	0.25	[-0.22 ; 0.75]	0.85	*0.85*	[0.84 ; 0.86]
		P	1.48	[1.18 ; 1.90]					

Significant Δ estimates and large effect sizes are highlighted in bold font, while tendencies for Δ and small effect sizes are highlighted in italics. **Estimates & Δ**: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ. **Effect size**: probability of superiority (A) with its 95% confidence interval (CI), between the posterior distributions of each variable for each treatment within morphs.

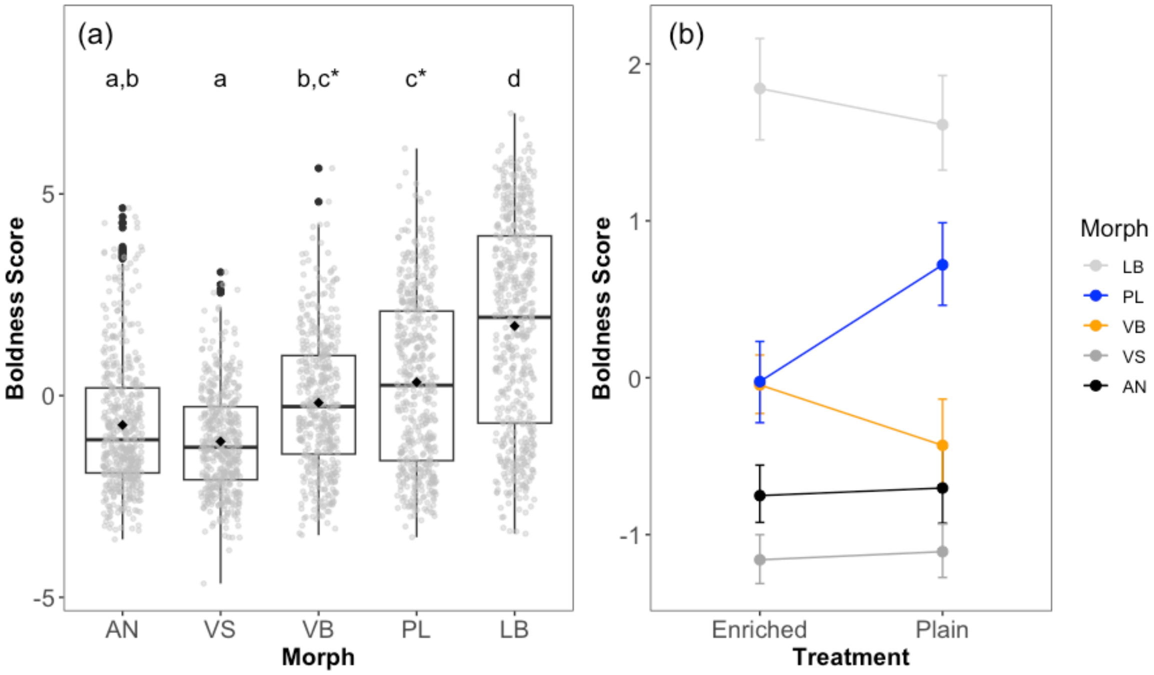


*Figure 1 – **(a)** Boxplots of boldness scores for each morph, from the least to the most diverged. The rhombus indicates the mean, grey dots represent scattered individual values, the central line is the median, ends of the boxes denote upper and lower quartiles, whiskers cover 95% of values. Significant differences between two morphs are present when they do not share a common letter on top of the boxes, the asterisk signifying a tendency to differ between VB and PL morphs. **(b)** Reaction norms of boldness scores across treatments for each morph. The dot represents the mean, vertical bars indicate one standard error, the slope of the line across treatments indicates the direction and amplitude of boldness plasticity. In this case, no difference was found to be significant between the treatments within morphs (see also Table 2).*

Paper II – Supplements

Table S1 – Sample size distribution between treatments and tank replicates for each morph, indicating the number of fish eventually included in the PCA and subsequent analyses.

Morph	Treatment Enriched			Treatment Plain		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
AN	44	46^{-1r}	37	44	37	30^{-1h}
VS	43^{-1h}	44	41^{-1t}	$35^{-1r,-2h}$	$33^{-1r,-1h}$	$34^{-1r,-1h,-1u}$
VB	47	50	48	/	44	32^{-1t}
PL	$41^{-1rd,-1u}$	41	39^{-1r}	46	$31^{-1r,-2t}$	35^{-1t}
LB	47^{-4r}	40^{-6d}	$40^{-7d,-3r}$	48	51^{-1t}	37^{-11t}

Fish from AN, PL and LB morphs were distributed over 6 replicate tanks (three replicate tanks per treatment) per morph. For the VS morph, there were initially five tanks (three enriched and two plain) because the sampled females did not provide enough eggs to create six tanks. Then, one enriched tank was lost due to technical issues, which reduced the VS morph to four tanks (two enriched and two plain). However, we created two pseudo-replicates (one enriched and one plain, see details in the main text), recovering the six tanks for the VS as planned. For the VB morph, there were initially six tanks, but a disease outbreak in the plain Replicate 1 drastically reduced the density in that tank, hence excluded from the experiment. Moreover, further mortality made it impossible to create a pseudo-replicate to replace the plain tank missing. The experiment for the VB morph was therefore performed on five tanks only (three enriched and two plain). Theoretically, a total of 1508 fish were meant to be tested: 52 fish per replicate tank * 6 tanks per morph, minus one lost plain tank in the VB * 5 morphs. Discrepancies between theoretical and actual sample size for each tank are either due to individuals excluded from analyses for not responding to the OFT (see also Table S2), or other reasons (superscripts) detailed hereafter. A total of 1495 fish were actually tested (13 tagged fish died before the beginning of the OFT tests (negative "d" superscripts)), of which 1185 were eventually included in the PCA analyzes. The 310 fish excluded are: 270 non-responding individuals; 6 fish not included for health concerns (negative "h" superscripts); 17 fish not included for technical issues happening during the OFT trials (negative "t" superscripts); 2 fish not included because not registered on ID lists (negative "u" superscripts); 15 fish not included because only participated in one OFT replication for various reasons due to mortality in between replications or to selection mismatches (negative "r" superscripts).

Table S2 – Proportion of non-responding individuals, i.e., fish that did not exit the OFT shelter after 20 min, per morph, per treatment and per batch.

		Morph	**AN**	**VS**	**VB**	**PL**	**LB**
		All treatments considered	*23.2 %*	*23.6 %*	*14.7 %*	*23.6 %*	*6.1 %*
Treatment	*All morphs considered*						
Enriched	*14.1 %*		18.1 %	16.8 %	7.1 %	20.9 %	6.6 %
Plain	*23.4 %*		28.4 %	30.6 %	26.2 %	26.3 %	5.6 %

N = 270 out of 1495 fish tested, see details in Table S1. As time spent in the shelter was a major component of shyness in the PCA (see Fig. S1), we considered the proportion of non-responding fish—technically spending the maximum time in the shelter—as a complementary group-level indicator of boldness. χ^2 tests showed that the proportions of non-responding fish depended on the morph (χ^2 (4, N=1455) = 46.13, $p < 0.001$), treatment (χ^2 (1, N=1455) = 20.95, $p < 0.001$) and batch (χ^2 (9, N=1455) = 77.57, $p < 0.001$). The treatment engendered a plastic response in boldness, with significantly fewer non-responding fish in the enriched treatment compared to the plain treatment. If non-responding individuals could have been attributed a (most likely low) boldness score, one can imagine stronger boldness score differences between treatments within morphs, hence the detection of plasticity in mean component (hypothesis H1.i). Interestingly, non-migratory “benthic” morphs VB and LB, considered the most derived from the ancestral form, have the highest proportions of responding fish. This is in line with the fact that those morphs are also overall bolder than their sympatric counterparts (Table 2, Fig. 1a in the main text).

Table S3 – Estimates per morph, pairwise differences in the posterior distributions between morphs (Δ) and their effect sizes, of boldness among-individual variance (Vamong) and within-individual variance (Vwithin).

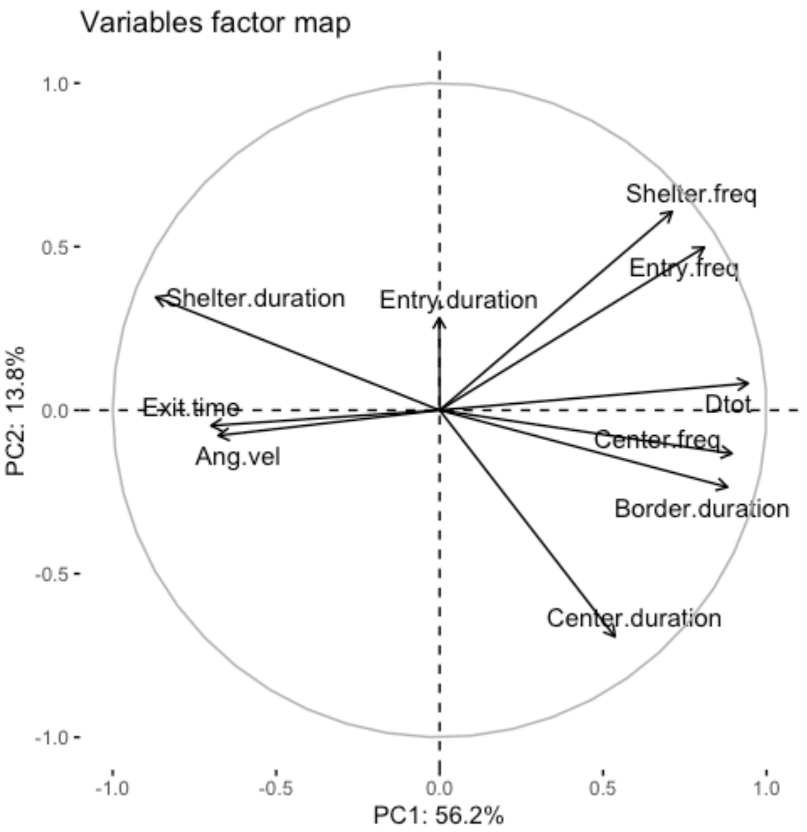
Variable		Estimates			Pairwise comparison	Δ				Effect size			
	Morph	Estimate	Lower CI	Upper CI		Estimate	Lower CI	Upper CI	PD	A	SE	Lower CI	Upper CI
Vamong													
	AN	0.66	0.30	1.02	VS - AN	-0.19	-0.62	0.22	0.82	*0.18*	0.00	0.17	0.19
					VB - AN	**0.60**	0.08	1.15	0.99	**0.99**	0.00	0.98	0.99
	VS	0.46	0.26	0.70	PL - AN	**0.84**	0.15	1.59	0.99	**0.99**	0.00	0.99	0.99
					LB - AN	**2.03**	1.13	3.03	1.00	**1.00**	0.00	1.00	1.00
	VB	1.25	0.87	1.70	VB - VS	**0.79**	0.33	1.29	1.00	**1.00**	0.00	1.00	1.00
					PL - VS	**1.04**	0.40	1.74	1.00	**1.00**	0.00	1.00	1.00
	PL	1.50	0.93	2.17	LB - VS	**2.22**	1.35	3.19	1.00	**1.00**	0.00	1.00	1.00
					PL - VB	0.25	-0.51	1.03	0.74	0.74	0.01	0.73	0.75
	LB	2.69	1.84	3.64	LB - VB	**1.43**	0.50	2.48	1.00	**1.00**	0.00	1.00	1.00
					LB - PL	**1.18**	0.13	2.30	0.99	**0.99**	0.00	0.98	0.99
Vwithin													
	AN	2.03	1.70	2.44	VS - AN	**-0.89**	-1.36	-0.48	1.00	**0.00**	0.00	0.00	0.00
					VB - AN	**-0.47**	-0.96	-0.02	0.98	**0.02**	0.00	0.02	0.03
	VS	1.13	0.95	1.37	PL - AN	**0.93**	0.30	1.62	1.00	**1.00**	0.00	1.00	1.00
					LB - AN	**1.98**	1.25	2.85	1.00	**1.00**	0.00	1.00	1.00
	VB	1.55	1.29	1.89	VB - VS	**0.43**	0.07	0.81	0.99	**0.99**	0.00	0.99	0.99
					PL - VS	**1.83**	1.28	2.47	1.00	**1.00**	0.00	1.00	1.00
	PL	2.96	2.48	3.58	LB - VS	**2.88**	2.23	3.71	1.00	**1.00**	0.00	1.00	1.00
					PL - VB	**1.40**	0.81	2.08	1.00	**1.00**	0.00	1.00	1.00
	LB	4.01	3.40	4.81	LB - VB	**2.45**	1.78	3.29	1.00	**1.00**	0.00	1.00	1.00
					LB - PL	**1.06**	0.20	1.97	0.99	**0.99**	0.00	0.99	0.99

Results were obtained from morph-specific multi-level structure Bayesian brms linear mixed models for z-scaled boldness score (model.morph), including the morph as fixed predictor, random effects for both the intercept and the morph-related effect nested within the individual IDs, and a sigma term accounting for potential variations in the residual standard deviation among morphs. See "Chapter III" in the R script accompanying the main text. Significant Δ estimates and large effect sizes are highlighted in bold font, while tendencies for Δ and small effect sizes are highlighted in italics. Estimates & Δ: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ. Effect size: probability of superiority (A) with its 95% confidence interval (CI), between the posterior distributions of each variable for each pair of morphs. The A statistic is a probability-based measure of effect size, indicating the probability that a randomly chosen member of group 1 scores higher on the response variable than a randomly chosen member of group 2, *i.e.*, in our case if A < 0.5, benthic fish outscore pelagic fish and *vice versa*.

Table S4 – Estimates per treatment within morphs, pairwise differences in the posterior distributions between treatments within morphs (Δ) and their effect sizes, of among-individual variance and within-individual variance (Vamong and Vwithin respectively).

Variable	Morph		Estimates			Δ (P - E)				Effect size			
		Treatment	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI	PD	A	SE	Lower CI	Upper CI
Vamong													
	AN	E	0.58	0.13	1.05	0.12	-0.63	0.94	0.62	0.63	0.01	0.61	0.64
		P	0.71	0.10	1.40								
	VS	E	0.51	0.24	0.86	-0.12	-0.55	0.37	0.69	0.31	0.01	0.30	0.32
		P	0.40	0.09	0.78								
	VB	E	1.29	0.86	1.85	-0.22	-1.14	0.72	0.69	0.31	0.01	0.30	0.32
		P	1.08	0.38	1.92								
	PL	E	1.59	0.83	2.51	-0.49	-1.73	0.75	0.79	0.21	0.00	0.21	0.23
		P	1.11	0.24	2.08								
	LB	E	2.94	1.69	4.44	-0.46	-2.26	1.32	0.69	0.31	0.01	0.30	0.32
		P	2.50	1.43	3.77								
Vwithin													
	AN	E	1.69	1.33	2.24	**0.82**	0.05	1.66	0.98	**0.98**	0.00	0.98	0.98
		P	2.51	1.94	3.27								
	VS	E	1.06	0.84	1.38	0.17	-0.26	0.64	0.78	0.78	0.01	0.77	0.79
		P	1.24	0.94	1.66								
	VB	E	1.44	1.15	1.84	0.36	-0.26	1.18	0.86	*0.86*	0.00	0.86	0.87
		P	1.80	1.33	2.58								
	PL	E	2.64	2.07	3.45	0.71	-0.40	1.91	0.89	**0.90**	0.00	0.89	0.90
		P	3.35	2.60	4.43								
	LB	E	4.21	3.32	5.41	-0.34	-1.79	1.09	0.69	0.32	0.01	0.30	0.33
		P	3.85	3.07	4.95								

Treatments: E – Enriched and P – Plain. Results were obtained from morph-specific multi-level structure Bayesian brms linear mixed models for z-scaled boldness score (models.treatment, one per morph), including the treatment as fixed predictor, random effects for both the intercept and the treatment-related effect nested within the individual IDs, and a sigma term accounting for potential variations in the residual standard deviation among treatments. See "Chapter IV" in the R script accompanying the main text. Significant Δ estimates and large effect sizes are highlighted in bold font, while tendencies for Δ and small effect sizes are highlighted in italics. **Estimates & Δ**: all estimates are medians of posterior distributions with their 95% credible interval (CI), along with probability of direction (PD) of the Δ, indicating the proportion of samples that are of the estimate's sign, *i.e.*, roughly the probability that the estimate is different from zero. **Effect size**: probability of superiority (A) with its standard error (SE) and 95% confidence interval (CI), between the posterior distributions of each variable for each pair of morphs. The A statistic is a probability-based measure of effect size, indicating the probability that a randomly chosen member of group 1 scores higher on the response variable than a randomly chosen member of group 2, *i.e.*, in our case if A < 0.5, benthic fish outscore pelagic fish and *vice versa*.



		Dimension									
		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Contributions											
	Dtot	15.861	0.487	1.522	1.246	3.986	1.985	7.570	4.491	62.853	< 0.001
	Center.freq	14.244	1.277	1.659	0.258	3.097	3.823	52.609	0.148	22.887	< 0.001
	Border.duration	13.809	4.004	0.006	0.943	2.085	31.500	4.906	4.866	3.316	34.565
	Shelter.duration	13.430	8.629	7.532	0.602	1.570	4.638	4.443	2.517	0.645	55.995
	Entry.freq	11.667	17.921	0.002	4.634	0.273	1.220	14.792	42.030	7.461	< 0.001
	Shelter.freq	9.005	26.651	0.516	6.856	0.978	10.218	1.003	44.517	0.256	< 0.001
	Exit.time	8.703	0.162	2.088	4.220	78.204	3.296	2.528	0.787	0.013	< 0.001
	Ang.vel	8.159	0.436	4.431	76.404	3.697	2.595	3.890	0.385	0.004	< 0.001
	Center.duration	5.123	34.672	2.404	1.755	1.490	40.326	5.447	0.259	2.386	6.139
	Entry.duration	< 0.001	5.672	79.840	3.084	4.621	0.399	2.813	< 0.001	0.180	3.301
Correlations											
	Dtot	0.944	0.082	-0.129	-0.085	0.150	-0.093	0.131	-0.053	0.166	< 0.001
	Center.freq	0.894	-0.133	-0.134	-0.039	0.132	0.128	0.344	-0.010	-0.100	< 0.001
	Border.duration	0.881	-0.235	0.008	-0.074	0.108	-0.369	-0.105	0.055	-0.038	0.009
	Shelter.duration	-0.869	0.346	-0.286	0.059	-0.094	0.142	0.100	-0.040	0.017	-0.011
	Entry.freq	0.809	0.498	-0.004	-0.165	0.039	0.073	-0.183	-0.162	-0.057	< 0.001
	Shelter.freq	0.711	0.607	-0.075	-0.200	-0.074	0.210	-0.048	0.166	0.011	< 0.001
	Exit.time	-0.699	-0.47	-0.151	-0.157	0.664	0.119	-0.076	0.022	-0.002	< 0.001
	Ang.vel	-0.677	-0.078	0.219	-0.668	-0.144	-0.106	0.094	-0.015	-0.001	< 0.001
	Center.duration	0.536	-0.693	0.162	-0.101	-0.092	0.417	-0.111	-0.013	0.032	0.004
	Entry.duration	-0.001	0.282	0.932	0.134	0.161	0.042	0.080	0.001	0.009	0.003
Eigenvalues		5.617	1.384	1.087	0.584	0.564	0.432	0.226	0.062	0.044	< 0.001
Variance proportion		0.562	0.138	0.109	0.058	0.056	0.043	0.023	0.006	0.004	< 0.001
Cumulative variance		0.562	0.700	0.809	0.867	0.924	0.967	0.989	0.996	1.000	1.000

Figure S1 – Principal Component Analysis (PCA) on the first OFT replication, used to obtain individual boldness scores (see Chapter I of the R script accompanying the main text). Dtot (cm): total distance travelled; Border.duration, Shelter.duration, Entry.duration and Center.duration (s): cumulative time spent respectively in the border, shelter, entry and centre zones; Center.freq, Shelter.freq and Entry.freq: frequency of visits in the centre, shelter and entry zone; Exit.time (s): latency to emerge from the shelter for the first time;

Ang.vel (deg.s^{-1}): absolute angular velocity. ***Top:*** *PCA Factor map on PC1 and PC2.* ***Bottom:*** *Contributions and correlations per variable, eigenvalue, variance proportion and cumulative variance, for each PC dimension.* ***Comment:*** *non-responding fish (see Table S1 and Table S2 for details) were not included in the PCA analyses. Instead, proportions of non-responding fish were compared between groups using χ^2 tests (see results Table S2).*

Paper III

Short communication published in Journal of Fish Biology

Article

Dellinger, M., Suret, L., Choblet, M., Jozet-Alves, C., & Benhaïm, D. (2024). Methodological approach to investigating spatial cognition in fishes: a case study on Arctic charr (*Salvelinus alpinus*). *Journal of Fish Biology*, 1-5. https://doi.org/10.1111/jfb.15936

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BRIEF COMMUNICATION

JOURNAL OF FISH BIOLOGY fsbi

Methodological approach to investigating spatial cognition in Arctic charr (*Salvelinus alpinus*)

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Abstract

Assessing cognitive traits poses consistent methodological challenges. We describe a method for testing Arctic charr by incorporating a T-maze into their housing, which reduced stress and improved the engagement of the tested fish in the task. We outline the phases of testing to evaluate learning efficiency and determine which orientation strategies, such as motor response versus beaconing, are prioritized by the animals. We emphasize the stress management aspects of the design, aiming to inspire other researchers tackling similar obstacles, rather than delving into the specific aspects of cognitive abilities assessment.

KEYWORDS
Arctic charr, egocentric strategy, learning, reward, spatial cognition, stress

Fish have become regular species in comparative cognitive studies because of their vast taxonomic diversity and associated life-history and ecological characteristics (Salena et al., 2021), allowing researchers to tackle a wide range of questions relevant to connected disciplines such as evolution and ecology. This also comes with many challenges because a relatively limited number of fish species have been studied so far and each species might require specific methodological adjustments. Spatial cognition, in particular, is the ability to acquire, process, and reorganize spatial information to make sense of an environment (Poucet, 1993). The role of learning and memory in fish orientation has been mostly studied in controlled experiments involving manipulations of spatial cues and/or testing procedures, whereby fish are trained to learn particular associations. Measuring cognitive traits comes with some recurrent methodological issues, including the evolutionary and ecological relevance of the stimuli used in such tasks (Dukas, 1998; Dukas & Ratcliffe, 2009), potential preexisting biases such as side-turning and/or eye-use preferences and preference for some stimuli (Salena et al., 2021), and, most importantly, the stress experienced by the subjects during the tests due to their capture, transport, handling, and confinement (Benhaïm et al., 2017; Davis et al., 2002; Salena et al., 2021). This is especially true for wild strains known to have higher emotional reactivity or responsiveness to fear-evoking stimuli compared with domesticated strains of fish (Price, 2002). We attempted to test Arctic charr (*Salvelinus alpinus*) spatial cognition, an unstudied species in this field, and encountered several failures where fish showed signs of possible stress (freeze or flight responses) or did not engage with the test within 7 days (six trials per day). This is a problem that many researchers working with other wild-caught fish species have encountered or will encounter. Here, we document an approach that worked for us and may be worth considering for other researchers. Previous work suggests that integrating an assay arena directly into housing aquaria can reduce stress, increase engagement time, and improve the proportion of individuals participating in trials within a limited time (Salena et al., 2021). We integrated our T-maze into the housing of individual fish as a way to overcome these latter issues. Relatively similar methods have been previously used on other species (e.g., Beri et al., 2014; Kuba et al., 2010). The setup introduced here consists of a living area with ecologically meaningful features (i.e. shelters and low light intensity), directly connected to a T-maze. We outline the various stages of the multi-phase testing procedure (Figure 1a). An example is provided to illustrate the learning process and cognitive strategies employed by the fish, such as motor response (turning right or left toward the reward's location) versus beaconing strategy (associating a visual landmark with the reward's location), and which strategy is prioritized when cues are set into conflict. In this short

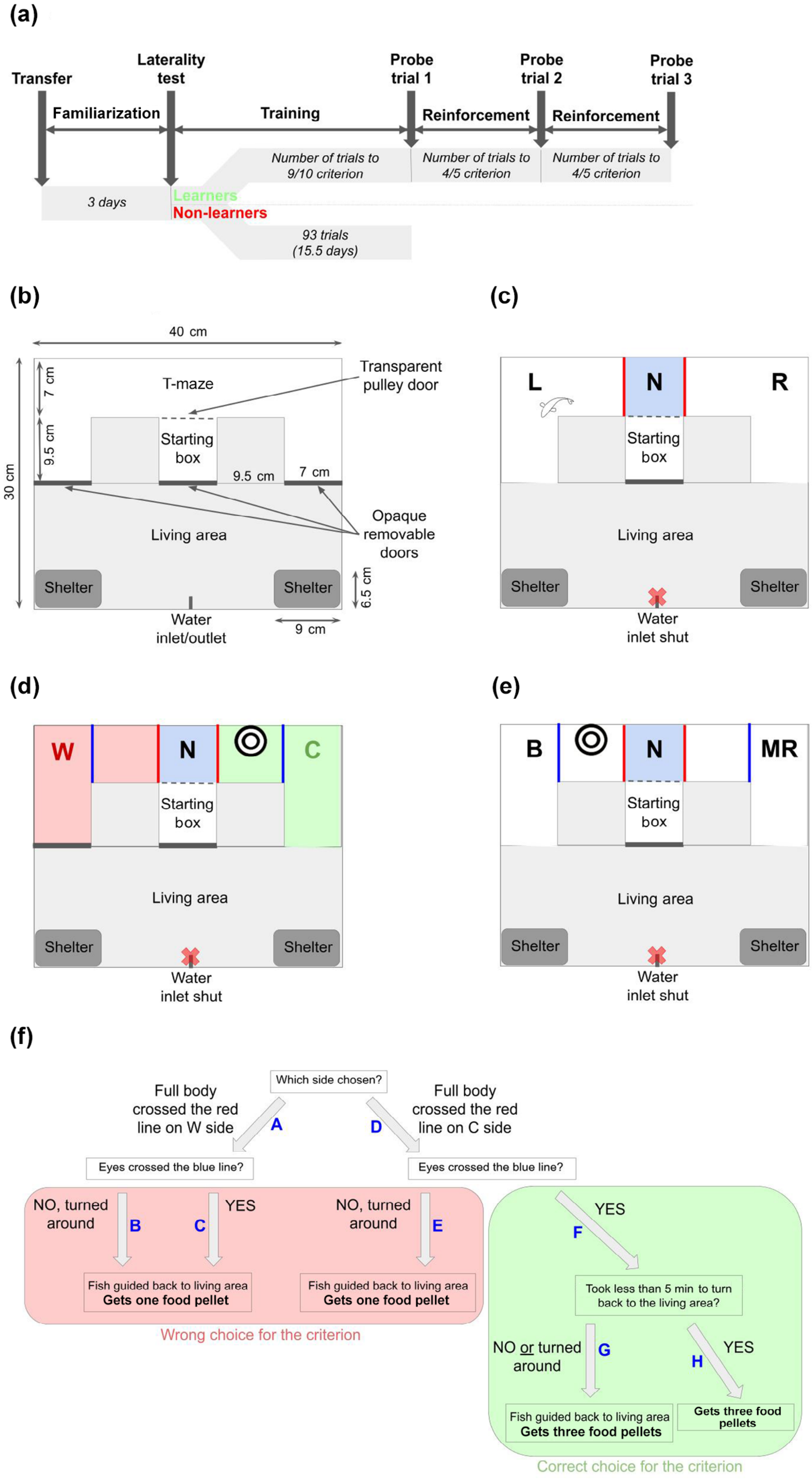

FIGURE 1 Legend on next page.

communication, our goal is to highlight management aspects of the design to increase fish engagement with the task, rather than describe the cognitive tests in detail. This may inspire other experimenters facing similar challenges.

This study was approved through an animal care permit (MAST – #2019-01.-09, Ref.190564). The cognitive setup included an opaque white-walled arena with strict axial symmetry, featuring a living area for the subject to reside in individually throughout the experiment. This area was connected to a T-maze's starting box via a removable opaque door system, with the extremity of both arms of the T-maze leading back to the living area through the same door system (Figure 1b). This design eliminates the stress associated with repeatedly capturing subjects from their home tanks (several times a day over prolonged periods) and transporting them to test arenas and back. It minimizes manipulations and reduces stress induced by netting and air exposure, as fish are gently guided by the experimenter from their living area to the adjacent starting box of the T-maze at the beginning of a trial, after which they can swim back on their own at the end of the trial.

The arena was set on a backlight table (Noldus) to ensure even lighting from below (12/12 light–dark cycle, 680 Lx) and was enclosed in a wooden box lined with black plastic sheets, referred to as the black box. A hole in the centre of the box roof allowed for a video camera to record trials from above, minimizing external disturbance by enabling remote observation. A panel of the box was removable to access the testing part of the setup. The black box also prevented fish from perceiving external visual cues during testing. The entire setup was installed in a dedicated room with no other source of lighting than the backlight table, in an unoccupied wing of the laboratory where only cognitive experimenters had access, ensuring absolute tranquility for the subjects throughout the experiment. In this experiment, we trained fish to solve a T-maze. Fish were rewarded with different types of positive stimuli to account for individual differences in motivation (Van Der Harst et al., 2003; Wood et al., 2011). First, the living area was designed to encourage the fish to view it as a reward: the floor of the living area was lined with a black plastic sheet to reduce light exposure and two shelters were provided (Figure 1b). Lower light intensity and the ability to shelter have been used as rewards or been beneficial in other species (Hossain et al., 1998; Stanbrook et al., 2020) and were confirmed as suitable rewards for Arctic charr in a pilot study, which showed that the fish spent the majority of their time in the shelter or in the darker area (unpublished data). When the fish returned to the living area at the end of the trial, they were immediately given food (Figure 1f) because they were not fed outside of testing periods to prevent overfeeding and

FIGURE 1 (a) Sequence of the different phases of the cognitive test (bold) with their durations (italics within corresponding gray bands). (b–e) The different configurations of the arena according to the testing phase, seen from above (surrounding black box not represented). The living area's darker shade represents its lower light exposure. Thick black lines, all opaque removable doors separating the living area and the T-maze; dotted lines, remotely pulley-operated transparent door; red and blue thick lines, virtual limits to be crossed by the fish to make and confirm its choices. Red crosses denote when the water inflow is shut. These figures give the example of a fish being trained to go on the right (all descriptions would be mirrored for a fish trained to go on the left). (b) Layout and measurements of the arena. (c) Laterality test: both exit doors leading back to the living area (left and right arms) were opened. In this example, when leaving the neutral zone of the maze (N, blue area), the fish first crossed the virtual red line with its whole body on the left side (L, left arm) as opposed to the right side (R, right arm). The left is hence considered its side-turning preference. (d) Training and reinforcement: In this same example, the fish had to learn to return to the living area on the opposite side of its side-turning preference, i.e., on the right. A target-shaped landmark was hence placed on the right side in front of the starting box, indicating the correct arm (C, green area) for which the removable door was withdrawn, allowing direct access to the living area. On the contrary, the removable door at the end of the wrong arm (W, red area) was left in place, forming a cul-de-sac. The fish was trained in this configuration (see (f)) until it would reach the learning criterion: Nine correct choices out of 10 consecutive trials during training. (e) Probe trial: spatial cues are set in conflict. The target-shaped landmark was placed in the opposite arm compared to the training configuration (on the left in this example). Removable doors at the end of both arms were withdrawn. The fish was allowed to freely navigate within the maze until its eyes would cross a blue line on either side (choice made). In this conflicting configuration, if the fish chose the side of the landmark, it learnt to associate the landmark with the reward during training, i.e., beaconing strategy (B, left arm). If the fish chose the previously rewarded arm, it learnt to associate a particular turning direction with the reward during training, i.e., motor response strategy (MR, right arm). (f) Decision tree describing the course of actions after a fish chose a side in the T-maze during training and reinforcements. Red and blue lines, W and C sides mentioned, respectively, refer to virtual lines and zones represented in (d). The fish was considered to have made a choice when its full body crossed a virtual red line. When a fish chose either the wrong (path A) or the correct side (path D), it always had the opportunity to confirm its choice (eyes crossing the subsequent blue line) and realize whether or not its choice led it back to the rewards, i.e., an open door to the living area on the correct side (path D–F) vs. a cul-de-sac on the wrong side (path A–C). However, the subject was not allowed to go backwards, hence if the fish turned around, that is, eyes crossing back the previous line (red line for paths A–B and D–E, or blue line for path D–F–G), the trial ended and the fish was guided back to the living area through the correct arm. All paths departing from path A were considered as wrong choices (unrewarded side). Path A–C was considered a confirmed wrong choice, which would end the trial and the fish would be guided back to the living area through the correct arm. Path D–E was considered a wrong choice as well because the fish did not confirm it by crossing the subsequent blue line with its eyes and turned around instead, which would end the trial and the fish would be guided back to the living area through the correct arm. In these cases (wrong choice, red box), the fish was fed only one food pellet (minimum food reward to make fish associate going back to the living area and access to a food reward). A choice was considered correct only when the fish confirmed its choice on the correct side by crossing the subsequent blue line with its eyes and noticed the open door (path D–F). From there, the fish was given 5 min to go back to the living area on its own (path D–F–H). If the fish turned around (eyes crossing back the blue line) or took more than 5 min (path D–F–G), the trial was ended and the fish was guided back to the living area through the correct arm. In both cases (correct choice, green box), the fish was given the maximum food reward of three pellets.

maintain motivation for the tasks. This served as an incentive to quickly return to the living area. As food was absent from the arena during testing, the subjects could not use olfactory cues from food to navigate the maze. When the fish made an incorrect choice in the maze, a slightly aversive reinforcement was applied by guiding it back to the living area through the correct arm with a plastic plate made from the same material used for the arena structure (see Figure 1f) to encourage the fish to learn how to exit the maze independently and quickly toward the safe living area in subsequent trials. The experiment was divided into six different phases: transfer, familiarization, laterality test, training, probe, and reinforcement (Figure 1a).

1 | TRANSFER AND FAMILIARIZATION PHASES

Transfer and familiarization phases: When a subject was to be introduced into the arena, to minimize the stress potentially caused by the transfer, it was gently netted from its home tank and anesthetized in a 200 ppm 2-phenoxyethanol solution at the home tank's temperature. It was weighed, measured (mean ± standard deviation 2.2 ± 0.7 g, total length 6.5 ± 0.7 cm, $N = 145$), and transported in the anesthetic solution to the cognitive testing room. Afterwards, the subject was immersed in water devoid of anesthetics in a bain-marie container (same temperature) which was placed inside the living area. This procedure avoids thermal shock by progressively equalizing the temperature inside the container to the temperature of the arena. After a 1-h recovery, the fish was released from the bain-marie directly into the living area without exposure to air for an additional acclimation hour. It was then fed, initiating the familiarization phase, which lasted 3 days, including the transfer day (Figure 1a). During this phase, the fish were fed three times a day and were left undisturbed otherwise.

All subsequent phases entail cognitive testing of the subject in the T-maze. Each trial began by gently guiding the fish to the starting box, followed by a 2-min acclimation period. The transparent door of the starting box was then lifted using a pulley system from outside the black box, ensuring no disruptive interaction with the subject during the trial. The black box was opened only to prepare for a trial or to conclude it. It was kept closed both during the trials while the fish was tested and outside of testing hours in its living area. To avoid overtesting the fish, only two testing sessions of three trials each were performed per day, with a minimum of 30 min of reward-time in the living area between trials and 1.5 h between sessions.

2 | LATERALITY TEST AND MAZE DISCOVERY PHASE

Just before the last feeding on the final familiarization day, the subject underwent a laterality test (Figure 1c), which enabled it to explore the maze for the first time and discover that an exit leading to the safe living area was available. The test also assessed the subject's side-turning preference, indicating a behavioral laterality (Bisazza & Brown, 2011). This one-trial laterality test has been effectively employed in other species (in cuttlefish: Alves et al., 2007; in rats: Andrade et al., 2001) and was validated as a reliable method for assessing lateralization in this species by a pilot study showing that the first trial was representative of the side-turning preference observed over 10 consecutive trials (unpublished data). The subject was free to explore the maze ad libitum until it returned to the living area on its own. The experimenter would then close the opaque doors of the extremity of the two arms of the maze and feed the fish for the last time of the day, for the subject to associate returning to the living area with food reinforcement.

3 | TRAINING PHASE

The phase aimed to train subjects to associate the arm opposite to their side-turning preference with positive rewards (Figure 1d). Subjects that did not leave the starting box within 20 min were deemed non-responsive and the trial was discounted. Non-responsive fish were returned to their living area without feeding. A fish failing to exit the starting box in six out of nine consecutive trials was considered unresponsive to the cognitive test, removed from the study, and replaced by another individual. If the fish made a choice (venturing in the left or right arm), the course of action would follow the decision tree presented in detail (Figure 1f). Each subject was trained as described until a learning criterion of nine correct choices out of 10 consecutive trials was reached. If a subject showed no sign of learning after 93 training trials (31 sessions), it was considered a non-learner, removed from the arena, and replaced by another.

4 | PROBE AND REINFORCEMENT PHASES

This part consisted of three probe trials (to confirm the strategy prioritization; Figure 1e), separated by reinforcement phases (regular training trials; Figure 1d) to reinforce learning until a learning criterion of four correct choices out of five consecutive trials was reached. During each probe trial, the intramaze landmark was displaced in the opposite arm to determine the strategy used to solve the maze (motor response versus beaconing). After returning to the living area, the experimenter fed the fish three pellets to reinforce learning, as there are no incorrect choices in probe trials.

Out of the 145 fish tested, 105 reached the 9/10 training learning criterion (72%), 26 were non-learners (18%), and 14 were removed (10%). Out of the 105 learners, two did not reach the first 4/5 reinforcement criterion and one did not reach the second. Out of 310 probe trials, 286 turned out to be motor responses (92%).

The presented approach was successfully used to train this fish species to turn to the correct side of the T-maze to be rewarded with access to their attractive living area. This is the result of several months of unsuccessful pretests where the fish were primarily transported several times a day from their home tank to the T-maze. We therefore intended with this paper to help researchers aiming to investigate spatial cognition in a previously unstudied fish species to avoid numerous biases induced by commonly used cognitive test procedures. The suggested method minimized the number of untrainable and non-responding fish. Fish never attempting the task have been shown to display the lowest activity, highest stress levels, and little to no motivation for the reward (Wood et al., 2011). We aimed to lower the level of stress of the tested fish and minimize interaction with them at every step of the cognitive tests. In the vast majority of cognitive tests on fish, food rewards are the primary incentive used and have, in most cases, proven successful (e.g., Baratti et al., 2022; Jones et al., 2023; Rodríguez et al., 2007). Food attractiveness and satiation level can vary among species, leading to inconsistent feeding and low motivation for cognitive tasks as a consequence (Daggett et al., 2019). This was evident in the targeted species, which did not show a clear and consistent motivation for the food reward. To overcome this issue, we used a combination of ecologically meaningful reinforcements in addition to the food reward, namely reduced luminosity and availability of shelters. This is in accordance with the results on zebrafish (*Danio rerio*) showing sustainable motivation when social stimuli and food are mixed (Daggett et al., 2019). These combined types of rewards also controlled for potential individual differences in motivation for a single type of reward (Van Der Harst et al., 2003; Wood et al., 2011).

This procedure appears efficient to determine which strategy was used by fish to solve the task. It could be adapted in the future to disentangle whether fish have learnt the two strategies in parallel and have prioritized one of them when information was conflicting, or whether only one type of information was learnt (motor response or beaconing).

AUTHOR CONTRIBUTIONS

M.D.: Conceptualization, methodology, investigation, data curation, formal analysis, original draft writing, funding acquisition. L.S. and M.C.: Methodology, investigation, review and editing. C.J.-A.: Conceptualization, review and editing. D.B.: Conceptualization, original draft writing, funding acquisition, project administration, supervision.

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Paper IV

Manuscript

Cognition-personality syndrome and underlying neural mechanisms shaped by eco-evolutionary background and developmental plasticity in response to environmental complexity in the Arctic charr

Authors

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Abstract

A growing body of evidence suggests that cognitive abilities and personality traits form a syndrome, but nothing is known about the proximate and ultimate mechanisms behind such consistent correlations. In this study, we explored the eco-evolutionary processes and developmental cues shaping spatial cognition, links of the latter with boldness personality trait, and underlying neural mechanisms. We used juveniles of five morphs of Arctic charr (*Salvelinus alpinus*) ranging along ecological and genetic gradients of divergence from a common ancestor. They were raised in common gardens split in enriched *vs.* plain treatments manipulating environmental structural complexity. In each group, we characterized individual boldness personality trait and spatial cognitive abilities, and subsequently quantified brain regional gene expression. We confirm the existence of a cognition-personality syndrome in this species, for which dopaminergic pathways and serine/threonine protein phosphatase 1 (*pp1*) genes might play a pivotal role. We provide the first empirical support for the co-evolution between cognition and personality, as we found that boldness increases while spatial learning speed and the plasticity of the syndrome in response to structural complexity decrease with divergence. All in all, spatial cognition, boldness personality trait, the syndrome they form together, and their underlying neural mechanisms are essentially dependent on the morph, the treatment, the morph-by-treatment interaction, the spatial cognitive ability, and the scale (global or individual) considered. This implies extremely intricate eco-evo-devo interactions governing their formation, in favour of a case-by-case model to explain their emergence.

Introduction

Spatial cognition involves the processes by which an organism perceives, interprets, transforms, mentally represents, stores and reuses information about spatial properties of its environment, such as distances, orientation and location (Waller & Nadel, 2013). All animals rely on spatial cognitive abilities to find food, locate mates, avoid predators, retrieve the nest, and in striking cases, migrate back and forth over worldwide distances. Therefore, spatial cognitive traits have crucial impacts on an individual's fitness, and are thus subject to natural selection (Morand-Ferron, 2017; Morand-Ferron et al., 2016). Cognitive traits are also subject to trade-offs due to the metabolic costs required for the development and maintenance of the necessary neural machinery (Dukas, 1999; Mery & Kawecki, 2003). This can lead to between- and within-species differences in cognitive styles, *i.e.*, the ways individuals consistently display differences in how they acquire, process, store and use information (Sih & Del Giudice, 2012). For example, differences in the rapidity and accuracy with which certain types of information are learned, or preferences in relying on new rather than past information for problem-solving. Indeed, spatial cognitive styles in each population within a species have been shown to be locally adapted to particular ecological conditions (Mettke-Hofmann, 2014; Rowe & Healy, 2014). For instance, when comparing chickadees (*Poecile atricapillus*) originating from Alaska and Kansas and raised in common gardens, Kansas birds had a lower number of hippocampal neurons and less accurate spatial memory compared to Alaska birds, where winter conditions are harsher and the retrieval of hoarded food is critical for survival (Pravosudov & Roth, 2013).

Animal personality is defined as among-individual differences in behaviour that remain consistent across time and/or contexts (Réale et al., 2007; Sih et al., 2004). The boldness-shyness continuum is the most studied animal personality trait (Conrad et al., 2011), boldness being defined as the individual propensity to take risks (D. S. Wilson et al., 1994). Cognition and personality are thought to be intrinsically interdependent (Lucon-Xiccato & Bisazza, 2017; Morand-Ferron et al., 2016) and could be so closely correlated that they might be coevolving Carere & Locurto, 2011). Within the last decade, a potential correlation between cognitive styles and animal personality has garnered scientific attention: this syndrome suggests that a speed-accuracy trade-off leads more "hasty" and "impulsive" individuals (bolder, more active, and more aggressive) to learn or make decisions faster but with lower accuracy than "careful" subjects (Chittka et al., 2009; Mettke-Hofmann, 2014; Sih & Del Giudice, 2012).

The relationship between personality traits and spatial behaviours (such as movement, dispersal, and migration patterns) is progressively galvanizing research in ecology (e.g., Beukeboom, 2023). For example, it has been shown in European hares (*Lepus europeaus*, Stiegler et al., 2022) and in bank voles (*Myodes glareolus*, Schirmer et al., 2019) that shy and bold individuals differed in their space use and home ranges. Stuber and colleagues (2022) recently set forth that spatial behaviours can exhibit a high among-individual consistency in animals, leading the authors to coin the term "spatial personality". Since spatial behaviours inherently rely on spatial cognitive abilities, this concept also implies a link between spatial cognition and personality. More specifically, given the mentioned examples linking boldness and movement patterns, correlation between boldness personality trait and spatial cognitive performances emerge with eco-evolutionary interests. To our knowledge, spatial cognition-boldness correlations have so far been investigated in only a handful of empirical studies per taxonomic groups: eightky in rodents (Brust & Guenther, 2015, 2017; Delacoux & Guenther, 2023; Guenther et al., 2014; Guenther & Brust, 2017; Kazlauckas et al., 2005; Mazza et al., 2019; Schuster et al., 2017); seven in birds (Bousquet et al., 2015; Chen et al., 2022; Garnham et al., 2019; Gibelli & Dubois, 2017; Medina-García et al., 2017; Titulaer et al., 2012; Watrobska et al., 2023); ten in fishes (Bannier et al., 2017; Bensky & Bell, 2020; Daniel & Bhat, 2020, 2023; Gibelli et al., 2019; Kareklas et al., 2017, 2018; Liu et al., 2022; Sommer-Trembo & Plath, 2018; S. L. White et al., 2017), three in reptiles (Carazo et al., 2014; De Meester et al., 2022; Goulet et al., 2018); and one in insects (Doria et al., 2019). Moreover, these studies provided contradictory results, reporting positive, negative, or no spatial cognition-boldness correlations. These inconsistencies are probably due to intrinsic inter- and intra-specific differences in biology, variations in cognitive abilities considered across studies, but also most likely to wide differences in the methodologies used to measure both boldness and spatial cognitive traits (Gibelli et al., 2019). As a result, a clear need for harmonisation and standardisation to allow comparisons is highlighted. All in all, this makes spatial cognitive styles-boldness a particularly promising syndrome to explore (Dougherty & Guillette, 2018). Now that such syndromes have been brought to light, attention should shift a step further, to investigate the proximate and ultimate mechanisms shaping their development. Indeed, the last two decades have seen the fields of animal personality and cognitive ecology flourish. Yet, despite the acknowledged implication of behaviour in diversification processes and speciation (Duckworth, 2009; Snell-Rood, 2013), there is a lack of clear empirical evidence about the role of cognition and personality in the picture of divergence processes. Scientific knowledge remains incomplete regarding how specific cognitive abilities and their correlation with personality traits might differ between and within species, and what evolutionary, ecological and physiological factors are involved in such differences.

Structural complexity–increased structural heterogeneity of the environment (Godfrey-Smith, 2002)–could be a key environmental factor governing the evolution and development of spatial cognition and its interplay with boldness personality trait. Environmental complexity in nature, or environmental enrichment in captivity, have been shown to influence a wide variety of behaviours, personality traits and cognitive abilities in animals (de Azevedo et al., 2023; Mikheev et al., 2010; Shumway, 2008; G. E. White & Brown, 2014; Zentall, 2021). For example, environmental complexity increased boldness and exploration in jumping spiders (Liedtke et al., 2015), or decreased boldness and exploration in mosquitofish (Xu et al., 2021). Because a structurally complex environment requires encoding and processing more spatial information, such habitats might select for higher spatial cognitive performances (environmental complexity thesis from Godfrey-Smith, 2002; Mettke-Hofmann, 2014). Differences in habitat complexity can also favour equal spatial learning abilities but differential cognitive navigation strategy use. For instance, three-spined stickleback fish (*Gasterosteus aculeatus*) from Putah creek, characterized by low water movement and denser vegetation, rely preferably on visual cues to locate food rewards, while sticklebacks from Navarro River, where intense water flow leaves no reliable visual, rely preferably on self-centred side-turning orientation (Bensky & Bell, 2018).

In line with Tinbergen's questions for thorough study of behaviours (Tinbergen, 1963), eco-evolutionary factors influencing the development of spatial cognition and its links with personality should be investigated alongside their underlying physiological processes (Mettke-Hofmann, 2014). The brain is highly responsive to all kinds of environmental stimuli experienced by the individual (Kolb, 2018), and these plastic alterations in the brain machinery could explain consistent differences in cognitive abilities and personality traits among-individuals. To study the neural mechanisms involved in cognition-personality syndromes, fishes are particularly interesting models: contrary to mammals showing neurogenesis mostly limited to the juvenile developmental period, fishes experience neurogenesis throughout their entire lifetime (Øverli & Sørensen, 2016). Consequently, differences in brain organisation and functioning might reflect lifelong neural plasticity in response to particular experiences and ecological conditions (Kotrschal et al., 1998), and correlate with differences in spatial cognitive styles and their link with personality. Focusing on neural plasticity in brain centres controlling spatial cognition bears obvious interest for the study of neural mechanisms governing spatial cognition-personality syndromes. Firstly, the telencephalon is one of the major neurogenetic zone in fishes brains and is involved in more complex spatial information processing such as allocentric map-like representation of space (Calvo & Schluessel, 2021; Ebbesson & Braithwaite, 2012; Øverli & Sørensen, 2016; Rodríguez et al., 2021). Secondly, the cerebellum is involved in simpler spatial information processes such as associative spatial learning and the use of egocentric navigation strategies (Calvo & Schluessel, 2021; Salas et al., 2017; Thompson & Steinmetz, 2009), such as beaconing – *i.e.*, using body position relative to landmarks to locate a goal – or motors responses – *i.e.*, performing sequences of movements to reach a goal (Arleo & Rondi-Reig, 2007; Darmaillacq et al., 2018). Finally, the optic tectum is also an important actor processing beaconing and motor response navigation strategies, as it is the region responsible for coordinating vision, postures, and movements, and hence an essential centre for generating egocentrically referenced actions in space (Rodríguez et al., 2021).

One can explore neural plasticity in response to structural complexity underlying spatial cognition-boldness syndromes by measuring gene expression patterns of markers of neurogenesis and neuronal development and plasticity (Sørensen et al., 2013). In this study, we will investigate different markers linked with personality and cognition (*bdnf*, *c-fos*, *egr1*, *neurod*, *pcna*, *crebbp*, *pp1*, *reelin*, *gr*, *mr*, *dbh*, *mao*), whose functions are presented in Table 1.

The expression patterns of those genes of interest and the related proteins levels were shown to be linked with cognitive abilities and personality traits, and influenced by environmental complexity/enrichment. Black rockfish (*Sebastes schlegelii*) reared in structurally complex conditions demonstrated faster spatial learning and higher BDNF protein levels in the telencephalon when compared to fish reared in barren conditions (Shen et al., 2023). In zebrafish, structural enrichment of the tanks induced an increase in the cerebellum size and in the number of c-Fos-marked cerebellar neurons (Flores-Prieto et al., 2024). In guppies (*Poecilia reticulata*), an increased cerebral expression of *neurod* was linked to bolder individuals (Pauli et al., 2023). In African cichlid fish (*Astatotilapia burtoni*) trained in spatial tasks, transcription of *bdnf* and *egr1* (but not *c-fos*) is increased in the telencephalon according to learning capacity (Wood et al., 2011). Atlantic salmon (*Salmo salar*) reared in enriched conditions displayed higher levels of *neurod* mRNA in the telencephalon, as well as faster spatial learning (Salvanes et al., 2013). Other epigenetic markers were also described as associated with cognitive abilities (Day & Sweatt, 2011), such as *crebbp*, (Alarcón et al., 2004; Guan et al., 2002), or memory-associated genes epigenetically modified in response to experience (Miller & Sweatt, 2007): *reelin*, a gene promoting memory, and the memory suppressor *pp1*. During context learning in rats, DNA methylation is decreased for *reelin* and increased for *pp1* (Miller & Sweatt, 2007). Particularly, PP1 entails several catalytic subunits (alpha, beta and gamma), and higher cerebral expression of alpha and beta subunits is thought to lead to deficient long-term memory in mandarin fish (*Siniperca chuatsi*, Cheng et al., 2015). Genes such as *gr* and *mr*, as well as genes such as *mao* and *dbh* encoding enzymes involved in monoamines neurotransmitters metabolism (serotonin, dopamine, noradrenaline and adrenaline), are major actors implicated in stress response and have also been shown to play a role in behaviour regulation, memory, and learning processes (Harley, 2004; Herrero et al., 2006; Luchiari & Maximino, 2023; Martorell-Ribera et al., 2020; Oitzl et al., 2010; Øverli et al., 2007; Sørensen et al., 2013). Brain dopaminergic activity has been shown to strongly correlate positively with spatial cognitive performances in the several species of teleosts (Calvo et al., 2023; Eddins et al., 2009; Levin, 2011; Messias et al., 2016; Naderi, Jamwal, Chivers, et al., 2016; Naderi, Jamwal, Ferrari, et al., 2016). In strains of rainbow trout selected for reactive and proactive coping styles, assimilable to shy and bold personalities respectively, reactive individuals displayed higher levels of monoamines and lower levels of monoamine metabolites in several brain regions (notably in the telencephalon and optic tectum) after exposure to stress compared to bold, presumably in part due to higher MAO activity (Øverli et al., 2001). Alfonso and colleagues demonstrated that bold European sea bass (*Dicentrarchus labrax*) displayed lower cerebral noradrenaline levels and lower brain plasticity, as bolder fish express less *mr*, *gr*, *egr1* and *neurod* in the whole brain compared to shy fish (Alfonso et al., 2019; see also Sadoul et al., 2022 regarding egr1 linked with boldness in this species). On the contrary, in the same species under chronic stress conditions, *neurod* was upregulated in bold compared to shy fish, but there were no differences in terms of *egr1*, *mr* and *gr* (Dellinger, 2019). In the olive flounder (*Paralichthyus olivaceus*), reactive (shy) fish displayed higher levels of *gr* and serotonin receptors mRNA in several parts of their central nervous system, notably in the telencephalon (and in the optic tectum in the case of *gr*), in comparison to proactive (bold) or intermediate fish (Rupia et al., 2023). A recent study (Cardona et al., 2022) shows that the transcription of *pcna*, *creb* and two genes related to monoamine pathways (but not of *bdnf*, *c-fos*, *egr1* and *neurod*) was increased in the forebrain of rainbow trout (*Oncorhynchus mykiss*) reared in structurally enriched conditions when compared to trout reared in barren tanks. All in all, we exposed here that there is relatively little knowledge in teleosts about the neural processes involved in personality and spatial cognition, let alone relative to their plasticity in response to environmental complexity, and the limited literature on this subject often presents contradicting results. When considering the results obtained by Rey and her team (2021) through comparative transcriptomics studies showing that gene expression patterns underpinning personality seem to be essentially species-specific in fishes, there is an exciting array of uncharted underlying neural processes to elucidate regarding fishes' personality, spatial cognition, and their interplay.

To our knowledge, no study so far has simultaneously investigated evolutionary, ecological, developmental, and neural aspects of spatial cognition and the syndrome it forms with personality, which is yet essential for a thorough understanding of the phenomenon. The goal of the present study was hence to implement such a multifactorial empirical investigation. For this, the Icelandic Arctic charr (*Salvelinus alpinus*), a teleost fish from the Salmonids family, is a particularly relevant model. All Arctic charr in Iceland have been shown to descend from a single ancestral colonising Lineage (Brunner et al., 2001; A. J. Wilson et al., 2004), putatively represented contemporarily by extant anadromous populations, such as the monomorphic anadromous population in Fljótaá River (AN) retaining higher genetic diversity (Brachmann et al., 2021). Isolated populations diverged from the common ancestor after being landlocked in separated freshwater lakes at the end of the last glaciation, further diverging *in situ* to form up to four distinct sympatric morphs within a given lake. Although broader sampling and more genetic data are needed to estimate the origin and history of each population, the degree of divergence from the ancestral form is assumed to depend on the age of the lakes' formation (Brachmann et al., 2021, 2022; Dellinger et al., 2023). This species consequently displays an evolutionary gradient of ecological, morphological and genetic divergence between and within populations across Iceland (Brachmann et al., 2021; Gíslason et al., 1999). In this study, we used five morphs of Arctic charr ranging along a gradient of evolutionary divergence from the common ancestor: the monomorphic anadromous population from Fljótaá River (AN) as the putative ancestral proxy, sympatric Silver (VS) and Brown (VB) morphs from Lake Vatnshlíðarvatn as moderately diverged from the ancestor (landlocked 6000–8000 years ago (Jónsson & Skúlason, 2000)), and sympatric Large Benthic (LB) and Planktivorous (PL) morphs from Lake Þingvallavatn as highly diverged forms (landlocked approximately 10 000 years ago (Saemundsson, 1992)). This represents an elegant model to study how different degrees of divergence might impact spatial cognition and its links with personality.

Working with sympatric morphs, hence genetically closely related, also allows to investigate how spatial cognition-boldness syndromes are influenced by differences in ecological niches within pairs of morphs. In Icelandic lakes, Arctic charr morphs tend to be either benthic, *i.e.*, feeding on the bottom with stocky dark-brown bodies and subterminal lower jaw seemingly adapted to benthic prey exploitation, or pelagic, *i.e.*, feeding in the water column with fusiform silvery bodies and pointy snouts with terminal mouth seemingly adapted to plankton filtration or even to piscivory (Gíslason et al., 1999; Malmquist et al., 1992; Snorrason et al., 1994). Consequently, even though they dwell in the same lake, sympatric morphs ultimately thrive in fundamentally different environments in terms of habitat structural complexity. In the large and deep Lake Þingvallavatn (84km^2, 34m mean depth, 114m max. depth), morphs LB (benthic) and PL (pelagic) are an extreme example of sympatric divergence, with obvious morphological and coloration differences, distinct specialized diets and spatio-temporally separated reproduction (Jonsson et al., 1988; Malmquist et al., 1992; Sandlund et al., 1987, 1992; Skúlason et al., 1989; Snorrason et al., 1994). The LB experience complex features amidst volcanic boulders beds, crevasses, and algae characterising the benthic zone of the lake, while the PL live in open waters where visual landmarks and stimuli are scarce (Jónasson, 1992; Malmquist et al., 2000). In Lake Vatnshlíðarvatn, a small, shallow (0,7km^2, 2-3 mean depth, 6m max. depth), and structurally simple lake composed in majority of a muddy bottom with gravels close to the shores, sympatric morphs VB and the VS display more subtle morphological and ecological differences (Jónsson & Skúlason, 2000): while VB are closer to the previous description of a benthic morph and VS are closer to the description of a pelagic morph, morphological divergence is not as pronounced as between LB and PL (Brachmann et al., 2021). VB specialise on one prey-type and spend their whole life cycle within the lake, reproducing in the littoral area. On the contrary, VS are generalists that alternate prey according to seasonal abundance, their diet overlapping the VB's in the summer, and migrate to adjacent streams to spawn (Brachmann et al., 2021; Jónsson & Skúlason, 2000).

In the present study, we investigated several hypotheses:

(H1) Cognitive styles and personality traits form a syndrome. This would be observed through correlations between individual spatial cognitive abilities and boldness personality trait. If there is an actual speed-accuracy trade-off (Sih & Del Giudice, 2012), we should expect these correlations to be negative.

(H2) Eco-evolutionary history influences (i) cognitive abilities and (ii) their correlation with personality traits. This would manifest through differences between morphs in terms of (i) spatial cognitive abilities and (ii) direction and/or amplitude of covariation between boldness and cognitive abilities.

(H3) Early environmental factors trigger differential development of (i) cognitive abilities and (ii) their correlation with personality traits (developmental plasticity, the property of a given genotype to produce different phenotypes depending on the environmental conditions under which development takes place (Lafuente & Beldade, 2019)). This would manifest through differences between individuals raised in plain *versus* structurally complex environments, either overall or within morphs, in terms of (i) spatial cognitive abilities and (ii) direction and/or amplitude of covariation between boldness and cognitive abilities.

According to the environmental complexity thesis (Godfrey-Smith, 2002), we specifically expect structural complexity in the natural habitat (H2) and in early developmental treatment conditions (H3) to enhance spatial cognitive abilities.

(H4) Mechanisms underlying differential development across eco-evolutionary factors of cognitive abilities, personality, and their interactions, involve neural plasticity. This would be highlighted through differential gene expression modulated by boldness, cognitive abilities, and their interactions with the different morphs and structural complexity treatments. Generally, we expect neurogenesis and neural plasticity to be correlated positively with higher spatial cognitive performances, and negatively with boldness.

Material and Methods

Biological Material and Housing Conditions

This study uses a subset of the same individuals screened for boldness personality trait in Dellinger, Caperaa, et al. (n.d.), *i.e.*, offspring of the five wild-caught Arctic charr morphs described above (see also Benhaïm et al., 2023; Dellinger et al., 2023). The crossing and rearing designs for those fish are already described extensively in Dellinger, Caperaa, et al. (n.d.), so only brief descriptions will be reported here. Eggs from each morph were kept separate and raised in common garden. New-borns were collectively transferred into six 20-L cylindrical tanks per morph, at a density of approximately 275 individuals per tank, between two common garden treatments modulating environmental structural complexity (Philip et al., 2022): plain treatment (bare tank) versus enriched treatment (with five volcanic rocks at the bottom and a plastic plant in each tank, mimicking natural features of these morphs' habitats, and providing diverse textures, colours, and vertical and horizontal structural heterogeneity). This hence created 10 morph-by-treatment batches (5 morphs x 2 treatments), and each treatment had three replicate tanks per morph (VB morph however missing a plain tank, see details in Dellinger, Caperaa, et al. (n.d.)). At 320 to 327 days post hatching (dph), 55 fish per tank chosen at random were intraperitoneally identified with a PIT tag (Oregon RFID FDX-B skinny tag: 8 x 1.4 mm), and the density was reduced to 85 fish per tank. Fish were fed three times a day with commercial aquaculture pellets (BioMar INICO, granulometry adapted to the growth of the fish, reaching 1.1 mm by the time of the behavioural experiments).

Boldness Personality Trait Assessment

Again, the method to assess boldness personality trait in those fish was fully described in Dellinger, Caperaa, et al. (n.d.). Briefly, 15 to 18 days after tagging (334 to 341 dph), 52 fish per tank (N=1508 in total) were recorded in an Open Field Test (OFT) with shelter replicated seven days later, which confirmed boldness repeatability, *i.e.*, personality (see also Dellinger et al., 2023 and Philip et al., 2022). After acclimating for five minutes in the closed shelter, the shelter's door was lifted. The fish was then free to emerge and navigate the arena at will for 20 minutes, during which a total of 12 risk-taking behaviours were collected. Individual boldness scores were calculated for each OFT replication as coordinates on the first axis of a Principal Component Analysis (PCA) including all risk-taking behavioural variables, taking repeated measures into account (Dellinger, Caperaa, et al., n.d.; Dellinger et al., 2023).

Cognitive Test

We used the cognitive test setup and protocol for spatial learning described in Dellinger, Suret, et al. (n.d.), especially developed to minimise stress for the fish, which would start for a morph between 379 and 393 dph (see details in *Methods S1*). The objective was to test a minimum of four subjects per triplicate tank (that is, 12 fish per treatment per morph, so 24 fish per morph, N=120) that had previously been screened for boldness personality trait with the OFT. As the VB morph was missing a plain replicate tank, we tested 6 fish per remaining plain tank instead of 4 for this morph. We also tested additional subjects within morphs (N > 24) when time allowed it or when a fish did not respond to the cognitive test, in order to maximize our sample size. Hence, a total of N=145 fish were submitted to the cognitive test (see *Table S1* for the exact distribution per morph, treatment, and tank). The cognitive test, divided into six phases (transfer, familiarisation, laterality test, training, probes, reinforcements), as well as the experimental setup, are described in a previous article (Dellinger, Suret, et al., n.d.), and detailed protocol and manipulations can be found in *Methods S1*. Only a brief description will be exposed here.

Experimental Setup

The cognitive test setup, in a dedicated isolated room, consisted of six opaque arenas in which each subject would live individually for the entire experimental period. Each arena was surrounded by a rectangular wooden box lined with black plastic sheets (referred hereafter as black box), granting full tranquillity to the fish by preventing any external disturbance both during and outside of testing, and allowing no external visual cues usable by the test fish outside the arena. Each black box could fit a camera on top, video recording each trial from above and allowing remote monitoring. All arenas were placed on a backlight table (Noldus, The Netherlands) set on 680 Lux, providing homogeneous light from the underside from 07:00 to 19:00 (12/12 photoperiod). Arenas had a strict axial symmetry and consisted in a living area which had two shelters, a flow-through water system, softened light exposure, and a net sealing the top to prevent the salmonid's natural propensity to jump out. The living area was connected directly to a T-maze by a starting box thanks to a removable opaque door system. The starting box opened on the T-maze arms thanks to a transparent pulley-operated door, while both left and right arms of the T-maze departing from the starting box directly led back to the living area thanks to the same removable opaque door system. The reward for solving the maze was returning to the safe living area with its two shelters, lower light exposure, and food. These combined types of rewards controlled for potential individual differences in motivation for a single type of reward (Van Der Harst et al., 2003; Wood et al., 2011), are ecologically relevant to the species, and were confirmed suitable rewards as they significantly attracted the fish in a pilot study (unpublished data).

Transfer and Familiarisation Phases

Subjects were successively introduced in an arena after a previous fish had completed the cognitive task (see *Methods S1* for randomisation details). For that, the new subject was gently netted from its home tank, anaesthetized in a 200 ppm 2-phenoxyethanol solution, weighed and measured, transported in the anaesthetic solution to the testing room and put to recover in a bain-marie container inside the living area. After recovering for an hour, the fish was released directly into the living area for an additional acclimation hour, after which it was fed five food pellets, marking the start of the three-day (transfer included) familiarisation phase, during which the subject would get used to the new living conditions. The fish was fed five food pellets three times a day, and left completely undisturbed otherwise.

Laterality Test and Maze Discovery

Just before the last feeding of the last familiarisation day, the subject performed a laterality test which allowed it to discover the maze and its exits for the first time, but also allowing assessment of the subject's side-turning preference, *i.e.*, behavioural laterality (Bisazza & Brown, 2011). For this, the subject was gently guided to the starting box with a dip net, and both exit doors leading back to the living area (left and right arms) were opened. After two minutes acclimation, the transparent starting box door was lifted from outside the black box. The first side (left or right) chosen by the fish when leaving the starting box was recorded as its individual side-turning preference (Alves et al., 2007). This one-trial laterality test was validated as a reliable assessment of side preference in this species by a pilot study showing that the side chosen on a first trial was representative of the side preference observed over ten consecutive trials (unpublished data). The subject was free to explore the maze *ad libitum*, until it returned to the living area on its own. Upon its return, the fish was immediately fed its last five food pellets of the day, for it to associate going back to the living area with receiving food. The fish was then left alone with its rewards (food, shelters, softer light, and peace being home) fully undisturbed until the next morning when it would start the training phase. The rewarded arm (right or left) during the following training and reinforcement phases was determined for each subject as the opposite of its side-turning preference, to ensure the fish would actually learn a new path during training, and did not only rely on unlearnt side biases.

Training Phase

From this phase on and until the subject completed the test, the food rewards distributed after a trial were the only feedings dispensed, to avoid over-feeding and maintain motivation to participate in the tasks. The training phase was composed of two training sessions of three trials each per day every day. The goal of this phase was to train the subjects until they learn to associate the opposite of their side-turning preference with a reward. In the T-maze, a target-shaped visual intramaze landmark (eight black-and-white rings, 5 cm diameter) was placed against the wall in front of the starting box on the side indicating the correct arm, *i.e.*, the rewarded arm having the door open to the living area. To exit the maze, the fish could choose a side through a turn (left or right, motor response strategy) and/or by following the landmark (beaconing strategy). During a training trial, the subject was gently guided to the starting box thanks to a hand net and the door leading back to the living area on the rewarded side was opened. After a two-minute acclimation, the transparent door was lifted and the fish was given 20 minutes to leave the starting box. If it did not come out after 20 minutes, the fish was considered not having responded to the trial, and this trial did not count. It was led back to the living area through the start box door and not fed. If a fish would not come out of the starting box for six trials out of nine consecutive trials, this subject would be considered non-responding to the cognitive test and was evicted (see *Table S1* for the number of fish evicted per morph, treatment, and tank), subsequently replaced by a new fish from the same home tank. If the subject came out of the starting box before the 20 minutes, the fish was given 20 minutes to choose a side. If the fish stayed for more than 20 minutes without making a choice, it was guided back to the living area through the correct arm with a plastic plate made from the same material used for the arena structure. It was not fed, was considered having not responded to the trial which did not count. If the fish made a choice, the course of action would follow the decision tree presented in Dellinger, Suret, et al. (n.d.). Briefly, a choice would be considered correct only when the fish would first choose the correct side, and would then confirm its choice by reaching the end of the arm without backing up. All other behaviours were considered wrong choices (see details in *Methods S1*). Once the subject was back in the living area (either by itself or guided back through the correct arm with the plastic plate, see *Methods S1*), the experimenter closed the exit door and fed the fish three food pellets for a correct choice (maximum food reward) or one pellet for a wrong choice. This minimum food reward was distributed, despite a wrong choice, for the fish to understand that going back to the living area was associated with receiving food. This way, as the food reward was added only once the fish was back home, the subject could not rely on food olfactory cues to navigate in the maze. Similarly, water inflow was shut during trials, preventing the fish to rely on sensory inputs (sounds, vibrations or currents) from water flow to navigate back to the living area. Each subject was trained as described until it would reach a learning criterion of nine correct choices out of ten consecutive trials. If a subject would not show any sign of learning after 93 training trials (31 sessions), it would be considered a non-learner, would be removed from the arena for dissection (*Cf. infra*), and replaced by a new subject introduced in the arena the next morning.

Probes and Reinforcements

This part followed the achievement of the 9/10 training criterion for the learners and consisted of three probe trials (to confirm the strategy used), separated by reinforcement phases (regular training trials) to reinforce learning until a reinforcement criterion of four correct choices out of five consecutive trials was reached. A probe trial was conducted like a training trial, except the fish was left to navigate freely in the maze until it reached the end of an arm on either side recorded as the choice of the subject, opaque doors at the end of both arms were open, and the landmark was placed in the opposite arm compared to training. Consequently, the choice of the subjects indicated which navigation strategy—beaconing or motor response—they preferentially use to locate the reward when navigation cues are set into conflict: if the subject chose the usual side of training, it learnt to associate the rewarded arm with a particular turn, hence prioritising motor response strategy; if the subject chose the landmark side, that is, the opposite side of the usual training, it learnt to follow a landmark to locate the rewarded arm, hence prioritising beaconing strategy. Once the subject was back in the living area (either by itself or being guided back through its chosen arm, *Methods S1*), both opaque exit doors were closed and the fish was fed a full ration of three pellets to reinforce learning as there is no wrong choice during a probe trial.

Brain Dissection

Twenty minutes after completing the cognitive test (either after probe trial 3 for the learners or after the 93rd training trial for non-learners), the subject was gently netted from its living area, anaesthetized in a 200 ppm 2-phenoxyethanol solution, quickly transported in the anaesthetic solution to the dissection lab bench where it was weighed and measured, and directly euthanized in a 1000 ppm 2-phenoxyethanol solution. The brain was then dissected out of the skull and immersed in a tiny petri dish filled with 0.5mL of DNA/RNA Shield (Zymo Research), and the telencephalon, the cerebellum, and the optic tectum were taken apart. Each isolated brain region was placed in a separate Eppendorf tube containing 1.25mL of DNA/RNA Shield (Zymo Research), which were then conserved at -20°C until RNA extraction. The whole brain dissection process, from the moment the fish was immersed in the euthanasia solution to the last brain part being deposited in the DNA/RNA Shield (Zymo Research) storage Eppendorf, lasted a maximum of 30 minutes (mean ± SD: 16.8 ± 3.6 minutes), minimising natural *post-mortem* mRNA decay. Fin clips were also collected on each subject, placed in individual Eppendorf tubes filled with 1.5mL of 95% ethanol and conserved at -20°C until DNA extraction for sexing (see details in *Table S1* for samples included in these analyses).

Regional Brain Gene Expression Quantification

The development, optimization and validation of the primers and methods described hereafter are available in the *Methods S2*.

RNA Extraction from Brain Regions Tissues

We extracted RNA from each brain region tissues sample using Monarch total RNA miniprep kits (New England Biolabs) following the manufacturer's instructions. After thawing the samples tubes at room temperature, the tissues were pipetted out and transferred to a new Eppendorf tube containing 300 µL of 1X DNA/RNA Protection from the kit in which tissues were minced. In brief, the kit steps consisted in firstly removing genomic DNA through a purification column, and secondly purifying RNA in the obtained flow-through with another purification column targeting RNA. The RNA was then eluted from the column with 100 µL of RNase-free water. After extraction, RNA concentrations were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific), and extracted RNA samples were stored at -80°C.

Reverse Transcription of Extracted RNA to cDNA

The cDNA synthesis was performed thanks to High-Capacity cDNA Reverse Transcription kits (Thermo Fisher) following the manufacturer's instructions. The reverse transcription mixture for each sample contained 4.2 µL milliQ water, 2 µL of 10X RT Buffer, 0.8 µL of dNTP mix, 2 µL of 10X RT random primers, 1 µL of reverse transcriptase and 10 µL of RNA solution for a final volume of 20 µL. It was heated according to the following protocol to activate the enzyme: 10' at 25°C, 120' at 37°C and 5' at 85°C. All synthesized cDNA samples were stored at -20°C.

Relative Quantification of Gene Expression by Real-Time Quantitative PCR

The relative quantification of the expression of 18 genes of interest (*Table 1*, *Table S2*), related to neural plasticity and its epigenetics (*bdnf*, two paralogs of *c-fos*, *egr1*, *neurod1*, *neurod1-like*, *pcna*, *reelin*, *crebbp*, and five sequences related to the catalytic subunits of *pp1*), to stress regulation (*mr*, *gr*), and to monoamine metabolism (*dbh*, *mao*), was conducted by qPCR performed with a QuantStudio 3 Real-Time PCR System (Thermo Fisher) using Thermo Fisher Connect. The genes *actb* (beta-actin) and *ub2l3* (ubiquitin-conjugating enzyme E2 L3) were used as reference (Ahi et al., 2013, *Table S2*). Each 96-well qPCR plate would test 12 samples (one per column) for four genes in technical duplicates (hence two lines per gene): the two reference genes *actb* and *ub2l3*, and two target genes tested in pairs formed at random (*Table S2*). The qPCR for LB and AN morphs samples were run in 2021 and for VB, VS and PL morphs in 2022. All samples within a year were fully randomized across plates within a pair of target genes plates. Each qPCR well contained 500, 350 or 200 nM of forward and reverse primers depending on the optimal concentration for the given primer (*Table S2*), 6.25 µL of SYBR Green master mix and 1 µL of cDNA for a final volume of 12.5 µL. Plates were thermocycled with an initial denaturation step of 2 minutes at 50°C and 10 minutes at 95°C followed by 40 cycles of: 15'' at 95°C and 1' at 60°C, followed by the melt curve step of: 15'' at 95°C, 1' at 60°C and 15'' at 95°C. Plates fluorescence reads were retrieved on Thermo Fischer Connect, and the app automatically calculated a plate-specific detection threshold for each of the four genes, determining the quantification cycle (Cq) value for a given well. However, as our method for a target gene expression quantification was assessed relatively to reference genes within a plate, target and reference genes on a same plate had to be set at the same threshold to hold relevant comparison. Hence, for each plate, we manually calculated the geometric mean of the four thresholds given by the app and used the result as the plate's overall threshold. The Cq value for each well was then recovered and the relative expression was measured according to the following calculus (inspired by Matlosz et al. (2022), and adapted to our design according to Ruijter et al. (2021)):

$$Cq_{p,x} = \frac{Cq_{p,x,well1} + Cq_{p,x,well2}}{2}$$

$$Q_{p,x} = Eff_p^{\ Cq_{p,x}}$$

$$Q_{refs,x} = \sqrt[2]{Q_{actb,x} \times Q_{ub2l3,x}}$$

$$RQ_{p,x} = \frac{Q_{refs,x}}{Q_{p,x}}$$

$$RE_{p,x} = \frac{RQ_{p,x}}{RQ_{p,c}}$$

Where $Cq_{p,x}$ is the mean Cq of the sample x for primer p over the technical duplicates well1 and well2; $Q_{p,x}$ represents the initial quantity of cDNA quantified with the primer p in the sample x (special case for the reference genes); Eff_p is the efficiency of primer p; $RQ_{p,x}$ is the quantity of cDNA quantified with primer p in the sample x, relative to the reference genes quantity; and $RE_{p,x}$ is the expression of the gene corresponding to primer p in sample x, relative to the calibrator sample (same calibrator sample for all RE calculations and arbitrarily designated). If the relative expression (RE) is equal to 1, the gene is equally expressed in the sample x as in the calibrator; if the RE is superior (or inferior) to 1, the gene is upregulated (or downregulated) compared to the calibrator.

Sex determination

We extracted DNA from subjects' fin clip samples using NucleoMag Tissue kits for DNA purification from cells and tissue (Macherey-Nagel) following the manufacturer's instructions. For this, an entire pectoral fin was cut to shreds with dissection scissors in Proteinase K supplied with the kit and left to be digested overnight at 56°C. The kit's subsequent steps are based on DNA purification with magnetic beads. Extracted DNA samples were stored at -20°C until sex determination by PCR amplification of the male-specific *SdY* gene (Matlosz et al., 2022), using the *etbr2* gene as a positive control for each sample (*Table S2*). Each well contained 2.75 µL of milliQ water, 0.5 µL of forward and reverse *SdY* primers, 0.25 µL of forward and reverse *etbr2* primers, 6.25 µL of Quick-Load *Taq* 2X Master Mix (New England Biolabs) and 2 µL of DNA for a final volume of 12.5 µL and was thermocycled with an initial denaturation step of 3 minutes at 95°C followed by 40 cycles of: 45'' at 95°C, 45'' at 57°C and 1'45'' sec at 72°C, and 7' at 72°C. The PCR product for each well was subsequently revealed, thanks to a 1.5% agarose gel in TAE 1X and Ethidium Bromide run 28 minutes at 90 V, samples showing a band for *SdY* being males, or females of this band was absent.

Statistical Analyses

As primary analyses, we verified with χ^2-tests that the proportions of males and females tested, and of left and right side-turning preferences, were equal between morphs. Still using χ^2-tests, we tested hypotheses (H2.i) and (H3.i) by verifying whether the proportions of learners *versus* non-learners (used as an indicator of spatial learning capacity), and of motor response *versus* beaconing during each probe (used as an indicator of navigation strategy prioritised), depended on the morph, the treatment, the morph-by-treatment batch, the sex and the side-turning preference. All other statistical analyses were performed with R v. 4.3.0 software (R Core Team, 2018). We fitted different linear models with the brms package v. 2.19.0 (Bürkner, 2017) in the Bayesian language Stan (Carpenter et al., 2017), run for 10 000 iterations with a thinning of 1, all using a dataset including learners only (N = 102, *Table S1*).

Firstly, we simultaneously tested hypotheses (H1), (H2.i,ii) and (H3.i,ii), with a model (henceforth referred to as Model.0) predicting the number of trials necessary for each subject to reach the 9/10 learning criterion (trials to training criterion, TTC, recorded as a proxy for individual spatial learning speed) under a Weibull family and a log link function. Model.0 included morph, treatment, boldness (boldness score obtained on the first OFT replication) and all combinations of their interactions as predictors. Sex was also included as a control factor (*i.e*, TTC ~ Morph*Treatment*Boldness + Sex).

Secondly, we tested hypothesis (H4) regarding spatial learning speed, by fitting separate models with a different gene RE within each brain region as the response variable, under Gaussian assumption (when the gene RE within a brain region did not reach normal distribution, it was boxcox-transformed). Each model's predictors included boldness, TTC, and all combinations of their interactions with morph and treatment. Again, sex was also included as a control factor (*i.e*, gene_x RE in brain region_y ~ Boldness + Morph:Boldness + Treatment:Boldness + Morph:Treatment:Boldness + TTC + Morph:TTC + Treatment:TTC + Morph:Treatment:TTC + Sex).

Diagnostics of all models were validated by inspecting potential scale reduction factors, effective sample sizes, tails heaviness and lightness, trace plots, densities, autocorrelation plots, trace rank plots and posterior predictive checks for each model parameter (the *pp1a.2* and *neurod1-like* genes did not satisfy the posterior predictive checks, and were therefore excluded from further analyses). We applied the functions emmeans and contrast under Tukey linear hypothesis for categorical variables or the function emtrends for continuous variables (package emmeans v. 1.7.8 (Lenth, 2023)), to perform post hoc tests on significant interactions for all models.

Note: Bayesian statistics do not use frequentist p-values. The significance of an estimate is obtained when its credible interval (CI) or its higher posterior density interval (HPDI) does not overlap zero. We considered a tendency for an estimate in the model predicting TTC when the CI overlapped zero no further than 0.09 units (tendencies were not considered for models predicting genes RE, for the sake of readability).

Finally, we further tested our hypotheses regarding spatial learning capacity by using two sample t-tests when distributional normality and homoscedasticity was reached (checked with Shapiro and Levene's tests respectively), or using Wilcoxon rank sum tests when not, comparing learners and non-learners in terms of: (H1) mean boldness (boldness score obtained on the first OFT replication) ; (H2.ii) mean boldness within morphs; (H3.ii) mean boldness within treatments overall, and within morph-by-treatment batches (except within batches where comparison was not possible: plain VB and plain LB where all individuals had learnt, and enriched AN where only one individual did not learn); and (H4) mean gene RE within each brain region.

Ethical Note

We obtained permission from landowners to fish in lakes and rivers. All procedures were designed to minimise stress for tested animals, respecting the European standard animal care protocols and the Icelandic Animal Protection Act (Act 15/1994, last updated with Act 11/2014), and were approved by a MAST animal care permit (#2019-01.-09, Ref.190564). Handling procedures involved optimal dosage of anaesthesia with 2-phenoxyethanol adjusted to individuals' reaction, as per the clauses of best practices for animal care and experiments of Hólar University Aquaculture Research Station (HU-ARC) operational licence.

Results

Proportions of males and females, as well as proportions of sides preferred, were not biased between morphs (*Table S4*). The cognitive test used here (Dellinger, Suret, et al., n.d.) proved to be a relevant training method for spatial learning in this species, as out of the 145 fish tested, 105 successfully reached the 9/10 training criterion (72%), 26 were non-learners (18%), and 14 were evicted (10%). Out of the 105 learners, two never reached the first 4/5 reinforcement criterion and one never reached the second, which were still counted as learners but not included in the gene expression analyses (see also *Table S1* for details per morph and treatment). There was no difference between morphs, treatments, morph-by-treatment batches, sexes, and side-turning preferences, in the proportions of learners *versus* non-learners, and of motor response *versus* beaconing strategies prioritised during probes (H2.i, H3.i, *Table S4*). Indeed, motor response turned out to be the strategy prioritized in vast majority (only 24 beaconings out of 310 probe trials; 10.5% of beaconings in probe 1; 5.1% of beaconings in probe 2; 9.7% of beaconings in probe 3), and among the 18 only individuals that used the beaconing strategy during their three probes, twelve individuals only used beaconing once, six individuals used beaconing twice, and no individual used beaconing the three times (one fish that used beaconing on probes 1 and 2 was the one evicted during the second reinforcement phase because it never reached the last reinforcement criterion).

Model.0 showed tendencies for males and bolder individuals overall to learn faster (H1). There was no effect of the treatment or the morph-by-treatment batch on learning speed (TTC, H3i, Figure 1B), but the morph (H2.i, Figure 1A) and all interactions involving boldness (H2.ii, H3.ii, Figure 2, Figure 3) had a significant impact on it (Table 2). Namely, AN individuals learnt significantly faster than VS and LB morphs, and tended to learn faster than VB and PL morphs. There was also a tendency for VB individuals to be faster learners than LB (Figure 1A).

The bolder individuals from VB and PL morphs were, the more slowly they learnt (this effect even tended to be more pronounced in the VB morph compared to the PL), while no differential effect of boldness on TTC was detected in the other morphs (Table 2, Figure 2). The bolder individuals from the plain treatment were, the more slowly they learnt, while no differential effect of boldness on TTC was detected in the enriched treatment (Table 2). Within morphs, boldness had a significantly different influence on TTC between treatments only within AN, where bolder AN from the enriched treatment tended to learn faster, while conversely bolder AN from the plain treatment tended to learn more slowly (Table 2, Figure 3).

Learning capacity was not influenced by boldness personality trait, neither overall, nor within morphs, within treatments, and within morph-by-treatment batches, except non-learners being shyer within the enriched VS batch (*Table S5*).

Boldness alone as well as its interaction with the treatment did not show significant effects on any of the genes relative expression (RE). Significant results are summarized on Figure 4. The *crebbp* gene (CREB binding protein, epigenetic marker associated with memory) did not show any relation to boldness or learning speed in any group and brain region. The majority of the significant signals across all genes and groups were found in the optic tectum and the telencephalon. Signals detected in the optic tectum were linked to boldness and to learning speed in similar proportions, while signals detected in the cerebellum and telencephalon were in large majority linked to learning speed.

The only significant effect of sex on gene expression was found in the optic tectum, where males showed higher expression of *dbh* (dopamine beta-hydroxylase) relative to females. Differences in *dbh* regulation were indeed particularly present in the optic tectum, both positively and negatively correlated to learning speed and boldness. Namely, slower learners showed an overall upregulation of *dbh* in the optic tectum, and this effect was even more pronounced in the AN morph. Interestingly, this effect was inverted in the VS morph and especially so in the enriched treatment. Still in the optic tectum, *dbh* expression decreased with boldness in VS morph overall, with an even more pronounced effect in the enriched batch, and in the PL enriched batch. The contrary was found for the VB morph overall, where *dbh* expression increased with boldness. The expression of *dbh* also positively correlated with boldness in the cerebellum of VS overall, and negatively correlated with TTC in the telencephalon of LB overall. The only other signal regarding the monoamine metabolism that was detected, was a downregulation of *mao* (monoamine oxidase) in the cerebellum of slower learners in the VB plain batch.

Interestingly, glucocorticoid receptor signals detected were solely morph-dependent, and were exclusively linked to boldness in the optic tectum, with a downregulation of *gr* for bolder AN and PL but an upregulation for bolder LB, while they were exclusively linked to learning speed in the telencephalon, with downregulation of *gr* for slower learners from AN and LB morphs. On the contrary, mineralocorticoid receptor signals were exclusively detected in the cerebellum, correlating with learning speed: *mr* expression for slower learners was downregulated in LB overall, but upregulated in the PL plain batch.

Differential expression of *pp1* (serine/threonine protein phosphatase 1, learning and memory suppressor) involved only the alpha (*pp1a.1*) and gamma (*pp1g.1*) subunits. The *pp1g.1* subunit signals were exclusively linked to learning speed in the plain treatment: in the optic tectum of plain-raised fish overall, *pp1g.1* was upregulated for slower learners, but interestingly this effect was reverted in the VB and PL morphs; this same gene was also downregulated in the cerebellum of VB plain slower learners. The *pp1a.1* subunit on the contrary, was positively correlated to boldness in the telencephalon of VS enriched fish.

Genes linked to neurogenesis and neural plasticity were almost exclusively linked to learning speed, and their signals were in major part detected in the telencephalon. The only two occurrences where such genes were linked to boldness were negative correlations between boldness and *bdnf* in the optic tectum of AN morph (the only significant signal for this gene actually), and between boldness and *egr1* in the telencephalon of VS plain batch. Otherwise, slower learners' expression of *egr1* was downregulated in the cerebellum and telencephalon of the LB morph overall and in the telencephalon of the VS plain batch, but upregulated in the cerebellum of PL plain fish. Significant signals for *c-fos.1*, *neurod1* and *pcna* were found exclusively in the telencephalon, the former two being downregulated for slower learners overall, and the latter being upregulated for VS and VB slower learners. The effect found for *c-fos.1* was also stronger in the plain treatment for VS and LB morphs, while the effect found for *neurod1* was stronger in AN and LB morphs overall. The expression of c-fos.2 was downregulated for slower learners, in the optic tectum of morphs VS and VB overall, and in the telencephalon of the VS plain batch.

All in all, other than the above-mentioned *pp1g.1* upregulation with TTC in the optic tectum in the plain treatment overall, AN showed no plasticity in response to structural complexity in terms of genes-boldness and genes-learning covariations. Morph VS, on the contrary, garnered the greatest number of such plastic modulations of these covariations.

Finally, results regarding differences in mean RE between learners and non-learners within each brain region are summarized in Figure 5 (Wilcoxons and t-tests outputs in *Table S5*). As much as *dbh* showed many significant signals related to learning speed within learners, none of the genes related to monoaminergic pathways (*dbh* and *mao*) showed differences in average expression between learners and non-learners, in any brain region. This was also the case for *c-fos.2* and *pp1a.2*. *Neurod1* was significantly more expressed in the telencephalon of non-learners relative to learners, along with tendencies for *gr*, *crebbp* and *egr1*. *Pcna* only tended to be more expressed in learners than non-leaners in this brain region, while this difference was significant in the cerebellum. *Pp1a.1* was more expressed for non-learners than learners, significantly in the optic tectum and as a tendency in the cerebellum. In the cerebellum, *egr1* was significantly more expressed in non-learners, while *c-fos.1* and *bdnf* tended to be more expressed in learners. In the optic tectum, *gr* and *pp1g.1* tended to be more expressed in non-learners.

The expression of *reelin*, *pp1b* and *pp1g.2* did not show any relation to boldness, learning speed or learning capacity in any group and any brain region.

Discussion

A Focus on Navigation Strategies

Nearly all probe trials, revealing the navigation strategy prioritised when navigational cues are set in conflict, turned out to be motor responses, irrespective of the morph, the treatment, or their interaction. Our results contradict the hypothesis relative to (H2.i) that animals from more complex environments with higher visual landmarks availability would rely preferably on beaconing strategy to navigate while animals from simpler habitats with few or no reliable visual cues would preferably use motor responses, as was shown in the three-spined stickleback (*Gasterosteus aculeatus*, Bensky & Bell, 2018; Odling-Smee & Braithwaite, 2003), in gobies (G. E. White & Brown, 2015b, 2015a), or in the climbing perch (*Anabas testudineus*, Sheenaja & Thomas, 2011). However, even though not consistently over their three probes, a few individuals still used the beaconing strategy, demonstrating the capacity of the Arctic charr to use both egocentric strategies. In the study by Sheenaja & Thomas (2011), fish were shown to learn to solve the spatial task faster in the presence of visual landmarks compared to a barren maze. It is hence possible that our subjects might have been using both strategies concomitantly during the process of learning how to solve the maze. However, once the place-reward association was understood, and given the extended number of trials needed for the fish to acquire the learning criteria, reliance on motor response alone was probably sufficient to solve the task most efficiently. Indeed, motor response is allegedly the least cognitively costly strategy as the more the path is repeated, the more the motor sequence is reinforced, eventually becoming a habit requiring minimal attention to execute (Darmaillacq et al., 2018). Such strategy switching to motor response over learning time has already been shown in rats (Chang & Gold, 2003; Packard & McGaugh, 1996). In the present study, we could assess which strategy (motor response or beaconing) was prioritised for navigation. Further research could focus more specifically on differences in learning ability when each strategy is used independently. For that, an experimental design in which each unique strategy is tested separately, similar to Bensky & Bell (2018) experiment, would be more adapted.

Eco-Evolutionary History Influence on Spatial Cognitive Abilities

Relative to (H2.i), we found that the morphs do not differ in their spatial learning capacity or in the navigation strategy used. Equal proportions of individuals from all morphs were able to reach the 9/10 learning criterion, and they all used the motor response strategy in majority when navigational cues where set in conflict. The spatial learning speed nevertheless differed between morphs, *i.e.*, the ancestral proxy AN was overall the faster learning morph, with a general trend for spatial learning speed to decrease with divergence.

Spatial learning speed did not differ between sympatric morphs within lakes. One possibility is that our sample size was not sufficient to detect differences that might be subtler within than among populations. Another explanation could be that the genetic background of these morphs is the predominant factor shaping spatial learning, surpassing potential effects of environmental complexity effects (Godfrey-Smith, 2002). This explanation is plausible, considering that we previously showed that this was likely the case for boldness personality trait in the Arctic charr (Dellinger, Caperaa, et al., n.d.; Dellinger et al., 2023) and that these two traits are shown here to form a syndrome in this species (see further paragraph).

Anadromous Arctic charr usually spend their first three to seven years in their native rivers before migrating to the sea (Klemetsen et al., 2003). AN's riverine origin, as opposed to the lacustrine origin of the other morphs, could explain their faster spatial learning ability. Climbing perch were shown to be faster spatial learners when originating from rivers than from ponds, irrespective of the presence or absence of visual landmarks in the maze, which was related to the openness of riverine systems, compared to enclosed ponds offering less opportunity to explore new routes (Sheenaja & Thomas, 2011). However, this argument is not sufficient to explain our results, as Þingvallavatn is the largest lake in Iceland, with consequent exploration opportunities, yet its morphs are the slowest spatial learners, at least compared to the AN. AN is also a migratory morph, traveling extensive distances (about 7 km) to the sea and back for reproduction. The migratory behaviour of anadromous morphs probably necessitates sharp spatial cognitive abilities to efficiently learn and remember the way to and from the sea whilst migrating (Mettke-Hofmann, 2014). Maintaining higher spatial cognitive abilities likely became an unnecessary cost when migratory behaviour got prevented as the anadromous ancestor became landlocked (Dukas, 1999; Dunlap & Stephens, 2016). Hence, relaxing selective pressures on spatial cognition would explain the progressive decrease in spatial learning speed with divergence time. The learning speed pattern of the VS seems in accordance with this interpretation. Firstly, VS is a recently diverged morph that has retained numerous ancestral characteristics, such as pigmentation, morphology, migration (Jónsson & Skúlason, 2000), resource use (Brachmann et al., 2021) and even mean boldness (Benhaïm et al., 2023; Dellinger, Caperaa, et al., n.d.; Dellinger et al., 2023). Secondly, this morph is a generalist and has a mixed riverine and lacustrine origin, still migrating but on a much smaller scale (about 700 m) from tiny adjacent native streams into the small lake at one or two years of age and back (Jónsson & Skúlason, 2000). Hence, with such a generalist lifestyle sharing characteristics with the other four morphs, it is interesting to note that the VS range of spatial learning speed among-individuals overlaps those of the other four morphs (Figure 1A), including the AN whose mean learning speed is however significantly faster.

No Developmental Plasticity in Response to Structural Complexity in Spatial Cognitive Abilities

Relative to (H3.i), the structural complexity treatment did not significantly impact any of the spatial cognitive abilities (spatial learning speed, spatial learning capacity, and navigational strategy used), neither alone nor in interaction with the morph. This contradicts the environmental complexity thesis (Godfrey-Smith, 2002) as well as most of the studies showing positive impacts of complexity/enrichment on spatial cognition in fishes (*e.g.*, Arechavala-Lopez et al., 2020; Carbia & Brown, 2019; Montalbano et al., 2022; Shen et al., 2023, for recent examples in different teleosts species). However, other studies have found no spatial cognitive effects of environmental complexity on teleosts (three-spined stickleback *Gasterosteus aculeatus*, Brydges & Braithwaite, 2009) and specifically other Salmonids (Chinook salmon *Oncorhynchus tshawytscha*, Cogliati et al., 2019). It is important to consider that the occurrence, direction and magnitude of changes in cognitive traits mediated by environmental enrichment are highly dependent on the characteristics of the enrichment treatment employed (as shown for fishes in Carbia & Brown, 2019; Cogliati et al., 2019; Shen et al., 2023), but also dependent on the particular cognitive ability considered (*e.g.*, in fishes Carbia & Brown, 2019). The idea that the structural complexity treatment used presently was not adapted to trigger cognitive plastic changes can be rejected, as the treatment had a significant impact on the relationship between spatial cognition and boldness (discussed later). When considered in parallel of the fact that the differences in structural complexity in the morphs natural habitat of origin did not seem to cause differences in cognitive abilities either within sympatric morphs' pairs, *i.e.*, genetically closely related, it appears as mentioned before that the genetic component in this species is probably preponderant in the development of the spatial cognitive phenotype. Hence the apparent lesser sensitivity to environmental complexity, as we showed is likely the case for boldness personality too in this species (Dellinger, Caperaa, et al., n.d.).

Spatial Cognition-Boldness Personality Syndrome

We presently confirm (H1) by showing correlations between spatial cognitive abilities and boldness personality trait, and so on different scales. Firstly, when considering a global scale, it is particularly interesting to note that in our previous study, mean boldness across these morphs increased with divergence from the ancestor (Dellinger, Caperaa, et al., n.d.), which is here related to mean learning speed across morphs decreasing with divergence. This overall negative correlation between spatial learning speed and boldness personality trait over an evolutionary gradient (the more diverged, the bolder and the slower learner), not only contradicts the speed-accuracy predictions (Sih & Del Giudice, 2012), but additionally brings empirical support, for the first time to our knowledge, for the idea that these traits could be coevolving (Carere & Locurto, 2011).

When considering the present results at the individual scale, we also found correlations between spatial cognitive abilities (spatial learning speed and spatial learning capacity) and boldness personality trait. In terms of spatial learning speed, relationships with boldness are complex, as all interactions including boldness score (boldness-morph, boldness-treatment, and the triple interaction morph-treatment-boldness) had significant impact on learning speed in our model. There was only a tendency for bolder individuals to be faster learners overall. This tendency seems to be the opposite of the global trend, and would somewhat match the speed-accuracy trade-off hypothesis predictions (Sih & Del Giudice, 2012). However, as this effect is just a trend, its biological relevance is debatable. In fact, the cognition-personality syndrome at individual scale was mostly dependent on the morph (H2.ii), the treatment, and the morph-by-treatment interaction (H3.ii). This is in line with Daniel & Bhat (2023) showing that populations of zebrafish differing in their habitats of origin also differed in how boldness correlated with spatial memory (in this study, boldness was however not repeated, hence cannot confidently be considered a personality trait (Dingemanse & Wright, 2020)). In our study, bolder individuals were slower learners than shy ones in the plain treatment overall (significant treatment-boldness interaction), which was not the case in the structurally complex treatment where boldness did not influence learning speed. This parallels the results from De Meester and colleagues (2022) showing that a boldness personality trait proxy negatively influenced spatial reversal learning ability, but only in lizards (*Podarcis erhardii*) originating from simple habitats, not from complex ones. The selective mechanisms invoked by the authors to explain such differences between environments are however not applicable to our common garden study. The processes related to our treatments are purely developmental, as no selective pressures were applied here. Nonetheless, we also found morph-specific boldness influence on learning speed (significant morph-boldness interaction), for which evolutionary processes are most likely involved. Namely, only VB and PL morphs showed a negative relationship between learning speed and boldness, while boldness had no impact on learning speed for the other morphs (other than the overall tendency for bolder individuals to be faster learners). Hence, it seems that speed-accuracy trade-off predictions (hastier individuals learning faster (Sih & Del Giudice, 2012)) are poorly supported when considering AN, VS and LB, and even contradicted when considering VB and PL morphs. This stresses the fact that one-size-fits-all generalisations regarding cognition-personality syndromes most likely do not capture the complexity of natural processes at their origin. Our results showing a significant effect of the triple morph-treatment-boldness interaction, both on spatial learning speed and capacity, underline intricate interdependencies between ecological, evolutionary, and developmental factors engendering and shaping those syndromes. Additionally, we found that the morph-treatment-boldness effect on spatial cognition was also cognitive ability-specific, as the influence of boldness on spatial learning speed differed between treatments within the AN morph only, while the influence of boldness on spatial learning capacity differed between treatments within the VS morph only. If even slight differences in evolutionary histories and/or environmental conditions can engender variations in correlations between cognitive abilities and personality traits, and those correlations differ depending on the cognitive ability considered, this would in major part explain the amount of contradictory results regarding cognition-personality syndromes exposed in the introduction.

The reason why VB and PL were the only morphs in which a clear spatial cognition-boldness personality trait syndrome was specifically found is still an open question. A thorough investigation referencing the differences between morphs in genetics, as well as biotic and abiotic environmental factors that could influence personality and cognition, would help providing answers. However, it is interesting to note that VB and PL morphs have the common trait of both being the more specialized morphs from their respective sympatric pairs, and both exploiting niches with lower structural complexity in the wild compared to their sympatric counterparts (PL living in the homogeneous water column of a deep lake; VB being sedentary in a small, simple, muddy-bottom lake). In a previous study, we also showed that those two morphs displayed a particularly high plasticity in boldness variability in response to structural complexity compared to the other three (Dellinger, Caperaa, et al., n.d.). The way this could relate to the formation of cognition-personality syndromes is yet unclear.

It is also worth noting that the cognition-personality syndrome showed developmental plasticity in response to structural complexity only in the ancestral proxy AN (significant difference in the slopes of boldness effect on TTC between enriched and plain AN), and the least diverged morph VS (non-learners being shyer than learners in enriched VS but not in plain VS). This resonates with the “plasticity-first hypothesis” (Levis & Pfennig, 2016) stating that ancestral phenotypic plasticity induced by environmental perturbations provides opportunity for adaptive divergence when colonizing a new environment, describing ancestral plasticity as a key for rapid evolution, predicted to be reduced after acclimation to the new habitat. Further investigations, notably with a higher sample size and a crossing design involving families to accurately assess differences in variances among morphs, are needed to look deeper into this hypothesis.

Neural Mechanisms Underlying Spatial Cognition, Boldness Personality Trait, and Their Syndrome

The expression of each gene relative to spatial learning or to boldness differed depending all at once on the morphs, the treatments, their interaction, and the brain region considered. Therefore, there were only rare occasions where a neural marker was found as a common denominator for patterns highlighted in the previous paragraphs. We will interpret these results with the hypothesis that mRNA quantities are proxies for protein levels.

It is worth comparing our results with those of Vindas and colleagues (2017). Their study showed upregulated expression of *gr* and *mr* in the hypothalamus, but not in the telencephalon, of proactive Arctic charr (proactivity being often related to boldness (Koolhaas et al., 1999; Sørensen et al., 2013)). Even though they used non-repeated behavioural measures of proactivity making the implication of a personality trait uncertain (Dingemanse & Wright, 2020), and despite using slightly different primers, our results support their findings by showing that *gr* and *mr* expressions were not related to boldness in the telencephalon. We also complete their results by showing that the expression of *gr*, but not *mr*, was also related to boldness in the optic tectum, and that the direction of the relationship was morph-dependent (Figure 4). The mechanisms behind those morph-specific links between *gr* expression and boldness personality trait are yet to be elucidated, but this expands on the fact that gene expression patterns underpinning personality in fishes are not only species-specific as shown by Rey and colleagues (2021), but could very well be population-, strain- or even genotype-specific as well.

The dopamine beta-hydroxylase (*dbh*) converts dopamine into noradrenaline, so that inhibition of *dbh* engenders increased levels of dopamine and a lack of noradrenaline (Thomas et al., 1998). Expression levels of this gene appear to be pivotal in our study. Particularly, *dbh* expression decreased with spatial learning speed overall in the optic tectum, indicating a potential decrease in dopamine levels while noradrenaline levels increase as time required to learn the task increases. This aligns with studies showing that higher levels of dopaminergic activity are positively correlated with spatial cognitive performances in other teleosts (Eddins et al., 2009; Levin, 2011; Messias et al., 2016). Higher dopamine levels could enhance the appraisal of the rewards' positive value (Hamilton et al., 2017), notably through the dopaminergic reward system, consequently increasing the motivation to obtain them (Lewis et al., 2021), and/or favouring the acquisition and memory of the place-reward association (Naderi, Jamwal, Ferrari, et al., 2016). This hypothesis is further supported by the fact that (1) this negative correlation between *dbh* expression and learning speed in the optic tectum was even stronger within the AN, the fastest learning morph, and (2) this correlation was on the contrary positive within the VS morph that was learning significantly slower than the AN (not found in the LB though). Here, *dbh* regulation was not directly linked with overall boldness at the individual scale. However, it was also related to boldness in some occasions (VS, VB and PL, see Figure 4), and it is interesting to note that we found levels of noradrenaline could potentially be higher in slower learners, and higher levels of noradrenaline are usually reported in bold individuals (Koolhaas et al., 1999; Sørensen et al., 2013), which still hints towards a possible implication of this pathway in the spatial cognition-boldness syndrome we found at the global scale (more diverged morphs being bolder and slower learners). The tendency for males to be faster learners might be related to males' differential regulation of *dbh* with learning speed compared to female, but the exact mechanism linking both phenomena is yet unclear.

The regulation of the protein phosphate 1 gene (*pp1*), a learning and memory suppressor (Miller & Sweatt, 2007), could be an indirect explanatory factor of why bolder individuals were found to be slower learners in the plain treatment overall. The gene coding the gamma subunit of PP1 (*pp1g.1*) was upregulated in the optic tectum of slower learners when reared in plain tanks. Additionally, within-morph differential expression of *pp1g.1* relative to learning speed was always linked to the plain treatment (Figure 4). Although *pp1g.1* expression was not directly linked to boldness herein, shy individuals are described as more prone to gather and retain information from their surroundings than bold individuals (Koolhaas et al., 1999; Sørensen et al., 2013). In a barren environment, the lack of cognitive stimulation for already "inattentive" bold individuals could lead to even further inhibition of the learning and memory systems, explaining why slower spatial learning accompanies boldness in the plain treatment. This hypothesis finds some additional support in the fact that the gene coding the alpha subunit of PP1 (*pp1a.1*) was upregulated in the telencephalon of bolder VS in the enriched treatment. To our knowledge, this is the first time that *pp1* gene expression in the brain is shown to be related to boldness personality trait, and the first time it is shown to be modulated by environmental complexity, with repercussions on spatial learning ability.

The *pp1* genes seemed to be important elements in spatial learning capacity as well. Impairment of learning and memory processes due to significantly higher *pp1a.1* expression in the optic tectum, as well as a tendency to higher expression of *pp1a.1* in the cerebellum and of *pp1g.1* in the optic tectum, could explain the incapacity of non-learners to reach the 9/10 training criterion. As expected, learners showed greater neural plasticity compared to non-learners, observed through significantly higher *pcna* expression in the cerebellum, and tendencies to higher expression of *pcna* in the telencephalon and *c-fos.1* in the cerebellum. However, contrary to our predictions, *neurod1* in the telencephalon and *egr1* in the cerebellum, markers of neurogenesis and neuronal activity respectively, were significantly more expressed in non-learners than in learners (with a similar tendency for *egr1* in the telencephalon). This can be paralleled with the tendency for glucocorticoid receptors gene (*gr*) to be expressed at higher levels in the telencephalon and in the optic tectum of non-learners compared to learners. This receptor's ligand, the stress hormone cortisol, is known to inhibit learning and memory (Pavlides et al., 1996; Sørensen et al., 2013). One possibility is that relative to learners, non-learner individuals were slightly more sensitive to cortisol which slowed down their learning to the point where 93 trials were not sufficient to reach the training criterion. But at the moment the experiment stopped for non-learners, they might still have been in the process of learning the task, which would explain the higher levels of neurogenesis and neural activity than the learners who had already acquired the association. Another possibility is that non-learners experienced the cognitive test procedures as more stressful than learners (which could be a cause or a consequence of their tendency to higher expression of *gr*) and that the increased neurogenesis and neural activity seen in their cerebellum and telencephalon could consequently be linked to fear conditioning rather than spatial learning, as those two brain regions were shown to be involved in emotional learning (Broglio et al., 2005; Rodríguez et al., 2005).

The gene expression patterns of VB and the PL morphs did not reveal commonalities that could explain the negative correlation between boldness and learning speed found in these morphs but not in the others. It is possible that different combinations of genes expressions can lead to a similar phenotype, or that this phenotype is governed by other genes not tested here. Similarly, no clear explanation could be found as to why boldness had a significantly different influence on learning speed between treatments within AN, as none of the genes tested were differentially expressed in relation to TTC and boldness between treatments in this morph. Also, we could not identify neural markers directly linked to both boldness personality trait and spatial cognitive performances, or genes linked to cognitive performances for the ones and boldness for the others systematically co-expressed, that could be the physiological causation of the highlighted cognition-personality syndrome. All in all, further investigations using a higher sample size and thorough transcriptomics approaches could help pinpoint all the genes involved in these cases.

Conclusion

In this study, we show that a syndrome between spatial cognition and boldness personality trait is present in the Arctic charr. Spatial cognition, boldness personality trait, the syndrome they form together, and their underlying neural mechanisms were essentially dependent on the morph, the treatment, the morph-by-treatment interaction, the spatial cognitive ability, and the scale (global or individual) considered, implying extremely intricate eco-evo-devo interactions governing their formation. This underscores that general theories modelling cognition-personality syndromes, like the speed-accuracy trade-off (Sih & Del Giudice, 2012) for instance, are most likely too restrictive for such a complex phenotype. Our results rather support a model in which cognition-personality syndromes development is a "case-by-case" phenomenon, shaped by finely intertwined influences of evolutionary, ecological and developmental factors. Nonetheless, we found that spatial cognition and its relationships with personality could be predominantly under strong genetic influence, most likely dictated by genetic divergence from the common ancestor. Indeed, learning speed decreasing and boldness increasing with divergence overall, we show the first empirical support to our knowledge for co-evolution between cognition and personality (Carere & Locurto, 2011). We even found hints of evolution of the syndrome's plasticity in response to structural complexity, with the ancestral proxy and the least diverged morph being the only two morphs showing between-treatments differences in the cognition-personality relationships, which echoes the "plasticity-first hypothesis" (Levis & Pfennig, 2016). Finally, we found that the dopamine metabolic pathway and the PP1 memory suppressor seemed to play preponderant roles in spatial cognition and its relationship with boldness. However, our results regarding underlying neural mechanisms showed just as much complexity as the cognition-personality relationship itself. Our pioneering gene expression results encourage further researcher on the subject, which would benefit from the combined use of immunohistochemistry techniques to pinpoint exact cerebral subregions involved, and transcriptomic screening to thoroughly assess all genes involved in these complex phenotypes.

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Paper IV - References

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Paper IV - Tables and Figures

Table 1 – Genes of interest linked to spatial cognition and boldness personality trait, investigated in the qPCR analyses.

GENE	MARKER	ROLE
actb	Reference	Beta-actin: cytoskeletal structure protein (Ahi et al., 2013)
ub2l3	Reference	Ubiquitin-conjugating enzyme E2 L3: protein degradation enzyme (Ahi et al., 2013)
bdnf	Neural Plasticity	Brain-derived neurotrophic factor: neurotrophin implicated in neuronal survival, growth, and differentiation; also involved in the regulation of learning, memory, and cognitive and emotional behaviours (Bergami et al., 2008; Cunha et al., 2010; Leal et al., 2017; Yamada et al., 2002)
c-fos	Neural Activity	Cellular Fos proto-oncogene: immediate early gene (IEG) only produced immediately after external stimulation, controls neural differentiation and proliferation, learning and cognition (Calvo & Schluessel, 2021; Lara Aparicio et al., 2022; Watson & Tsai, 2017)
egr1	Neural Activity	Early growth response protein: IEG implicated in cell differentiation and proliferation, synaptic plasticity, and more particularly in learning and memory (Burmeister & Fernald, 2005; Calvo & Schluessel, 2021)
neurod	Neurogenesis	Neurogenic differentiation factors: neural differentiation, neural survival, synaptic maturation (Ince-Dunn et al., 2006; Korzh et al., 1998; Steiner et al., 2006)
pcna	Neurogenesis	Proliferating cell nuclear antigen: characterizing new-born cells (Mandyam et al., 2007)
crebbp	Learning & Memory	cAMP-responsive element binding protein-binding protein: transcription factor co-factor promoting acquisition and storage of memories (Alarcón et al., 2004; Guan et al., 2002)
pp1	Learning & Memory	Serine/threonine protein phosphatase 1: inhibits memory by neuronal long-term depression and depotentiation (Miller & Sweatt, 2007; Munton et al., 2004)
reelin	Learning & Memory	Reelin: responsible for neural migration during cerebral development, promoting memory by controlling synaptic transmissions and long-term potentiation of neurons (Miller & Sweatt, 2007; Weeber et al., 2002)
gr	Stress Response	Glucocorticoid receptor: binds with cortisol, normalises stress response, induces neuronal long-term depression and suppresses synaptic plasticity, participates in storage and recovery of memories, implicated in personality and spatial cognition (Herrero et al., 2006; Joëls et al., 2008; Oitzl et al., 2010; Øverli et al., 2007; Pavlides et al., 1996; Sørensen et al., 2013)
mr	Stress Response	Mineralocorticoid receptor: binds with cortisol with higher affinity than gr, determines onset thresholds of stress response and related behaviours, promotes long-term potentiation and excitability of neurons, implicated in personality and spatial cognition (Herrero et al., 2006; Joëls et al., 2008; Oitzl et al., 2010; Øverli et al., 2007; Pavlides et al., 1996; Sørensen et al., 2013)
dbh	Monoamines Metabolism	Dopamine beta-hydroxilase: synthesises noradrenaline from dopamine, two neurotransmitters linked with learning and personality (Harley, 2004; Koolhaas et al., 1999; Sørensen et al., 2013; Thomas et al., 1998)
mao	Monoamines Metabolism	Monoamine oxidase: participates in the stress response by degrading monoamines, notably dopamine, noradrenaline, adrenaline and serotonin, actors in personality and cognitive processes (Harley, 2004; Koolhaas et al., 1999; Martorell-Ribera et al., 2020; Shih & Thompson, 1999; Sørensen et al., 2013)

*Table 2 - **Left**: output of Model.0 in log scale, Bayesian brms linear model predicting TTC, including the morph (AN from Fjlótaá River taken as reference, sympatric VS and VB from Lake Vatnshlíðarvatn, and sympatric PL and LB from Lake Þingvallavatn), treatment (Enriched or Plain, Enriched being taken as reference), boldness, and all combinations of their interactions (AN morph and Enriched treatment taken as references), and sex (females taken as reference) as predictors. **Right**: results of the post hoc tests caried out on Model.0, investigating the differential effects on TTC of the morph, of boldness per morph, and of boldness per morph-by-treatment batch. Significant estimates highlighted in bold black font, while tendencies are highlighted in bold grey font. Estimate: median of posterior distribution for each variable, contrast or slope; Error: Estimate Error; CI: Credible Intervals; HPDI: 95% Highest Posterior Density Intervals.*

Model.0				**Post Hoc**			
Predictor	**Estimate**	**Error**	**CI**	**Comparison**	**Contrast or slope**	**Estimate**	**HPDI**
Intercept	**3.93**	0.15	[3.66 ; 4.24]	**TTC Between morphs**	VS - AN	**0.35**	[0.04 ; 0.68]
VS	0.26	0.22	[-0.17 ; 0.71]		VB - AN	0.22	[-0.06 ; 0.50]
VB	-0.01	0.19	[-0.40 ; 0.36]		PL - AN	0.28	[-0.02 ; 0.55]
PL	0.14	0.22	[-0.29 ; 0.57]		LB - AN	**0.45**	[0.15 ; 0.74]
LB	**0.43**	0.22	[0.01 ; 0.88]		VB - VS	-0.13	[-0.44 ; 0.19]
Treatment Plain	-0.25	0.20	[-0.64 ; 0.14]		PL - VS	-0.08	[-0.40 ; 0.25]
Boldness	-0.11	0.10	[-0.30 ; 0.08]		LB - VS	0.10	[-0.22 ; 0.43]
Males	-0.14	0.10	[-0.34 ; 0.07]		LB - VB	0.05	[-0.22 ; 0.34]
VS Plain	0.21	0.31	[-0.41 ; 0.82]		PL - VB	0.23	[-0.06 ; 0.51]
VB Plain	0.44	0.28	[-0.11 ; 0.98]		PL - LB	0.18	[-0.12 ; 0.47]
PL Plain	0.26	0.30	[-0.33 ; 0.86]	**Boldness effect on TTC per morph**	AN	0.03	[-0.10 ; 0.16]
LB Plain	0.07	0.30	[-0.53 ; 0.65]		VS	-0.06	[-0.06 ; 0.10]
VS:Boldness	0.02	0.16	[-0.29 ; 0.33]		VB	**0.16***	[0.03 ; 0.28]
VB:Boldness	**0.27**	0.12	[0.02 ; 0.51]		PL	**0.09***	[0.01 ; 0.18]
PL:Boldness	**0.24**	0.11	[0.02 ; 0.46]		LB	-0.01	[-0.09 ; 0.06]
LB:Boldness	0.09	0.11	[-0.14 ; 0.31]	**Boldness effect on TTC between treatments within morphs (Enriched - Plain)**	AN	**-0.29°**	[-0.56 ; -0.02]
Plain:Boldness	**0.29**	0.14	[0.02 ; 0.56]		VS	-0.06	[-0.37 ; 0.24]
VS:Plain:Boldness	-0.22	0.21	[-0.63 ; 0.19]		VB	-0.00	[-0.26 ; 0.24]
VB:Plain:Boldness	-0.29	0.18	[-0.66 ; 0.06]		PL	0.07	[-0.12 ; 0.24]
PL:Plain:Boldness	**-0.35**	0.16	[-0.66 ; -0.04]		LB	-0.02	[-0.16 ; 0.12]
LB:Plain:Boldness	-0.27	0.15	[-0.57 ; 0.03]				

* Boldness slope tended to be higher in VB than in PL (VB - PL estimate: 0.06, HPDI: [-0.09 ; 0.21], see also Figure 2).

° Bolder AN in the enriched treatment tended to learn faster (Estimate: -0.11, HPDI: [-0.30 ; 0.08]), while bolder AN in the plain treatment tended to learn more slowly (Estimate: 0.18, HPDI: [-0;00 ; 0;36]) (see also Figure 3).

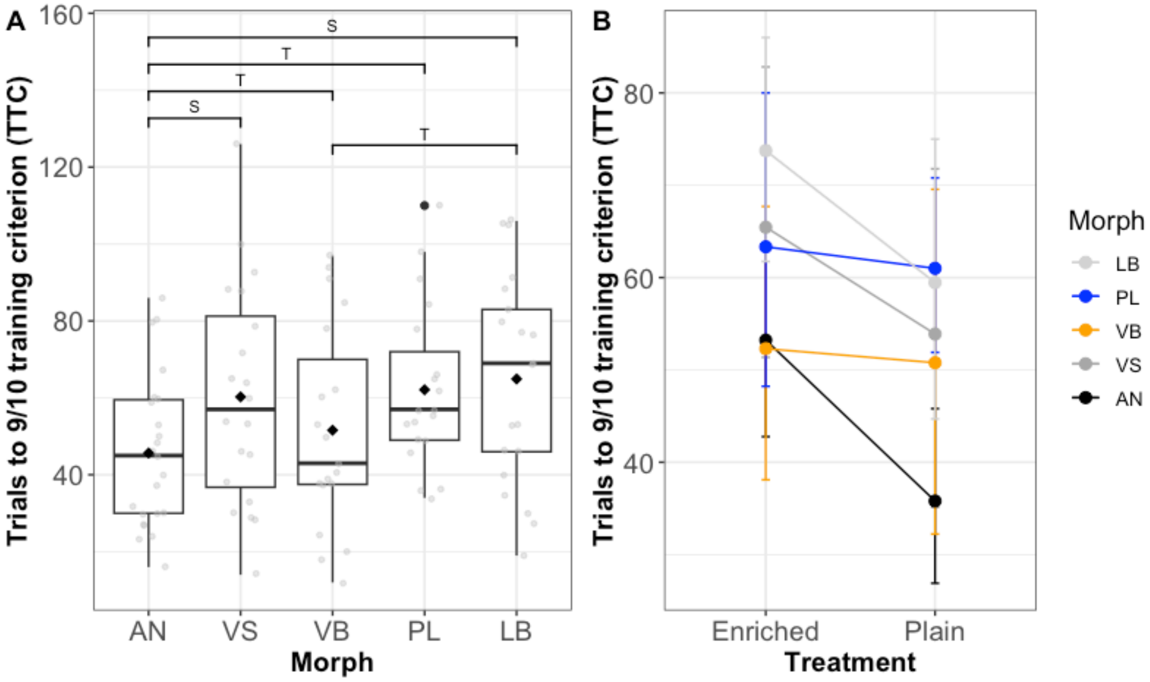

Figure 1 – Learning speed, i.e., number of trials necessary for each subject to reach the 9/10 learning criterion (trials to training criterion, TTC), across the five morphs of Arctic charr, from the least to the most diverged: AN from Fljótaá River, sympatric VS and VB from Lake Vatnshlíðarvatn, and sympatric PL and LB from Lake Þingvallavatn. ***(A)*** *Boxplots of learning speed for each morph: the rhombus represents the mean, grey dots are scattered individual values, the central line indicates the median, ends of the boxes denote upper and lower quartiles, whiskers cover 95% of values. Significance levels from Tukey post hoc pairwise comparisons of TTC between morphs, carried out on Model.0: S – significant; T – tendency (see also Table 2).* ***(B)*** *Reaction norms of learning speed across treatments (enriched and plain) for each morph: the dot represents the mean, vertical bars indicate 1 standard error, the slope of the line across treatments indicates the direction and amplitude of boldness plasticity. There was no effect of the treatment overall or within morphs (see Table 2).*

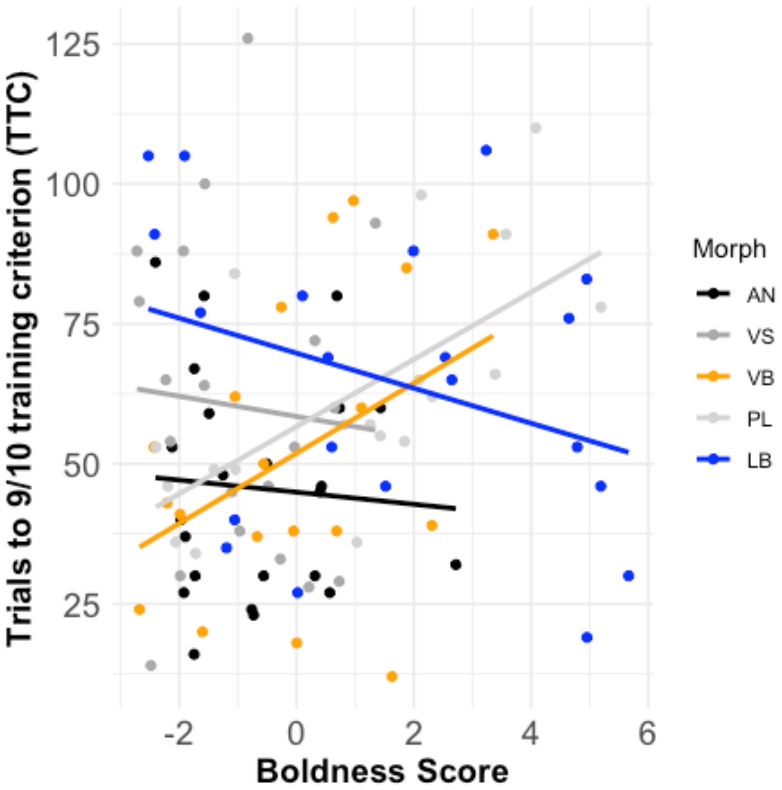

Figure 2 – Effect of boldness (boldness score on the first OFT replication) on learning speed, i.e., number of trials necessary for each subject to reach the 9/10 learning criterion (trials to training criterion, TTC), across the five morphs of Arctic charr, from the least to the most diverged: AN from Fljótaá River, sympatric VS and VB from Lake Vatnshlíðarvatn, and sympatric PL and LB from Lake Þingvallavatn. For each morph, dots are scattered individual values, and the line represents the linear regression. NB: only the slopes for VB and PL morphs were significantly different from zero, and VB slope tended to be higher than PL (see Table 2).

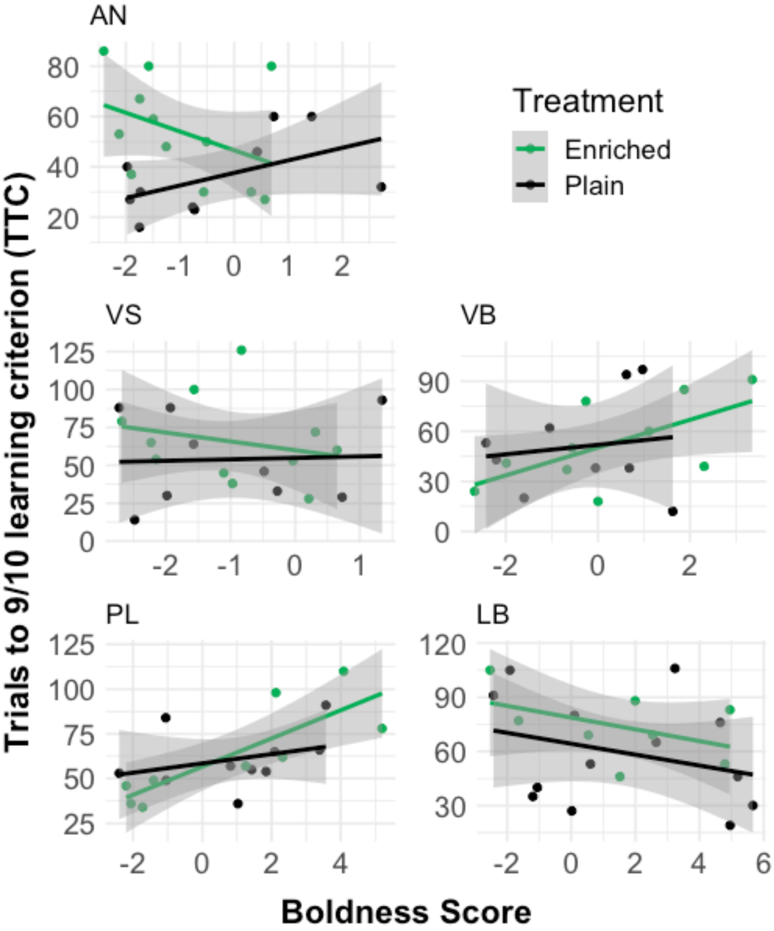

Figure 3 - Effect of boldness (boldness score on the first OFT replication) on learning speed, i.e., number of trials necessary for each subject to reach the 9/10 learning criterion (trials to training criterion, TTC), between treatments (enriched vs. plain) within each morph of Arctic charr, from the least to the most diverged: AN from Fljótaá River, sympatric VS and VB from Lake Vatnshlíðarvatn, and sympatric PL and LB from Lake Þingvallavatn. For each treatment, dots are scattered individual values, the line represents the linear regression, and the grey buffer represents its standard error. NB: between the two treatments, the slopes were significantly different from each other only within the AN morph, and they both tended to be different from zero (see Table 2).

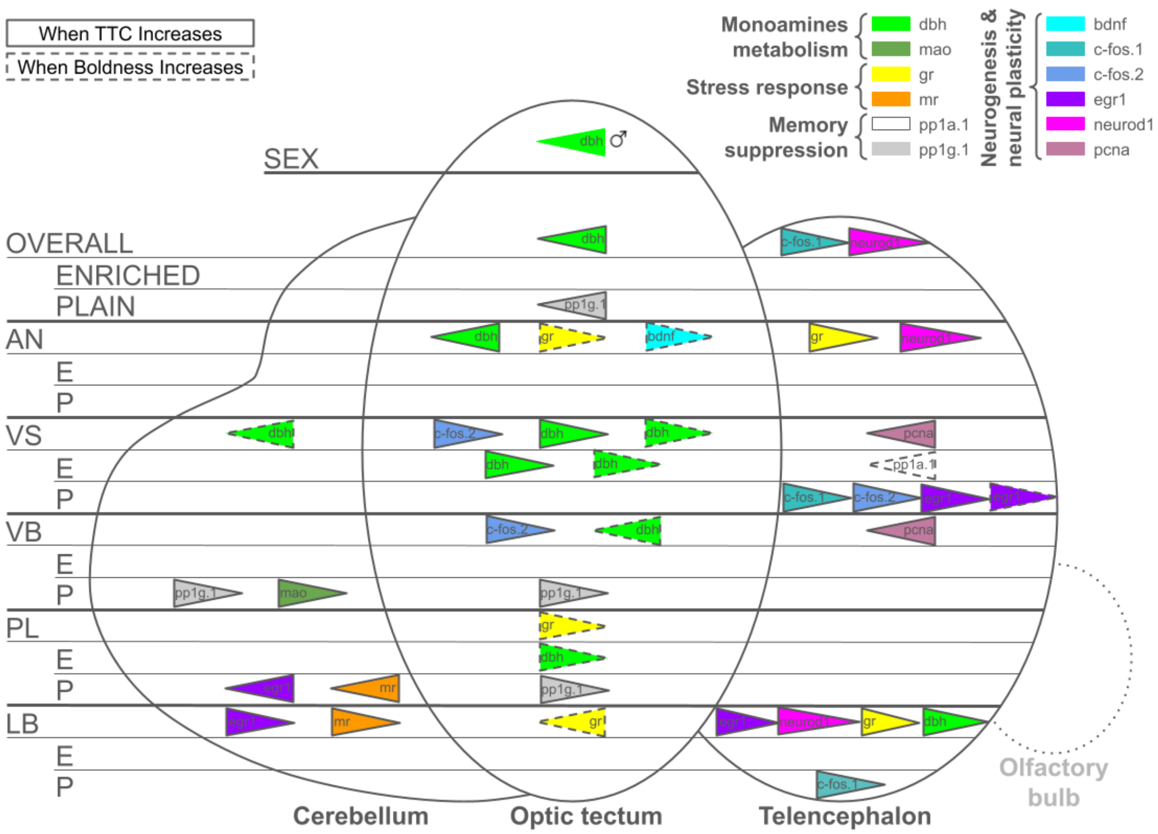

Figure 4 – Gradients of relative expression (RE) for the different genes quantified in three brain regions (cerebellum, optic tectum, and telencephalon; fish brain in lateral view) as a function of increasing number of trials needed to reach the 9/10 learning criterion (trials to training criterion, TTC) or as a function of increasing boldness. Interpretation of triangular gradients in right-reading: pointing left = upregulation; pointing right = downregulation of the given gene's relative expression with increasing TTC (solid outline) or increasing boldness (dashed outline). Sex effect in the optic tectum: to be interpreted as males upregulating dbh compared to females. AN morph from Fljótaá River, sympatric morphs VS and VB from Lake Vatnshlíðarvatn, and sympatric morphs PL and LB from Lake Þingvallavatn. E: Enriched treatment. P: Plain treatment.

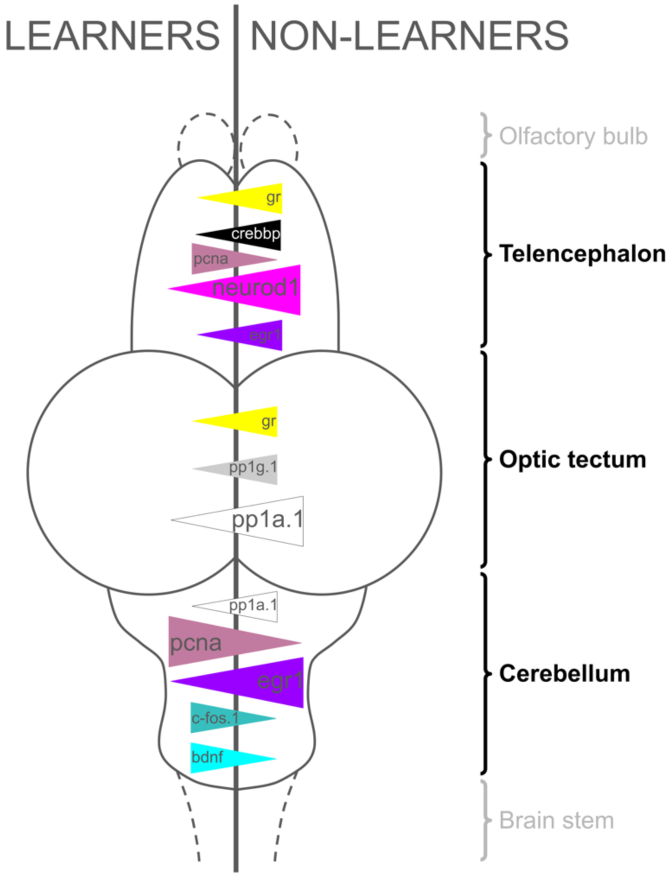

Figure 5 – Mean differences in genes relative expression (RE) between learners (left side) and non-learners (right side) in three different brain parts (cerebellum, optic tectum, and telencephalon; fish brain in dorsal view). Interpretation of triangular gradients for a given gene: pointing left = non-learners have a higher RE than learners; pointing right = learners have a higher RE than non-learners. Larger gradients symbolise significant differences, smaller gradients represent tendencies.

Paper IV – Supplements

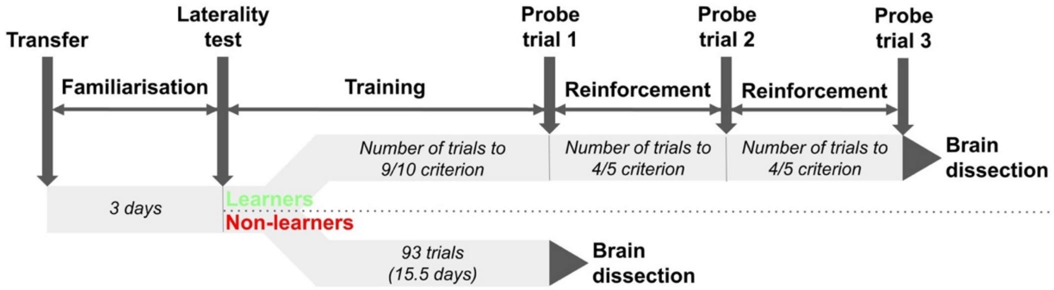

Figure S1 - Sequence of the different phases of the cognitive test. Phases are presented in bold font, with their durations in italics within the corresponding grey bands. Note the different courses of actions for learners (top band) and non-learners (bottom band). Figure modified from "Dellinger, M., Suret, L., Choblet, M., Jozet-Alves, C., & Benhaïm, D. (n.d.). Methodological approach to investigating spatial cognition in fishes: a case study on Arctic charr (Salvelinus alpinus). Journal of Fish Biology, in revision".

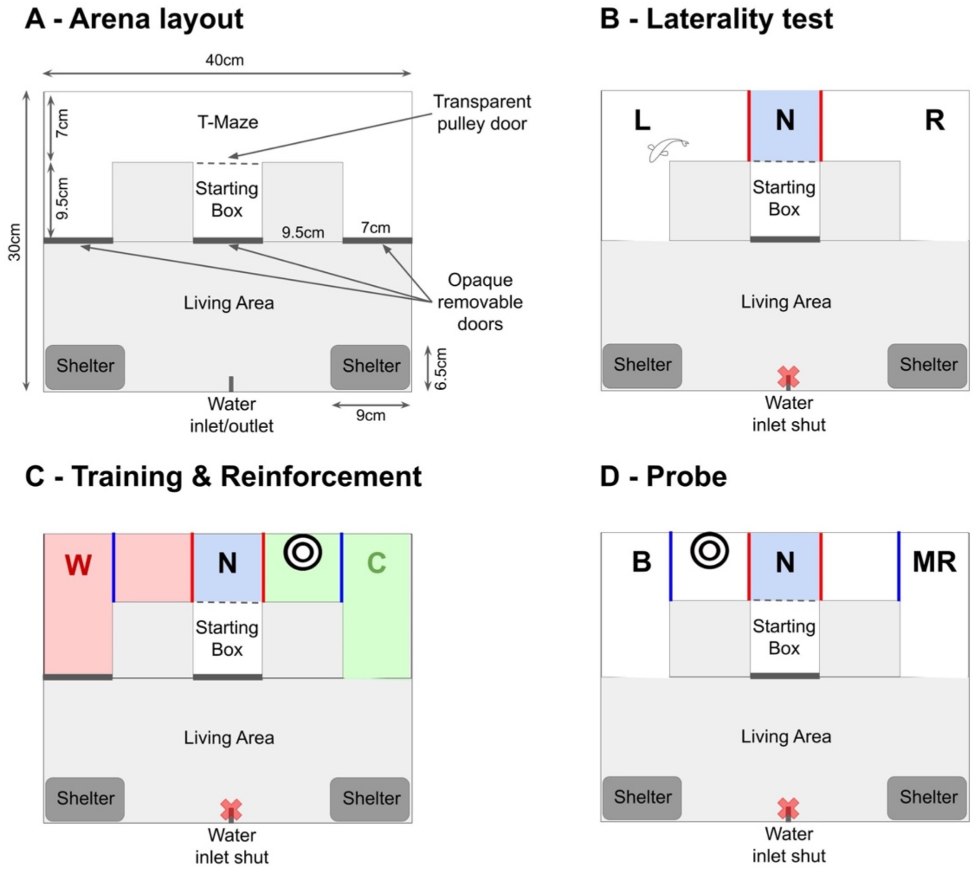

Figure S2 - Scheme of an arena and its configurations according to the cognitive test phase, seen from above. The surrounding wooden black box, and the net sealing the top of the living area, are not represented. Note that the living area is in darker shades, representing its lower light exposure. All opaque removable doors are represented by thick black lines *separating the living area and the T-maze, and the transparent door remotely operated with a pulley system is represented by the dotted line. Thick red and blue lines in the T-maze are virtual and denote limits to be crossed by the fish to make and confirm its choices. Note that when the eyes of the fish would cross the virtual blue lines, and only then, it could see a cul-de-sac of the closed arm, or the living area and one of its shelters entry from the maze through the open doors (realising there is a way back to the living area during laterality test and maze discovery; realizing whether it made a correct choice or not during trainings, reinforcements and probes). A red cross denotes when the water inflow would be shut. This figure gives the example of a fish being trained to go on the right, hence all descriptions would be simply mirrored for a fish trained to go on the left.* ***A*** *– Layout and measurements of an arena.* ***B*** *– Laterality test configuration: in this example, when leaving the neutral zone of the maze (N, blue area), the fish first crossed the virtual red line with its whole body on the left side (L, left arm) as opposed to the right side (R, right arm). The left is hence considered its lateralization side.* ***C*** *– Training and Reinforcement configuration: in this same example, the fish had to learn to return to the living area on the opposite side of its lateralization side, i.e., on the right. The target-shaped landmark was hence placed on the right side in front of the starting box (normally stuck up vertically against the wall), indicating the correct arm (C, green area) for which the removable door was withdrawn, allowing direct access to the living area for the fish. On the contrary, the removable door at the end of the wrong arm (W, red area) was left in place, forming a cul-de-sac. The fish was trained in this configuration until it would reach the learning criterion, that is, nine correct choices out of ten consecutive trials during training, and four correct choices out of five consecutive trials during reinforcement.* ***D*** *– Probe trial configuration: after reaching either the training or the reinforcement learning criterion, the fish was submitted to a probe trial assessing which orientation strategy it prioritizes when orientation cues are set in conflict. The target-shaped landmark was placed on the opposite side compared to the training configuration, here on the left, and the removable doors at the end of both arms were removed, so that the fish could access the living area on both sides. In this conflicting configuration, if the fish would choose the side of the landmark, it learnt to associate the landmark with the rewarded arm during training, hence it uses a beaconing strategy (B, left arm). If the fish would choose the usual training side, it learnt to associate a particular turn with the rewarded arm during training, hence it uses a motor response strategy (MR, right arm). Figure modified from "Dellinger, M., Suret, L., Choblet, M., Jozet-Alves, C., & Benhaïm, D. (n.d.). Methodological approach to investigating spatial cognition in fishes: a* case study on Arctic charr (Salvelinus alpinus). Journal of Fish Biology, in revision".

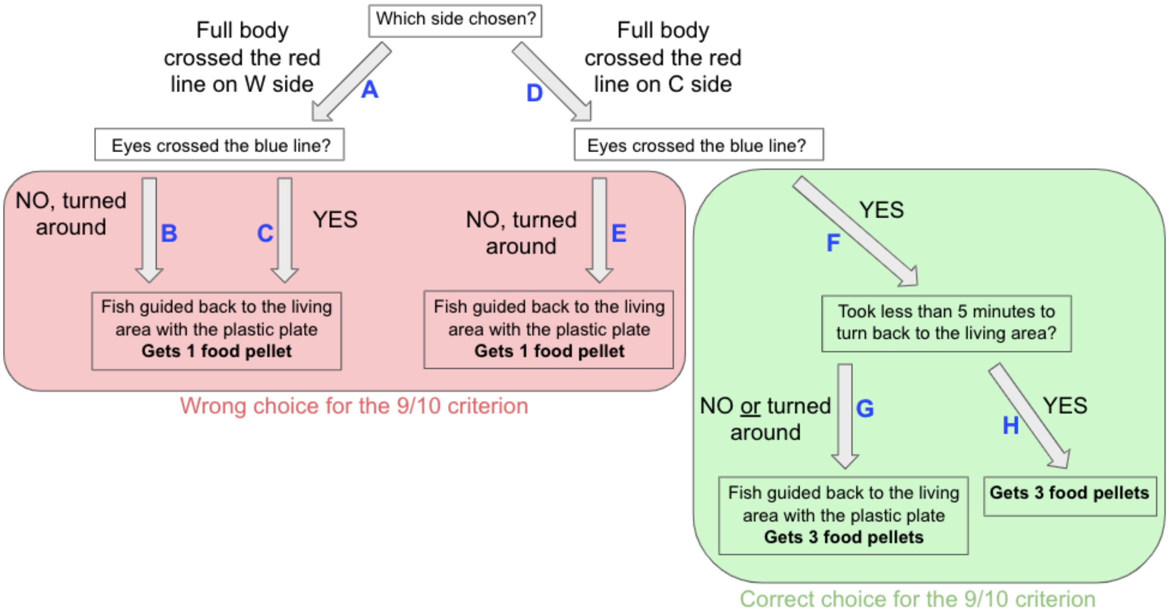

Figure S3 – Decision tree describing the course of actions after a fish chose a side in the T-maze during training or reinforcements. Red and blue lines, W and C sides mentioned respectively refer to virtual lines and zones represented on Figure S2C. The fish was considered to have made a choice when its full body crossed a virtual red line. When a fish chooses either the wrong (path A) or the correct side (path D), it always has the opportunity to confirm its choice (eyes crossing the subsequent blue line) and realise whether or not its choice led it back to the rewards, i.e., an open door to the living area on the correct side (path D-F) vs. a cul-de-sac on the wrong side (path A-C). However, the subject was not allowed to go backwards, hence if the fish turns around, that is, eyes crossing back the previous line (red line for paths A-B and D-E, or blue line for path D-F-G), the trial ends and the fish is guided back to the living area through the correct arm. All paths departing from path A were considered as wrong choices (unrewarded side). Path A-C was considered a confirmed wrong choice, which would end the trial and the fish would be guided back to the living area through the correct arm. Path D-E was considered a wrong choice as well, because the fish did not confirm it by crossing the subsequent blue line with its eyes and turned around instead, which would end the trial and the fish would be guided back to the living area through the correct arm. In these cases (wrong choice, red box), the fish was fed only one food pellet (minimum food reward to make fish associate going back to the living area and access to a food reward). A choice was considered correct only when the fish confirmed its choice on the correct side by crossing the subsequent blue line with its eyes and notice the open door (path D-F). From there, the fish was given 5 minutes to go back to the living area on its own (path D-F-H). If the fish turns around (eyes crossing back the blue line) or takes more than 5 minutes (path D-F-G), it ends the trial and the fish is guided back to the living area through the correct arm. In both cases (correct choice, green box), the fish is given the maximum food reward of three pellets. Figure modified from "Dellinger, M., Suret, L., Choblet, M., Jozet-Alves, C., & Benhaïm, D. (n.d.). Methodological approach to investigating spatial cognition in fishes: a case study on Arctic charr (Salvelinus alpinus). Journal of Fish Biology, in revision".

Methods S1 – Complete and detailed protocol of the spatial cognitive test

This test was aimed at determining in each morph and treatment groups (1) the spatial learning performances of each fish regarding navigation towards a rewarded location in a T-maze, using a motor response strategy (turning right or left towards the reward's location) and/or a beaconing strategy (associating a visual landmark with the reward's location), and (2) which of those two strategies the fish prioritise when cues are set in conflict. The cognitive test consisted in 6 phases (Figure S1) was run between 15/11/2020 and 26/02/2021 for the LB (starting at 384 days post hatching (dph)), 14/02/2021 and 27/05/2021 for the AN (starting at 384 dph), 26/12/2021 and 20/04/2022 for the VB (starting at 379 dph), 26/01/2022 and 26/06/2022 for the VS (starting at 387 dph) and 02/03/2022 and 13/08/2022 for the PL (starting at 393 dph).

Transfer phase: introducing a new fish in an arena

When a fish is taken out of an arena (Figure S2A) after completion of the experiment, delicately scrap all of the surfaces of the arena as well as the water outlet's grid with a brush, completely empty the water by siphoning all the water, dry every surfaces of the arena with paper towel (including the sliding doors and the shelters) and leave it to fully dry before you add water again at the end of the day for the next fish to be placed in the day after.

We created a list randomising the testing order of the 24 individuals within morphs, as well as between morphs on the overlapping periods. When a subject was to be introduced in an arena, at around 09:00 one fish was chosen at random from the tank designated by the randomisation list, gently netted, identified thanks to its PIT tag, anaesthetized in a 200 ppm 2-phenoxyethanol solution to avoid any stress potentially caused by the transfer, weighed and measured, and transported in the anaesthetic solution to the cognitive testing room.

There, a small container was filled with the water from the fish's tank of origin, and placed in the living area, the arena being filled with water and the inlet flowing already since the evening before (*Cf. supra*). The fish was transferred from the anaesthetic solution to the container, the removable net was then placed above the container to prevent jumps, the black box closed back, and the fish was left to recover in this bain-marie, undisturbed for an hour. After one hour, at around 10:00, the fish was released by tilting the container and pouring all its content in the living area, the removable net placed above the living area and the black box closed for an additional acclimation hour. After this hour, at around 11:00, the experimenter opened the black box and hand fed the fish five food pellets in the living area, marking the start of the familiarization phase. The familiarization phase would last for three days, transfer included, during which the subject would get used to the new water conditions and get to learn to feed and live in this new home. The fish was fed three rations of five food pellets a day (at approximately 09:00, 13:00 and 17:00, except the very first meal given at 11:00 as described above). Outside those three feedings per day, the subject was left completely undisturbed with the black box kept shut at all times.

Laterality test and maze discovery

From this phase on, trials monitoring is done remotely through live videos on a computer screen. Just before the last ration of the last day of familiarization, the subject performed a laterality test which allowed it to discover the maze for the first time, understand that there was a way out towards the safe living area, but also allowing to assess the subject's preferred turning side. This one-shot laterality test was confirmed a reliable lateralization assessment thanks to a pilot study similar to "*Alves, C., Chichery, R., Boal, J. G., & Dickel, L. (2007). Orientation in the cuttlefish Sepia officinalis: Response versus place learning. Animal Cognition, 10(1), 29–36. https://doi.org/10.1007/s10071-006-0027-6*". This pilote study showed that when fish were submitted 10 consecutive times to such a laterality test, the side chosen on the first trial was consistent with the individual laterality index, in other words, the side chosen the most across all 10 trials.

1) Place the camera in the top hole of the black box.
2) Open the black box door.
3) Gently guide the fish in the starting box with the hand net and close the door behind it. NB: no need to chase the fish, they usually avoid the net from a distance, swimming away in the opposite direction.
4) Make sure there is no uneaten food pellets or faeces lying around in the maze or the living area. If so, siphon them all out to prevent potential odours hinting the fish out. This also allows the food rewards distributed only once the subject solves the maze to be recognized as such and not mistaken with previous remains.
5) Shut the water inlet to prevent potential currents hinting the fish out.
6) Open both exit doors of the maze leading back to the living area (left and right arms).
7) Close the black box door to leave the fish fully undisturbed and launch the video recording.
8) Leave the fish for acclimation for 2 minutes.
9) After the 2 minutes, delicately lift the transparent door of the starting box thanks to pulley rope. Hook the pulley rope on the black box side.
10) The fish has 20 minutes to come out of the starting box and venture in the maze. If it doesn't come out after 20 minutes, guide it back through the starting box opaque into the living area door with the plastic plate (opaque plastic plate made out of the same material used to build the arenas) and leave it alone

for 30 minutes. Start all over again after the 30 minutes. If the fish did not come out after 3 attempts, this fish is considered as not responding to the test and it should be evicted and put back in its home tank of origin. *NB: this never happened during our experiment.*

11) When the fish comes out of the starting box, gently close the transparent pulley door directly behind the fish very cautiously not to frighten it, to prevent the fish from going back into the starting box.
12) Record which side it first chooses (*i.e.* the whole body crossing one of the virtual red lines on Figure S2B) as its side-turning preference.
13) Leave the fish to explore the maze *ad libitum* until it gets back by itself into the living area. Once it is back, open the black box door and the put the exit doors of the maze back in place. If the fish returned into the maze meanwhile, gently guide it out with the plastic plate and close the exit doors.
14) Give the fish the 5 pellets of its last food ration of the day, for the subject to understand that going back to the living area is associated with receiving food.
15) Stir the water in the maze from left to right at least six times with the plastic plate, in order to equally distribute the olfactory cues potentially deposited by the fish during the trial, thus preventing potential olfactory navigation in subsequent trials.
16) Close the black box door, stop the video recording, and leave the fish alone for 30 minutes with its rewards (food, shelters, lower light exposure and peace being home)
17) After the 30 minutes, open the black box to re-open the water inflow. This operation is delayed this way because it necessitates to introduce the hand beneath the net sealing the living area, which could disturb the subject in its interactions with the rewards.
18) Close back the black box and leave the fish fully undisturbed until the next morning when it would start the training phase.

Training and reinforcement phases

The goal of the training phase was to train the subjects until they learn to associate the opposite of their side-turning preference with a reward. From this phase on and until the subject completed the test, the food rewards distributed after a trial were the only rations dispensed, in order to avoid over-feeding and maintain motivation to participate in the tasks. The goal of reinforcement phases was to maintain and reinforce learning. Both training trials and reinforcement trials were conducted the same way. The fish were individually tested in rounds, one after the other from arena 1 to arena 6. This would give each subject at least 30 minutes to interact with their rewards while the other fish were being tested before starting a new trial. Three consecutive rounds completed one session. The training phase was composed of two training sessions per day, one in the morning and one in the afternoon, every day. Hence the subjects were trained six times a day, with a minimum of 1.5-hour break between the two sessions to let them recover from previous trials and maintain their motivation to perform the tasks. Also, the black boxes were opened only to set up or conclude a trial, otherwise kept shut at all times. To exit the maze, the fish could choose a side through a turn (left or right, motor response strategy) and/or by following the landmark on the wall (beaconing strategy). The pulley-operated transparent door, between the starting box and the T-maze, allowed the fish to see a part of the maze while in the starting box. In this configuration, the subject could see the landmark and the two possible directions when still in the starting box, but could not see which arm of the maze was rewarded (open door) from the starting box or from the centre of the maze (Figure S2C).

1) Place the camera in the top hole of the black box.
2) Open the black box door and place the target-shaped landmark (8 black-and-white rings, 5 cm diameter) against the wall facing the starting box, on the opposite side of the side-turning preference recorded for the individual. The proximal edge of the landmark should touch the virtual red line on Figure S2C, the distal edge of the landmark is facing the virtual blue line. The target should be fully immersed in the water, upper edge grazing the surface.
3) Gently guide the fish in the starting box with the hand net and close the door behind it. NB: no need to chase the fish, they usually avoid the net from a distance, swimming away in the opposite direction.
4) Make sure there is no uneaten food pellets or faeces lying around in the maze or the living area. If so, siphon them all out to prevent potential odours hinting the fish out. This also allows the food rewards distributed only once the subject solves the maze to be recognized as such and not mistaken with previous remains.
5) Shut the water inlet to prevent potential currents hinting the fish out.
6) Open the exit door of the maze on the same side as the landmark, *i.e.*, on the **opposite side of the side-turning preference** recorded for the individual, doing the same hand gesture on both exit sides so that the fish cannot infer which door was opened.
7) Record the temperature in the maze, in the middle of the neutral zone (Figure S2C), with a thermometer.
8) Close the black box door and launch the video recording and leave the fish for acclimation for 2 minutes.
9) After the 2 minutes, delicately lift the transparent door of the starting box thanks to pulley rope. Hook the pulley rope on the black box side.
10) The fish has 20 minutes to come out of the starting box and go in the maze.

- If it doesn't come out after 20 minutes, guide it back through the starting box opaque door into the living area with the plastic plate, the fish is considered not having responded to the test and this trial does not count. Leave this fish alone, do not feed it, and go on testing the next fish.

***NB*:** if the fish doesn't come out for 6 out of 9 consecutive trials, this fish is considered as not having responded to the test and it should be discarded. If the fish goes out of the starting box before the 20 minutes, continue with the next steps.

- When the fish comes out of the starting box, close the transparent door directly behind the fish, very cautiously not to frighten it. The fish has 20 minutes to make a choice.

***NB*:** If the fish stays for more than 20 minutes in the neutral zone (Figure S2C) without making a choice, guide it back through the correct arm into the living area with the plastic plate. The fish did not respond to the test and this trial doesn't count. Leave this fish alone, do not feed it, and go on testing the next fish. If the fish makes a choice (full body crossing one of the virtual red line on Figure S2C), follow the decision tree in Figure S3.

12) Once the fish is back in the living area, close the exit door and feed it the ration indicated in the decision tree (Figure S3).
13) Take the landmark out and use the plastic plate to stir the water in the maze from left to right at least six times with the plastic plate, in order to equally distribute the olfactory cues potentially deposited by the fish during the trial, thus preventing potential olfactory navigation in subsequent trials.
14) Close the box door, stop the video recording, record the fish choice for the trial (correct or wrong), and let the fish alone with its rewards (food, shelters, lower light exposure and peace being home) for at least 30 minutes before starting a new trial with it.
15) Start the whole process again on the next fish, until all fish are tested (1 round). Do 3 consecutive rounds to complete one session.
16) At the end of the session, wait for 30 minutes and then open the black box to re-open the water inflows in the living areas from maze 1 to maze 6. This operation is delayed this way because it necessitates to introduce the hand beneath the net sealing the living area, which could disturb the subject in its interactions with the rewards.
17) Close back the black box and leave the fish fully undisturbed until the next morning for the next session.

NB: Learners' training criterion = 9 correct choices out of 10 consecutive trials; Reinforcement criterion = 4 correct choices out of 10 consecutive trials; Non-learner = a fish that did not show any sign of learning after 93 training trials.

Probes

Probe trials follow directly the trial at which the fish reached the training or reinforcement criterion (see further). During a probe trial, the fish must solve the task based on what it learnt during the training phase. Navigational cues are set in conflict and the choice of the subjects indicates which orientation strategy they preferentially use to locate the reward (beaconing strategy vs. motor response strategy). Note that choosing one strategy during a probe trial does not imply that the fish did not learn to use the other strategy as well during training. That's why we refer to prioritising one strategy over the other to orient during probe trials, as the subject can only use one strategy at a time to solve the maze when orientation cues are set in conflict. Learners performed 3 probe trials to confirm the strategy prioritised, with reinforcement sessions in-between each (Probe 1 — Reinforcement — Probe 2 — Reinforcement — Probe 3).

1) Place the camera in the top hole of the black box.
2) Open the black box door and place the target-shaped landmark (8 black-and-white rings, 5 cm diameter) against the wall facing the starting box, on the training side, *i.e.*, the side-turning preference recorded for the individual. The proximal edge of the landmark should touch the virtual red line on Figure S2D, the distal edge of the landmark is facing the virtual blue line. The target should be fully immersed in the water, upper edge grazing the surface.
3) Gently guide the fish in the starting box with the hand net and close the door behind it. NB: no need to chase the fish, they usually avoid the net from a distance, swimming away in the opposite direction.
4) Make sure there is no uneaten food pellets or faeces lying around in the maze or the living area. If so, siphon them all out to prevent potential odours hinting the fish out. This also allows the food rewards distributed only once the subject solves the maze to be recognized as such and not mistaken with previous remains.
5) Shut the water inlet to prevent potential currents hinting the fish out.
6) Open both exit doors of the maze leading back to the living area (left and right arms both rewarded, in order to reinforce learning; no mistake possible to prevent the subject from learning a rewarded location in the probe configuration that could interfere with the next trials)
7) Record the temperature in the maze, in the middle of the neutral zone (Figure S2D).
8) Close the black box door and launch the video recording and leave the fish for acclimation for 2 minutes.
9) After the 2 minutes, delicately lift the transparent door of the start box thanks to the pulley rope. Hook the fishing rope on the black box side.

10) The fish has 20 minutes to come out of the starting box and go in the maze.
    - If it doesn't come out after 20 minutes, gently guide it back through the opaque door of the starting box into the living area with the plastic plate, the fish is considered not having responded to the test and this trial does not count. Leave this fish alone, do not feed it, and go on testing the next fish.
    - If the fish goes out of the starting box before the 20 minutes, continue with the next steps.
11) When the fish comes out of the starting box, close the transparent door directly behind the fish very cautiously not to frighten it. The fish has 20 minutes to make a choice.
    - If the fish stays for more than 20 minutes in the neutral zone (Figure S2D) without making a choice, guide it back to the living area with the plastic plate. The fish did not respond to the test and this trial doesn't count. Leave this fish alone, do not feed it, and go on testing the next fish.
    - If the fish makes a choice (full body crossing one of the virtual red lines on Figure S2D), leave the fish to navigate freely in the maze until its eyes would cross a blue line on either side, regardless of whether the subject would turn around after crossing red lines several times on either side. (*i.e.*, do not guide the fish to the living area if it crosses a red line and turns back, wait until it crosses a blue line). Record the choice when the eyes cross a blue line (Figure S2D), *i.e.*, motor response if the eyes cross the blue line on the training side, or beaconing if the eyes cross the blue line on the landmark side. Then, guide the fish back to the living area on the side it chose with the plastic plate only if it turned around (eyes crossing the previous blue line) or took more than 5 min to go back in the living area on its own.
12) Once the fish is back in the living area, close both exit doors (so that the fish does not see that the door on the opposite side was also open) and feed it a full ration of 3 pellets.
13) Take the landmark out and use the plastic plate to stir the water in the maze from left to right at least six times with the plastic plate, in order to equally distribute the olfactory cues potentially deposited by the fish during the trial, thus preventing potential olfactory navigation in subsequent trials.
14) Close the box door, stop the video recording, record the fish choice for the trial (correct or wrong), and let the fish alone with its rewards (food, shelters, lower light exposure, and peace being home) for at least 30 minutes before starting a new trial with it.

NB: A fish always performs 2 sessions of 3 trials a day. The only exception is when the fish reaches the training or reinforcement criterion on the last trial of the afternoon session: in this case the probe trial follows directly in an additional trial after the afternoon session, to standardise the probe following directly the criterion achievement. Wait for at least 30 minutes before performing this additional trial, so that the fish can interact with its rewards as usual between two trials.

Table S1 - Distribution of the fish that participated in the cognitive test, per morph, treatment, tank replicate and learning capacity (Y: learners, i.e., reached the 9/10 training criterion; N: non-learners, i.e., did not reach the 9/10 training criterion after 93 trials, E: evicted, i.e., for not responding to the test in 6 out of 9 consecutive trials, or for technical reasons indicated in the footnote).

Morph	**Status**	**Treatment Enriched**				**Treatment Plain**				**TOTAL**
		Replicate 1	Replicate 2	Replicate 3	**Total**	Replicate 1	Replicate 2	Replicate 3	**Total**	
AN	Y	4	4	5	**13**	2	4	4	**10**	**23**
	N	0	1	0	**1**	1	0	1	**2**	**3**
	E	1	0	0	**1**	4	1§	1	**6**	**7**
	Total	**5**	**5**	**5**	**15**	**7**	**5**	**6**	**18**	**33**
		Replicate 1	Replicate 2	Pseudo-replicate		Replicate 1	Replicate 2	Pseudo-replicate		
VS	Y	3	4	4	**11**	3	2	4	**9**	**20**
	N	1	0	1	**2**	1	2	0	**3**	**5**
	E	0	0	0	**0**	0	0	0	**0**	**0**
	Total	**4**	**4**	**5**	**13**	**4**	**4**	**4**	**12**	**25**
		Replicate 1	Replicate 2	Replicate 3			Replicate 2	Replicate 3		
VB	Y	3	3	4	**10**		6	5€#	**11**	**21**
	N	1	1	0	**2**		0	1#	**1**	**3**
	E	0	0	0	**0**		1	1	**2**	**2**
	Total	**4**	**4**	**4**	**12**		**7**	**7**	**14**	**26**
		Replicate 1	Replicate 2	Replicate 3		Replicate 1	Replicate 2	Replicate 3		
LB	Y	4#	3	2	**9**	5°	4	4	**13**	**22**
	N	0	3	3	**6**	0	0	0	**0**	**6**
	E	1*	0	1*	**2**	1	1*	0	**2**	**4**
	Total	**5**	**6**	**6**	**17**	**6**	**5**	**4**	**15**	**32**
		Replicate 1	Replicate 2	Replicate 3		Replicate 1	Replicate 2	Replicate 3		
PL	Y	4	3	2£$	**9**	4	2	4	**10**	**19**
	N	0	2	3	**5**	1	2	1	**4**	**9**
	E	0	0	0	**0**	0	0	1	**1**	**1**
	Total	**4**	**5**	**5**	**14**	**5**	**4**	**6**	**15**	**29**
TOTAL		**22**	**24**	**25**	**71**	**22**	**25**	**27**	**74**	**145**

§ — Evicted due to time constrains.

€ — One of these fish did not have a boldness score as it did not come out of the shelter during both OFT replications.

— One of these fish did not exit the shelter in one of the two OFT replications.

NB: Fish from € and # were counted as learners or non-learners where appropriate but were not used in the analyses related to boldness.

* — Evicted because of a snowstorm preventing access to the lab, hence interrupting these fish's training for 2 days.

° — One of these fish had reached the 9/10 training learning criterion but had to be evicted because of the snowstorm as well. This fish was counted as a learner but did not participate in probes and reinforcements and was not included in genetics analyses.

£ — One of these fish had reached both the 9/10 training criterion and the first 4/5 reinforcement criterion but never reached the second 4/5 reinforcement criterion and was evicted after 58 trials in the second reinforcement phase. This fish was counted as a learner with its probe trials included in analyses but was not included in genetics analyses.

$ — One of these fish had reached the 9/10 training learning criterion, but never reached the first 4/5 reinforcement criterion because it reached the 6/9 eviction criterion during the first reinforcement phase. This fish was counted as a learner but was not included in genetics analyses.

Finally, the cerebellum of one VB enriched fish from the tank replicate 3 was too damaged during the microdissection and was hence excluded from gene expression analyses.

Methods S2 – Primers' design and optimization methods

To design the primers for our genes of interest, we used total mRNA extracted from the entire body of new-born Arctic charr from the VB morph, as new-born individuals are expected to express all genes during early development. New-borns were euthanized in a 1000 ppm 2-phenoxyethanol solution, rinsed, immediately preserved in DNA/RNA shield (Zymo Research), and stored at -20°C until analyses. To extract RNA from these tissues, we used the Monarch total RNA miniprep kit (New England Biolabs) as described in the main text, except we used 600 µL of 1X DNA/RNA Protection Reagent for newborns instead of the 300 µL used for brain tissues. At the end of the extraction, all RNA concentrations were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific) and all extracted RNA were stored at -80°C.

The cDNA synthesis was performed thanks to the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) as described in the main text. All synthesized cDNA were stored at -20°C.

Arctic charr orthologs of all genes of interest were found on the NCBI database and checked thanks to BlastN alignments against other salmonids, such as Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). For each gene of interest, two primer pairs were designed thanks to NCBI's Primer-BLAST tool with the following parameters:

- amplicon size between 70 and 150 bp
- melting temperature between 57°C and 63°C
- primers must span and exon-exon junction or the pair must include an intron of at least 1000 bp to avoid any genomic DNA amplification
- the pair does not amplify another part of the Arctic charr genome

Newly designed primers were tested with qPCR (as detailed in the main text) using the new-borns' cDNA. The primers' efficiencies were measured by amplifying new-born cDNA at five different concentrations by qPCR: 1, 1/10, 1/100, 1/1000 and 1/10000. Two negative control were used, a milliQ water negative control and an RNA control to check if the pair amplifies genomic DNA. The common logarithm of the initial amount of cDNA for each concentration used as a function of the Cq value was traced and the slope was recovered. The efficiency (E) was calculated according to the following formula:

$$E = 10^{-1/slope}$$

As the melting curve represented the melting temperature of the amplicon, several peaks meant the presence different amplicons. Hence, for a primer pair to be validated, its melting curve had to present only one peak that had to be at the same melting temperature for all dilutions of cDNA. Moreover, the pair had not to amplify the RNA control. Finally, we wanted a pair's efficiency to range between 1.8 and 2.2. In cases where the efficiency was higher than 2.2 but the melting curve was accepted, the primers were tested with a lower concentration respecting the limit range of the SYBR Green master mix. After that, if the efficiency was still higher than 2.2 at 200 nM, the primers were designed again.

Eventually, only *c-fos.1* and *reelin* primers were validated with efficiencies higher than 2.2 as last resort (2.29 and 2.30 respectively, deemed acceptable deviation). Final primers' sequences validated and their efficiencies can be found in Table S2.

Table S2 – Primers used for qPCR and for sex determination PCR, with their mRNA accession number on GenBank. Primer's nomenclature: "gene name(+subunit for pp1: a= alpha; b = beta; g = gamma).paralog number" Tm: melting temperature. Eff: primer's PCR efficiency. nM: optimal concentration of the primer used in the qPCR mix (in nM). A sample on a qPCR plate would always be tested for both reference genes (actb and ub2l3, Ahi et al., 2013*), and two target genes consistently tested in randomly assigned pairs across all samples. Hence, the last column indicates the other primer paired with the target (not applicable for sex determination primers, for which "Sexing" is than indicated).*

MARKER	PRIMER	ACCESSION NUMBER	PRIMER SEQUENCE (5' → 3') Forward	Reverse	Eff	nM	PAIRED WITH
References	*actb*	JR540730	GAAGATCAAGATCATCGCCC	CAGACTCGTCGTACTCCTGCT	1.95	500	*ub2l3*
	ub2l3	JR540740	CGAGAAGGGACAGGTGTGTC	ACCAACGCAATCAGGGACT	1.93	500	*actb*
	etbr2		GAGCTGTCCTTGGCTTTGTC	ACGCCCTGGTCATCAACTAC	-	-	Sexing
Sex	*SdY*		TTGGGCCTATGAATTTCTGATGTTG	TTCATATCACTCACCCTGTCTGAAG	-	-	Sexing
Neurogenesis & neural plasticity	*bdnf*	XM_023995638.2	TGGGCGATCTACTGAGCAC	ACTCTTCTAACCTGGTGGAACATTT	2.00	200	*mr*
	c-fos.1	XM_024140347.1	GCTTGCGACAACCTGGCTTA	CAGGTCTGTGAAGTCCTGAGA	2.29	200	*crebbp*
	c-fos.2	XM_024138101.1	CTCCCCAATCTCAGGACTTCAC	TGTGAGAAGGTGCCACAGAA	2.00	350	*mao*
	egr1	XM_024140455.1	GACTCCCTTCCTCTCTGCATCA	GATATCAGGTAACGTATCTCCAGC	2.20	500	*pp1a.1*
	neurod1	XM_024007224.2	AGTCGAGTCGTATTTCATTTCACAC	ATTGCTAAGGCAACCGATTTGT	2.19	200	*gr*
	neurod1-like	XM_023997937.1	ACATACTCTGCATTAAAAAGAGGAC	CGCCGTCCTTACTCATGGTT	2.20	200	*pp1a.2*
	pcna	XM_023978361.1	TGGGAGTCAATCTTAGCAGTATGT	CGAGTGTGTCTGCGTTGTC	2.18	500	*pp1g.2*
Learning & Memory	*crebbp*	XM_024142785.1	CTGGCCATCGGGCTTCATT	CAACCTCGTCGTCTGTAGCC	2.10	500	*c-fos.1*
	pp1a.1	XM_024007673.1	TTTGCGAAGAGGCAGCTAGT	CAGGCTTGAGGATCTGGAAGG	2.00	500	*egr1*
	pp1a.2	XM_024010867.1	GACTATTGTCAAATCAAAGCAACAC	CTGGCCGAGAGCCCTTAAC	1.95	200	*neurod1-*
	pp1b	XM_023973977.1	CTCTGCTGGTGAGCACGAC	GGACGACATCCTCGCACTTC	1.93	500	*reelin*
	pp1g.1	XM_024002585.1	CCCTCATGTGCTCTTTTCAGATTT	AGCAGAATAGAGAGCAGGACATA	2.20	500	*dbh*
	pp1g.2	XM_023978738.1	ACCAGCACTAGATTTGCACT	AGGTTTTGCACCTCTGACTTCT	2.10	500	*pcna*
	reelin	XM_024134606.1	AGTTCTCGTTGGTCATGGGC	TGGTAGGCAGGCCTCTTTGA	2.30	500	*pp1b*

Table S3 – Continued

MARKER	PRIMER	ACCESSION NUMBER	PRIMER SEQUENCE (5’ → 3’)		Eff	nM	PAIRED WITH
			Forward	Reverse			
Stress Response	*gr*	XM_024011109.2	AACCATTCAGCGTTACACGAG	ATCCATTTCTTGATCCTGCTTAGTC	1.80	500	*neurod1*
	mr	XM_024136927.1	ACAGGCTTGGTTTCGTGTCT	CGCTGGGTCATTGATTTACCTTA	1.84	200	*bdnf*
Monoamines Metabolism	*dbh*	XM_024010730.1	AACGTTGACCCTGGATACCTG	CTGACCACAGCTACACGCAT	2.02	200	*pp1g.1*
	mao	XM_024010934.1	CGATTGGCTAAAGAGTGCGG	CATCAGGGCGAATGGGTTCC	2.10	200	*c-fos.2*

Table S4 – Results of χ^2-tests comparing proportions of different variables between different groups. Df: degrees of freedom; P: p-value.

PROPORTIONS OF	BETWEEN	Df	χ^2	P
Males *vs.* Females	Morphs	4	4.288	> 0.05
Left *vs.* Right side-turning	Morphs	4	5.373	> 0.05
Learners *vs.* Non-learners	Morphs	4	4.648	> 0.05
	Treatments	1	1.205	> 0.05
	Morph-by-treatment	9	12.870	> 0.05
	Sex	1	1.403	> 0.05
	Side-tuning preference	1	1.862	> 0.05
Motor response *vs.* Beaconing navigation strategies in Probe 1	Morphs	4	5.362	> 0.05
	Treatments	1	0.485	> 0.05
	Morph-by-treatment	9	7.916	> 0.05
	Sex	1	0.009	> 0.05
	Side-turning preference	1	1.685	> 0.05
Motor response *vs.* Beaconing navigation strategies in Probe 2	Morphs	4	2.250	> 0.05
	Treatments	1	0.190	> 0.05
	Morph-by-treatment	9	5.795	> 0.05
	Sex	1	0.154	> 0.05
	Side-turning preference	1	1.831	> 0.05
Motor response *vs.* Beaconing navigation strategies in Probe 3	Morphs	4	3.235	> 0.05
	Treatments	1	0.169	> 0.05
	Morph-by-treatment	9	4.178	> 0.05
	Sex	1	0.855	> 0.05
	Side-turning preference	1	1.097	> 0.05

Table S5 – Results of either two sample t-tests or Wilcoxon rank sum tests comparing mean boldness (boldness score on the first OFT replication) or relative expression (RE) of all genes of interest (except neurod1-like presenting an incompatible distribution) within different groups. Morphs: AN from Fljótaá River, sympatric VS and VB from Lake Vatnshlíðarvatn, and sympatric PL and LB from Lake Þingvallavatn. Treatments: enriched and plain. Df: degrees of freedom. P: p-value. Significant terms are highlighted in bold black font, while tendencies are highlighted in bold grey font.

VARIABLE COMPARED	**GROUPING FACTOR**	**TWO SAMPLE T-TEST**			**WILCOXON RANK SUM TEST**	
		t	**Df**	**P**	**W**	**P**
Boldness	**Overall**	—	—	—	1176	0.550
	AN	—	—	—	39	0.762
	VS	—	—	—	68	0.243
	VB	-0.338	19	0.739	—	—
	PL	0.297	26	0.769	—	—
	LB	—	—	—	41	0.216
	Enriched	—	—	—	309	0.148
	Plain	—	—	—	275	0.352
	AN Enriched	*Cannot be tested: only one non-learner*				
	AN Plain	-0.184	10	0.858	—	—
	VS Enriched	1.486	11	0.166	—	—
	VS Plain	0.517	10	0.616	—	—
	VB Enriched	-0.084	10	0.935	—	—
	VB Plain	*Cannot be tested: all learners*				
	PL Enriched	-0.378	12	0.712	—	—
	PL Plain	1.070	12	0.306	—	—
	LB Enriched	-1.171	12	0.264	—	—
	LB Plain	*Cannot be tested: all learners*				
RE *bdnf*	**Cerebellum**	—	—	—	**310**	**0.082**
	Optic tectum	0.417	110	0.678	—	—
	Telencephalon	—	—	—	832	0.123
RE *c-fos.1*	**Cerebellum**	—	—	—	**792**	**0.069**
	Optic tectum	—	—	—	937	0.235
	Telencephalon	—	—	—	970	0.234
RE *c-fos.2*	**Cerebellum**	—	—	—	886	0.127
	Optic tectum	—	—	—	948	0.214
	Telencephalon	—	—	—	948	0.238
RE *crebbp*	**Cerebellum**	—	—	—	1043	0.739
	Optic tectum	—	—	—	1090	0.868
	Telencephalon	—	—	—	**892**	**0.089**

Table S5 – Continued

VARIABLE COMPARED	**GROUPING FACTOR**	**TWO SAMPLE T-TEST**			**WILCOXON RANK SUM TEST**	
		t	**Df**	**P**	**W**	**P**
RE *dbh*	**Cerebellum**	—	—	—	1077	0.800
	Optic tectum	—	—	—	974	0.314
	Telencephalon	—	—	—	1035	0.404
RE *egr1*	**Cerebellum**	—	—	—	**728**	**0.020**
	Optic tectum	—	—	—	977	0.225
	Telencephalon	—	—	—	**818**	**0.072**
RE *gr*	**Cerebellum**	—	—	—	1013	0.592
	Optic tectum	—	—	—	**889**	**0.074**
	Telencephalon	—	—	—	**823**	**0.096**
RE *mao*	**Cerebellum**	—	—	—	915	0.205
	Optic tectum	-0.051	120	0.810	—	—
	Telencephalon	-1.649	119	0.102	—	—
RE *mr*	**Cerebellum**	—	—	—	1029	0.778
	Optic tectum	-0.538	110	0.591	—	—
	Telencephalon	—	—	—	859	0.175
RE neurod1	**Cerebellum**	—	—	—	1051	0.465
	Optic tectum	—	—	—	1104	0.699
	Telencephalon	—	—	—	**822**	**0.044**
RE *pcna*	**Cerebellum**	—	—	—	**1279**	**0.027**
	Optic tectum	—	—	—	1248	0.158
	Telencephalon	—	—	—	**1376**	**0.064**
RE *pp1a.1*	**Cerebellum**	—	—	—	**731**	**0.095**
	Optic tectum	—	—	—	**673**	**0.018**
	Telencephalon	—	—	—	915	0.488
RE *pp1a.2*	**Cerebellum**	—	—	—	1215	0.804
	Optic tectum	—	—	—	1067	0.806
	Telencephalon	—	—	—	939	0.113
RE *pp1b*	**Cerebellum**	—	—	—	1105	0.650
	Optic tectum	—	—	—	1129	0.883
	Telencephalon	—	—	—	1128	0.760
RE *pp1g.1*	**Cerebellum**	-0.531	120	0.596	—	—
	Optic tectum	—	—	—	**867**	**0.086**
	Telencephalon	—	—	—	926	0.139
RE *pp1g.2*	**Cerebellum**	—	—	—	1164	0.685
	Optic tectum	—	—	—	997	0.279
	Telencephalon	—	—	—	981	0.236

Table S5 – Continued

VARIABLE COMPARED	**GROUPING FACTOR**	**TWO SAMPLE T-TEST**			**WILCOXON RANK SUM TEST**	
		t	**Df**	**P**	**W**	**P**
RE *reelin*	**Cerebellum**	—	—	—	1153	0.805
	Optic tectum	—	—	—	1119	0.922
	Telencephalon	—	—	—	1081	0.497