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Année universitaire : 2023 - 2024 Spécialité : **Sciences Halieutiques et Aquacoles** Spécialisation (et option éventuelle) : **AQUA**	**Mémoire de fin d'études** ☒ d'ingénieur de l'Institut Agro Rennes-Angers (Institut national d'enseignement supérieur pour l'agriculture, l'alimentation et l'environnement) ☐ de master de l'Institut Agro Rennes-Angers (Institut national d'enseignement supérieur pour l'agriculture, l'alimentation et l'environnement) ☐ de l'Institut Agro Montpellier (étudiant arrivé en M2) ☐ d'un autre établissement (étudiant arrivé en M2)

Assessing sociability in juvenile Arctic charr (*Salvelinus alpinus*) using the mirror test for aquaculture applications

Par: Chedi Hebaieb

Soutenu à Rennes, le 12/09/2024

Devant le jury composé de :

Président : Bastien Sadoul

Enseignant chercheur à l'Institut Agro Rennes-Angers

Maître de stage : David Benhaim, professeur à Hólar Université

Enseignant référent : Bastien Sadoul

Autres membres du jury

Pablo Brosset : Enseignant chercheur à l'Institut Agro Rennes-Angers

Elodie Merlot : Directrice de recherche à l'INRAE

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Acknowledgments

I would like to express my sincere gratitude to all those who supported me throughout my research and the writing of this thesis.

First and foremost, I would like to thank my advisor, David Benhaim, for his invaluable guidance, support, and encouragement. Your expertise and insights have been instrumental in shaping my research and helping me navigate the challenges I faced during this journey, especially as someone who wasn’t initially interested in research.

I am also grateful to Sabine Lobligeois, my colleague and second supervisor, for the unmatched kindness and professionalism she demonstrated during my time in Iceland. It has been a pleasure to brainstorm with you. I wish you all the success for your PhD.

Many thanks also go to Gabrielle Ladurée, a colleague in the behaviour team, who helped me tackle many difficulties in statistical programming, particularly with the statistical model I was working on for the first time.

I would like to extend my thanks to my dear colleague and roommate, Floor Kuhn, for sharing so many memories together and for the support and professionalism we exhibited during this project. I am proud of what you have achieved and wish you the best of luck in your future endeavors.

Special thanks to all my friends in Hólar, especially Bjarni Dagur Johannsson, for being an amazing friend during my stay.

I would like to thank my advisor in Rennes, Bastien Sadoul, for the time he gave to offer guidance for this project.

I also appreciated the Innovation Grant from The Icelandic Centre for Research (RANNÍS) that helped me cover some of my expenses during my internship in Iceland.

Finally, and most importantly, I would like to express my gratitude to my family, who were far away but always showed their support and belief in me. All that I have achieved is thanks to the unconditional efforts that they have put.

Summary

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Ethical note

The design of the housing and of the experimental procedures were put in place respecting the European standard animal care protocols and the Icelandic law on Animal protection (Law 15/1994, last updated with Law 55/2013). The protocols were also approved through an animal care permit (MAST – 1905641).

Introduction

Aquaculture offers seemingly optimal conditions for farmed fish, providing essential needs like food and care (Macaulay et al., 2021). However, this environment can become stressful due to various factors throughout the production cycle. Routine procedures, when conducted inadequately, may lead to conditions that compromise welfare (Folkedal et al., 2010). These procedures can be biological, physical, or chemical in nature (Davis, 2006), but the housed species may also be unable to exhibit their natural behavioural repertoire in certain housing conditions (Martins et al., 2012). Inappropriate aquaculture practices and a lack of understanding of species' domestication techniques affect not only fish welfare but also diminish productivity for fish farmers (Bagni et al., 2007). Therefore, there is significant interest in developing new methods to alleviate poor welfare conditions and promoting practices that enhance the psychological and biological well-being of farmed species (Fife-Cook & Franks, 2019). For instance, recent insights have shown that selecting fish based on behavioural traits can ensure optimal productivity, aligning with ethical aquaculture approaches (Prentice et al., 2022).

Welfare in aquaculture

Over the past two decades, a considerable interest has been accorded to the welfare of farmed fish from both scientific and social communities. As a result, this spurred a development of policies like Council Directive 98/58/EC[1] and the recommendation on the welfare of farmed fish by the Council of Europe in 2005[2] (Martins et al., 2012). Although this recognition has been accorded by different parties of the sector (Stakeholders, welfare NGOs and scientific communities), a clear consensus on how to assess and define animal welfare has been evolving (Huntingford et al., 2006).

The word "Welfare" is used in different definitions and contexts, necessitating an understanding of the different approaches employed by researchers (Appleby, 1999; Dawkins, 1998). Most

[1] **Council Directive 98/58/EC**: This directive concerns the protection of animals kept for farming purposes. You can find the full text here: Directive - 98/58 - EN - EUR-Lex (europa.eu)

[2] **Recommendation on the welfare of farmed fish (2005)**: This recommendation is issued by the Council of Europe. You can find the document here: Recommendation concerning farmed fish - European Committee on Legal Co-operation (coe.int)

definitions fall under one of the three general concepts adopted for the welfare of aquaculture fish (Figure 1) (Fraser, 2008).

Emotional definitions are set in terms of subjective mental states. Here the requirement for good welfare is that the animal should feel well, not suffer negative experiences such as pain or fear and have access to positive experiences such as companionship in the case of social species and tranquillity in the case of less social ones (Dawkins, 1998).

Functional definitions focus on an animal's ability to adapt to its current environment. In this case, good welfare requires that the animal is in good health and that its biological systems (particularly those involved in coping with the challenges of stasis) are functioning appropriately and are not forced to respond beyond their capacity (Huntingford et al., 2006).

Nature-based definitions arise from the view that each animal species has an inherent biological nature that it must express, which is normally acquired from its ancestors in the wild. Here, good welfare requires that the animal can live a natural life and express its natural behavioural repertoire. This approach reflects the view that what is natural is inherently good and focuses on something measurable: what animals do in the wild and in captivity (Huntingford et al., 2006).

The purpose of understanding various visions and concepts of fish welfare, or animal welfare in general, is to improve production practices and laboratory research beyond merely alleviating poor welfare, which includes physical health and biological needs. It is about recognizing the importance of positive welfare (i.e., mental, and physical states that exceed what is necessary for immediate survival), particularly in production and profitability contexts. Optimal housing procedures must consider the best social, mental, and emotional states of the species, inherited from its wild ancestors, which can be explored in laboratory conditions (Fife-Cook & Franks, 2019).

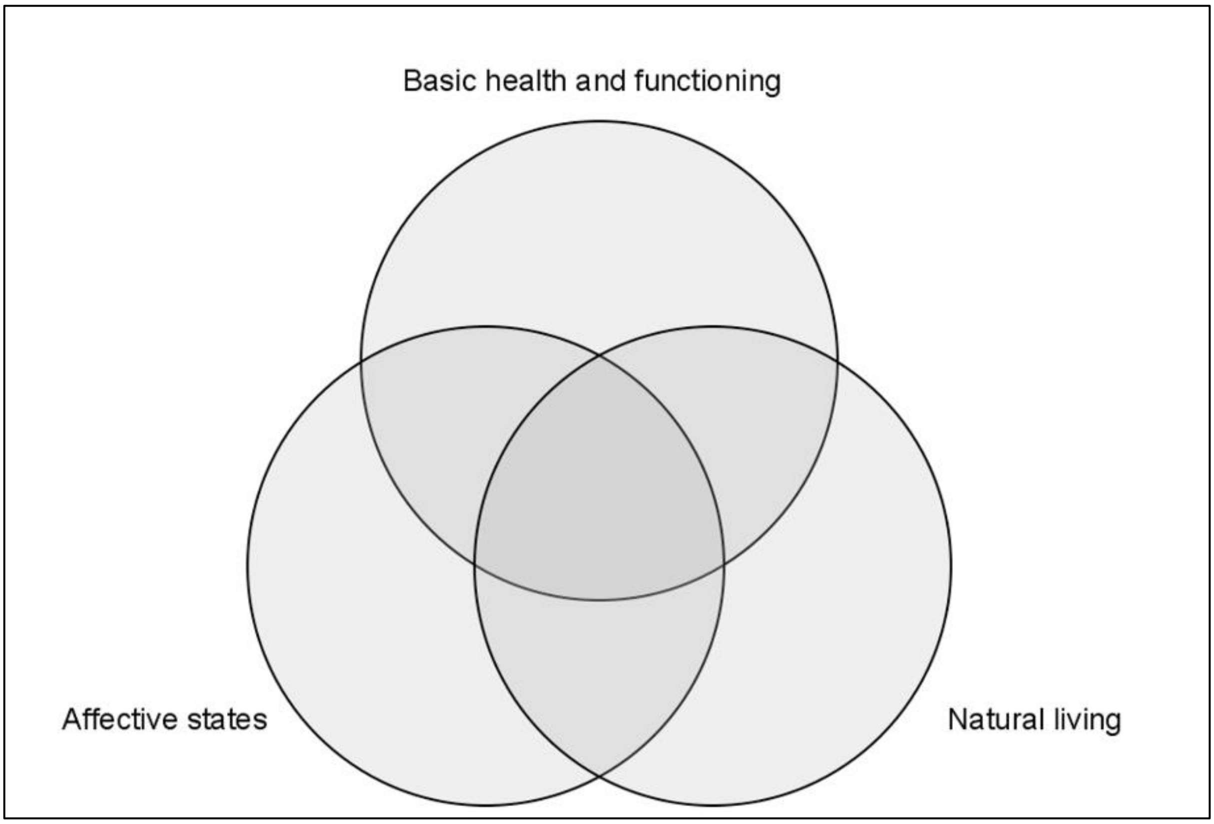


***Figure 1**: The three conceptions of animal welfare (Fraser, 2008)*

Stress Coping Style (SCS): Emergence of behavioural phenotyping in aquaculture

Stress is strongly linked to the different visions of welfare, being the main explanation for either reduced or enhanced welfare (Prentice et al., 2022). As the most used techniques to measure stress in fish are physiological (Davis, 2006), there has been an overemphasis on glucocorticoid physiology, leading to the conflation of "stress" with cortisol in some cases. Conceptual models like stress coping style (SCS) explicitly acknowledge the significance of behaviour (Castanheira et al., 2017). Coping styles are consistent sets of behavioural and physiological stress responses observed within groups of individuals over time. Shaped by evolutionary forces, these coping styles represent adaptive response patterns to the challenges encountered by fish in their natural habitat. Their predictability and consistency suggest they serve an adaptive function, aiding individuals in effectively navigating and surviving in their environment. Overall, coping styles reflect a species' ability to respond to stressors and adapt, contributing to their overall fitness[3] and survival (Koolhaas et al., 1999).

SCS suggests a functional integration of neuroendocrine and physiological pathways with individual behavioural profiles (Figure 2). According to SCS, a "proactive" stress response is characterised by behavioural immobility, such as freezing, coupled with lower glucocorticoid levels, while "reactive" types are expected to exhibit more active "fight-or-flight" behaviours along with higher glucocorticoid levels (Table 1) (Prentice et al., 2022).

Table 1: *Physiological and neuroendocrine differences between proactive and reactive coping styles (Koolhaas et al. 1999).*

	Proactive	Reactive
HPA axis activity	Low	Normal
HPA axis reactivity	Low	High
Sympathetic reactivity	High	Low
Parasympathetic reactivity	Low	High
Testosterone activity	High	Low

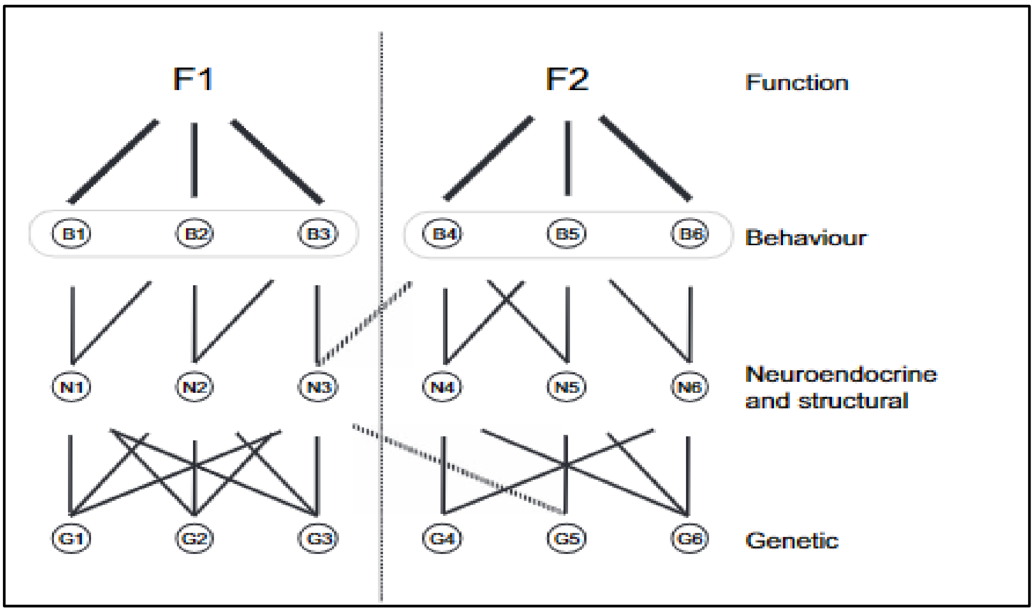


Figure 2: *Organisation of behavioural traits: F1 et F2 are two biological functions. B 1 to B6 are behaviours implicated in those biological functions. N1 to N6 are neurophysiological and structural traits explaining each behavioural trait. G1 to G6 are the genes coding for each neurophysiological and structural trait (Réale et al. 2007).*

[3] The fitness of species for aquaculture fish refers to their ability to thrive and reproduce in a controlled farming environment (Zhang et al., 2023). See more in Appendix A, Figure 1.

Behavioural changes can indicate both positive and negative states of welfare (Zhang et al., 2023), reflecting responses to stressors and other environmental conditions (Martins et al., 2012). In fact, recent research on fish behaviour has advanced significantly by exploring the genetic and neuroendocrine foundations of behaviours. This progress has been achieved through various methods including artificial selection, genetic mapping, candidate gene analysis, and functional genomics. These approaches have deepened our understanding of how genetic and physiological factors influence behavioural traits in fish populations (Conrad et al., 2011). In behavioural phenotyping, indicators such as foraging behaviour (Alanärä & Brännäs, 1996; Sánchez et al., 2009), ventilatory activity (Barreto & Volpato, 2004), swimming patterns both in individual (Herbert & Steffensen, 2005) and group context (Føre et al., 2009), stereotypic and abnormal behaviour are linked to both acute and chronic stressors in aquaculture (von Borell, 1995). Technological advancements in video surveillance and image processing offer promising opportunities for real-time monitoring of fish behaviour in aquaculture, providing a cost-effective and non-invasive means of assessing their welfare on farms. However, the study of coping styles (SCS) often reveals consistent individual behavioural patterns that can indicate underlying personality traits rather than merely responses to environmental disruptions. These coping behaviours, such as proactive or reactive tendencies, may persist across various situations, suggesting stable individual differences in how organisms perceive and react to challenges (Prentice et al., 2022).

Throughout this research, linguistic uniformity is maintained with the use of the term “Personality”, highlighting the individual identity of the fish and its reactions to social and emotional stimuli, with minimal emphasis on external stressors, whether physical or chemical in nature. The primary focus of our work on personality lies on the nature-based dimension of welfare.

Animal personality

Personality has become a major subfield of behavioural ecology over the last two decades and studies of fishes are gradually contributing to it (Bierbach et al., 2017; Houslay et al., 2018). The translation into concrete applied contexts is still in its infancy but is increasingly becoming clearer. The greatest potential for welfare gains likely to arise in housing fish species for scientific research, developing novel species for aquaculture and improving management practices in already housed species (Prentice et al., 2022).

Animal personality refers to the consistent differences in behaviour among individuals across time and/or contexts. This among-individual variation is attributable to the combined influences of genetic and environmental effects that affect the phenotype of an individual (Beukeboom, 2023; Prentice et al., 2022; Réale et al., 2010). Commonly studied personality traits include aggressiveness, activity, boldness, exploration, and sociability (Réale et al., 2007).

Réale et al. (2007) proposed these five personality axes to categorize temperament:

Boldness-Shyness Axis: This axis, initially observed by Darwin, reflects an individual's reaction to any risky situation that is not necessarily new.

Exploration-Avoidance Axis: This axis reflects an individual's response to new situations. It encompasses the tendency to either explore or avoid unfamiliar environments or stimuli.

Activity: This axis pertains to the general level of activity exhibited by an individual in a non-risky and non-novel environment.

Aggressiveness: This axis refers to an individual's agonistic reaction to the presence or absence of conspecifics, meaning how an individual responds to the presence or absence of members of the same species in terms of aggressive behaviour.

Sociability: This axis represents an individual's reaction to the presence or absence of conspecifics, excluding aggressive behaviour. It encompasses how an individual interacts with or seeks out the company of others of the same species.

In literature, the term "animal personality" is often used interchangeably with other terms such as temperament and coping style (Conrad et al., 2011). A behavioural syndrome is also a frequently used term in behavioural ecology and occurs when personality traits correlate with each other (Réale et al., 2007). The correlation observed between two behaviours within a population, known as a behavioural syndrome, is postulated to arise from a constraint which may be attributed to a shared underlying mechanism governing both behaviours, such as genetic or hormonal pleiotropy[4], or genetic linkage (Lacasse & Aubin-Horth, 2014).

Repeatability and heritability

Primary inquiries in animal personality research involve determining whether differences in behavioural types have a genetic basis, i.e., assessing heritability, and investigating the extent

[4] If a single gene affects multiple behaviors, changes in that gene can lead to correlated changes in those behaviors. For example, a gene that influences aggression might also affect exploration tendencies, leading to a behavioral syndrome where aggressive individuals are also more exploratory (Class & Brommer, 2015; Lacasse & Aubin-Horth, 2014).

to which individuals maintain consistency in their behavioural traits over time, i.e., assessing repeatability of behavioural responses (Chervet et al., 2011).

The identity of the subject tested refers to the consistent set of behaviours it continues to show among its congeners, quantified with repeatability i.e., the proportion of phenotypic variance due to among individuals' differences (Nakagawa & Schielzeth, 2010). Repeatability is a measure of the consistency of a trait within individuals across repeated measurements and provides initial evidence that variation in the trait is influenced by factors intrinsic to the individual (Dohm, 2002).

Heritability is the proportion of phenotypic variance explained by additive genetic effects. It determines how much of a trait's variation is due to genetics rather than the environment. Traits with high heritability are more reliably passed from parents to offspring. This influences the rate of evolution for traits under selection, as those with high heritability respond more strongly to selective pressures (Prentice et al., 2022).

Repeatability (R) often establishes the upper limit for the heritability of a trait. Both measures tend to be correlated when comparing across species or populations if the phenotypic variances in the compared units are governed by similar processes involving additive genetic variance (Chervet et al., 2011). In cases where a behavioural trait exhibits high heritability, it should also demonstrate high repeatability, as genes are more likely to play a role in determining behaviour (Réale et al., 2007). Conversely, high repeatability may coincide with low heritability if the behavioural type is influenced by factors such as the current internal state of individuals, their life-history strategy (Wolf et al., 2007), or social strategy (Bergmüller & Taborsky, 2010), which can introduce variation largely independent of genetic effects. Maternal or paternal effects, maternal additive genetic effects, and genotype × environment interactions may also lead to discrepancies between heritability and repeatability estimates, with scenarios where heritability exceeds repeatability (Dohm, 2002). Both low repeatability and low heritability would suggest that the population does not exhibit animal personalities, especially if repeatability decreases over time (Chervet et al., 2011).

Studying and measuring personality traits

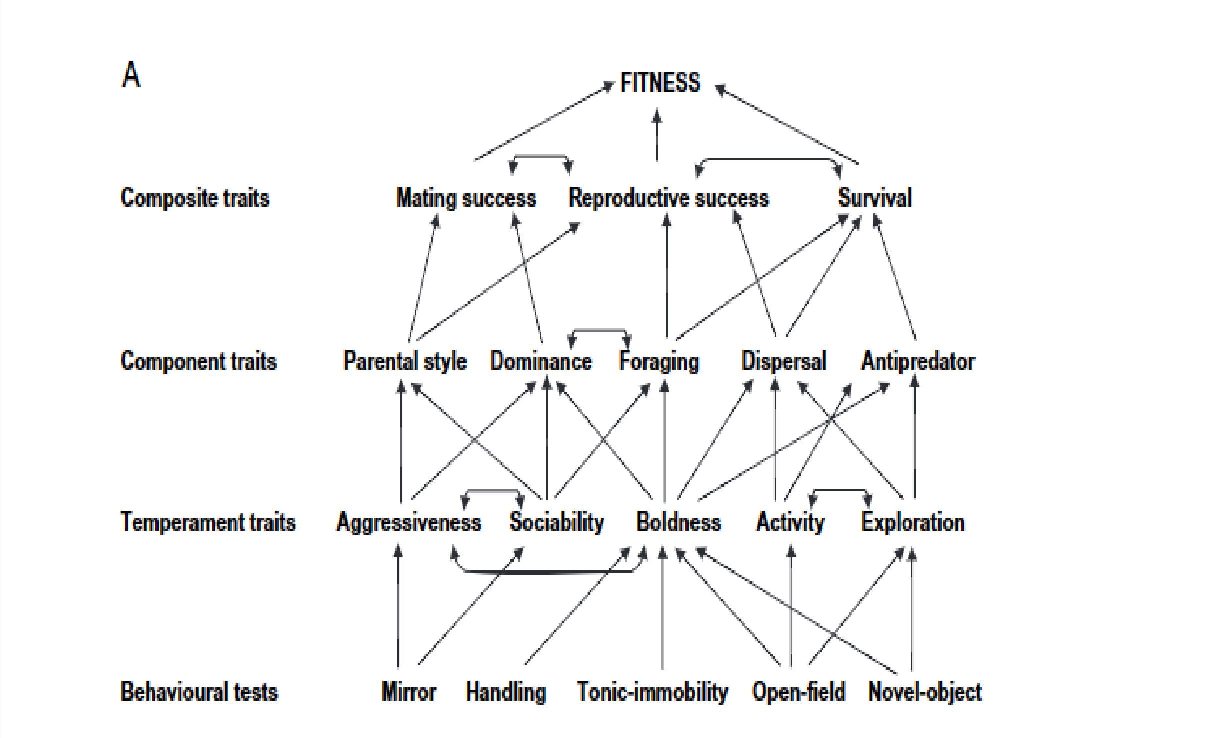


Figure 3: *Different personality traits, correlations and tests (Réale et al. 2007).*

Different tests are used in behavioural ecology to study behavioural traits (Figure 3). However, when testing personality, several issues arise. For instance, there are confusions in identifying personality axes. For example, Beukeboom (2022) showed that locomotion in a familiar versus unfamiliar environment doesn't mean the same thing in the case of Atlantic Cod (*Gadus morhua*). In this context, the personality axes "activity" and "exploration" should not be confused or treated as if they reflect the same personality trait.

Moreover, in the context of genetic selection for personality traits, individual and group tests on Arctic charr (*Salvelinus alpinus*) for the risk-taking trait have shown differences. Arctic charr exhibit different behaviours when alone *versus* in a group, indicating a lack of correlation between the two tests. Therefore, caution must be taken to select based on group tests (Philip et al., 2022).

In addition, scientists cannot extrapolate some tests between species, even when from the same family and genetically close. Balzarini and her colleagues (2014) showed that three cichlid species differed in responses to a Mirror Test when assessing aggressiveness. Aggression towards a mirror image has limited predictive value for intraspecific aggression in two of the three tested species. Therefore, it is important to investigate specific behaviours through specific tests.

In our case study, we focused on two axes: sociability and aggressiveness. The conspecific test is commonly used to measure sociability (Gartland et al., 2022) , and to standardize it, a Mirror Test is typically employed to eliminate size differences between the tested fish and the conspecific, as well as differences in stimuli caused by conspecifics (Cattelan et al., 2017). This Mirror Test is also used to measure aggressiveness. This can put in doubt the use of this technique when trying to simplify and standardize the Congener Test. Different behaviours can be exhibited and not necessarily related to sociability (Cattelan et al., 2017).

The use of the Mirror Test (MT) to measure either sociability or aggressiveness

Sociability and aggressiveness are often mixed or related to what is called a behavioural syndrome. It can be deduced that a less aggressive fish is more social and vice versa. However, Statistical approaches (Toms & Echevarria, 2014) and hormonal studies have shown that these two temperament traits do not share the same axis and are not gradients of one behavioural trait (Table 2) (Luchiari & Maximino, 2023).

Table 2: *Sociability and aggressiveness: traits, operational definitions and neurotransmitters involved (Luchiari and Maximino, 2023)*

Personality trait	Behavioural traits	Example of operational definition	Neurotransmitter system involved
Sociability	Social novelty test Social preference test Shoaling Conditional approach	Time spent near conspecific Time spent near novel conspecific Inter-fish distance Time spent inspecting predator when conspecific is present	Sexual steroids nonapeptides dopamine
Aggressiveness	Mirror Test Aggressive encounters	Aggressive display or contact Aggressive display or contact	Serotonin dopamine histamine

To study sociability, researchers often conduct laboratory experiments to control variables and manipulate contexts. In these experiments, a focal fish is placed in a tank with conspecifics separated by a transparent partition, and the time it spends near the group is measured as an indicator of sociability. Alternatively, a single fish can serve as a stimulus, but using a shoal as a stimulus is preferred for accurately reflecting the species' average behaviour (Cattelan et al., 2017).

The Mirror Test offers advantages over using live conspecifics for several reasons. It enables control over the variability in behaviour exhibited by live stimuli, as live stimuli may react differently based on behaviour, chemical cues and also when being familiarized with each other, meaning that they were raised in the same tanks (Cattelan et al., 2017). In contrast, the mirror provides a standardized stimulus image that behaves consistently but lacks the olfactive stimuli coming from a live individual. Consequently, this standardization allows for repeated measurements of the same focal individual, enhancing the reliability of experimental results (Rowland, 1999).

On the other hand, the measurement of aggressive behaviour in animals, particularly through staged encounters and Mirror Tests, follows a similar pattern. Aggression plays a vital role in both inter- and intra-specific competition and is commonly quantified through staged encounters between individuals (Huntingford, 1980). However, the use of live opponents can introduce variability due to individual differences in behaviours (Balzarini et al., 2014). To standardise the opponent behaviour, Mirror Tests are also employed here, assuming that fish do not recognize themselves in the mirror and treat their reflection as a conspecific (Kusayama et al., 2000).

Yet, the validity of using the Mirror Test to measure sociability in fish is still uncertain, as highlighted by several studies (Pham et al., 2012; Pitcher, 2012). While certain behaviours towards the mirror image, like the number of looks, correlate positively with shoaling tendency, others, such as the number of contacts, might be associated with traits like fear avoidance and activity rather than sociability (Budaev, 1997).

In addition, while Mirror Tests have been considered valid in quantifying aggression, recent studies have questioned this assumption, showing differences in hormonal responses and reactions between real opponents and mirror images (Drozdz et al., 2006; Hirschenhauser et al., 2008; May & Mercier, 2007). Moreover, in some species of cichlids, aggressive behaviours induced by a real opponent differ from those induced by an opponent simulated by the mirror (Balzarini et al., 2014; Elwood et al., 2014).

Many divergences may arise, as previously mentioned, between methods used to assess aggressiveness and sociability in fish as they might be closely related (Toms & Echevarria, 2014). In fact, due to multiple behaviours observed in front of the mirror that can be attributed to sociality and/or aggressiveness (Sverdrup et al., 2011), it's possible that the two personality traits can be observed depending on the species, age and environment (Beukeboom et al., 2023). The question is now about whether these behaviours change according to the test taken by the same fish for the same species, at the same age and environment.

Contextualization

A notable example for behavioural studies is the Arctic charr (*Salvelinus alpinus*) in Iceland, where these principles are actively applied and studied for wild species and aquaculture strains due to its ecological and economic importance for fish farmers and society. In recent years, the production of farmed fish in Iceland has significantly increased, reaching 51 thousand tonnes in 2022. Specifically, Arctic charr production totalled 4,931 tonnes, making it the second produced farmed species in the country (Figure 4).

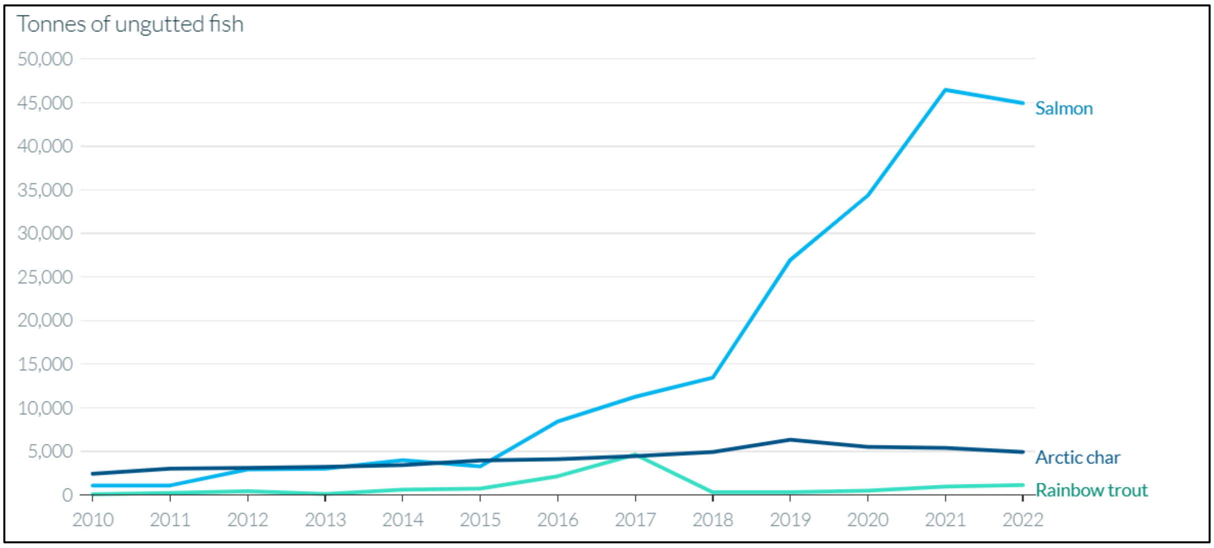


Figure 4: *Farmed fish production in Iceland (Statistics Iceland, 2022)*

The breeding station at Hólar, plays a pivotal role, supplying approximately 90% of the fingerlings required for charr farming operations throughout Iceland. Furthermore, it engages in collaborative breeding initiatives, commonly with counterparts in Norway and Sweden, fostering a shared effort in genetic improvement and strain development (Brännäs et al., 2011).

It has been known that Arctic charr is an atypic salmonid, preferring high stocking densities and exhibiting lower agonistic interactions when more individuals are reared (Brown et al., 1992). Moreover, this species was shown to gain more weight when not sorted according to similar size, suggesting that the fish are avoiding possible energy wasting on potentially lower energy gain, which would happen in case of size-sorting (Baardvik & Jobling, 1990).

These characteristics highlight that Arctic charr is already complex and atypical among salmonids, especially in aquaculture. This suggests that social welfare criteria may differ from other salmonids, thus warranting thorough investigation into this personality trait.

Despite the importance of the species in aquaculture and the development of research on Arctic charr personality traits, no standardized test has been set to measure sociability or aggressiveness for this species. Usually, encounters with different congeners are the preferred type of tests to assess sociability in fish species. However, some researchers use the Mirror Test to eliminate behavioural and size differences that might be induced by the presence of different congeners, which can have varying levels of stimulation (Cattelan et al., 2017).

In this study, we aimed to evaluate the reliability of the Mirror Test and video tracking for assessing sociability in juvenile Arctic charr. This assessment involved comparing conspecific testing (original technique for measuring sociability) with Mirror Test results (technique used to standardize the Congener Test). We went further into the details, and we separated the potential social interaction zones into a Contact Zone and an Interest Zone that might help optimizing the video tracking technique and identifying the best area to take in consideration for further studies. Additionally, given the species' social complexity, we explored the potential influence of social interactions on growth rates at juvenile stage.

We predicted that there would be correlations between the two tests for the time spent in one or both sociability zones (either strictly in contact or interested in the congener or the focal image). We also predicted that one or two traits linked to sociability and/or aggressiveness might be displayed and that the tests measure those behaviours combined. Should the findings indicate that the Mirror Test does not effectively measure sociability, a subsequent study conducted by a colleague, a bachelor student in applied biology, aimed to investigate whether this test could instead gauge aggressiveness using manual scoring techniques and a specific ethogram that explore specific behaviours displayed in both tests and their specific zones.

Another hypothesis was that growth could be influenced by aggressive-social behaviour, although we acknowledge that the results may be inconclusive due to the limited timeframe of the study.

Material and methods

Fish selection

The fish utilized in this research are progenies of an aquaculture strain originating from the breeding facility located in Hólar, Iceland. The sample tested was from the cohort 2023 and their parents from cohort 2019. A strategic approach was employed to ensure genetic diversity and optimize reproductive success. For this, a biparental mating design was realised generating 8 families from which only 5 were randomly chosen. Females selected for breeding were the least genetically related individuals within the cohort and were chosen based on their readiness on the designated breeding day, ensuring they had enough eggs. Males were selected based on their close genetic relation to the fewest number of ready females, with the additional criterion of having adequate sperm availability. Female selection occurred prior to crossing, and males were paired with the least related females who had not yet been fertilized. In cases where multiple non-fertilized females were available, selection was performed randomly. Both males and females were selected based on growth criteria (weight).

Rearing

A total of 2083 Charr juveniles were housed in 20-litre tanks in a flow-through system with 200% water renewal permitting a stable oxygen saturation above 90 % over the duration of the experiment. Five families were selected and allocated two tanks each (Table3). This design and replications allowed to mitigate potential tank effects. Rearing parameters were followed and recorded from day 0 to the end of the experiments (Table 4). Food granulometry (not shown on the table) was adapted based on guidelines from the Laxa and BioMar Denmark (BioMar, 2022) companies and the instructions from professionals at the breeding station of Hólar.

Table 3: *Number of fish per family and bucket*

Family	2		3		4		5		6	
Tank ID	26	8	35	16	18	34	9	36	7	25
Number of fish	198	189	190	196	203	203	190	192	262	260

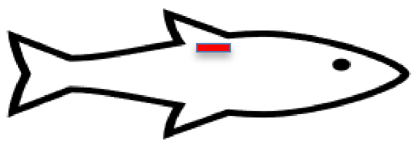
Table 4: *Rearing parameters used for the acclimation period and the experiments*

Period	Temperature (C°)	Photoperiod	Luminosity (Lux)	Feeding
Incubation (Hatchery)	5	Day: 7:30 – 19:30	0-5	5 meals with two-hour intervals.
Day 0	6	Night: 19:30 – 7:30	5-10	Food quantity calculated based on 150% of the fish needs due to food washout.
Day 12	7.5		50	
Day 20 to the end (including experiments)	8.5			

A daily follow-up, hygiene sheet and checklist were established to record all parameters and ensure a suitable rearing environment.

Tagging

For behavioural experiments, twelve fish per tank were individually tagged using Visible Implant Elastomer (VIE) tags. The tags were injected under the dorsal fin, to be clearly seen from above, and had a combination of simple or double colors (Figure 5). After the sociability tests, VIE tags vanished because of fish growth. So, they were retagged with VIE and with Pit tags. The pit tags were inserted intraperitoneally by making an incision in the abdominal wall with a scalpel.



Y left	Y right	PY
P left	P right	RY
O left	O right	RO
R left	R right	OY

Figure 5: *Position and label of the VIE tags. Capital letters refer to the colour (Y: Yellow, P: Pink, O: Orange, R: Red). For a double tag, the sides did not make a difference*

Experiments

Agenda

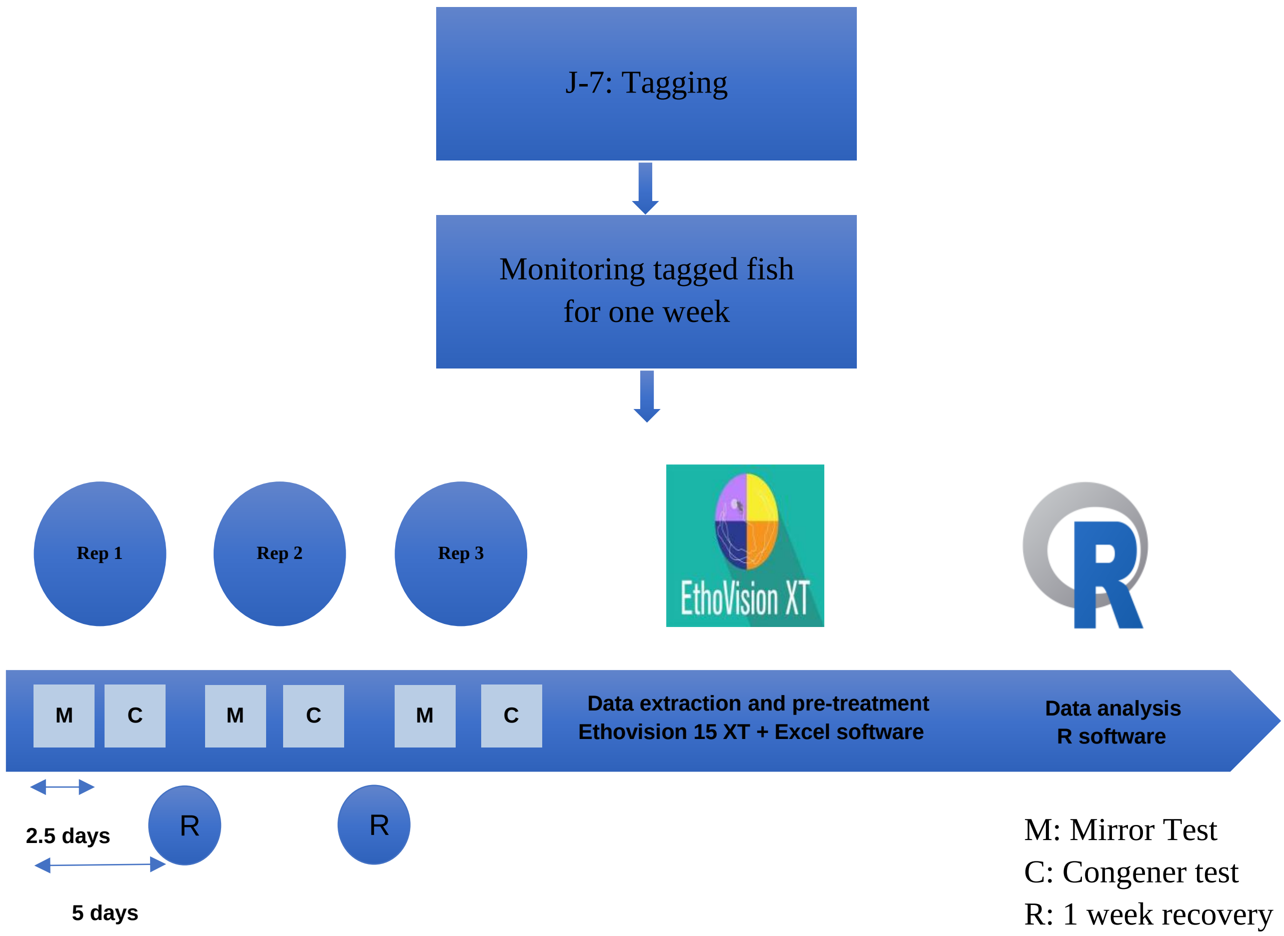


Figure 6: *Agenda of the behavioural experiments*

Mirror and Congener Tests were conducted over three weeks on 120 tagged fish. Three repetitions of the two tests were performed weekly, with each test lasting two days and a half). Each repetition was followed by a week of recovery and monitoring (Figure 6). Parameters were kept stable throughout the entire period. Afterwards, video footages from every trial were collected totalling 180 video footages. Eleven fish died during the experiment, equalling 6% of the total sample. Each fish performing less than 2 repetitions was removed from the statistical analysis.

After the experiments, videos were tracked using Ethovision software (XT 15 version).

Experimental design

For the experimental design (Figure 7), four arenas were used for each trial, displaying four tagged fish being tested (Figure 7, 1). Each arena has a length of 40 cm and a width of 30 cm.

During the Congener Tests (Figure 7, 2), a waterproof Plexiglas membrane was affixed about 10 cm from the wall on the edge of the four arenas. It did not allow water mixture, thus influencing only based on visual cues. Water changes were implemented before every experiment. A single congener was randomly selected, adhering to predetermined size criteria, with each candidate fish encountering a congener of approximately equivalent length. The selection was completely based on visual approximation and the environment where the congeners were picked was similar to that of the tested fish.

For the Mirror Tests (Figure 7, 3), a mirror was positioned at the same position as the Plexiglas membrane, just sliding over it to provide uniform space availability for each fish. The water level was fixed at 10 cm. Additionally, the water within the arena was renewed after each test session and taken from the same source used for rearing, ensuring the same quality, temperature and oxygen saturation as where the fish was reared.

A backlight (110 ×110 cm, Noldus, the Netherlands) was put under the arenas to ensure homogenous light and optimize the video footage quality.

A Basler camera was fixed exactly above the backlight and 98 cm to its center to enable clear vision of all areas.

(1)

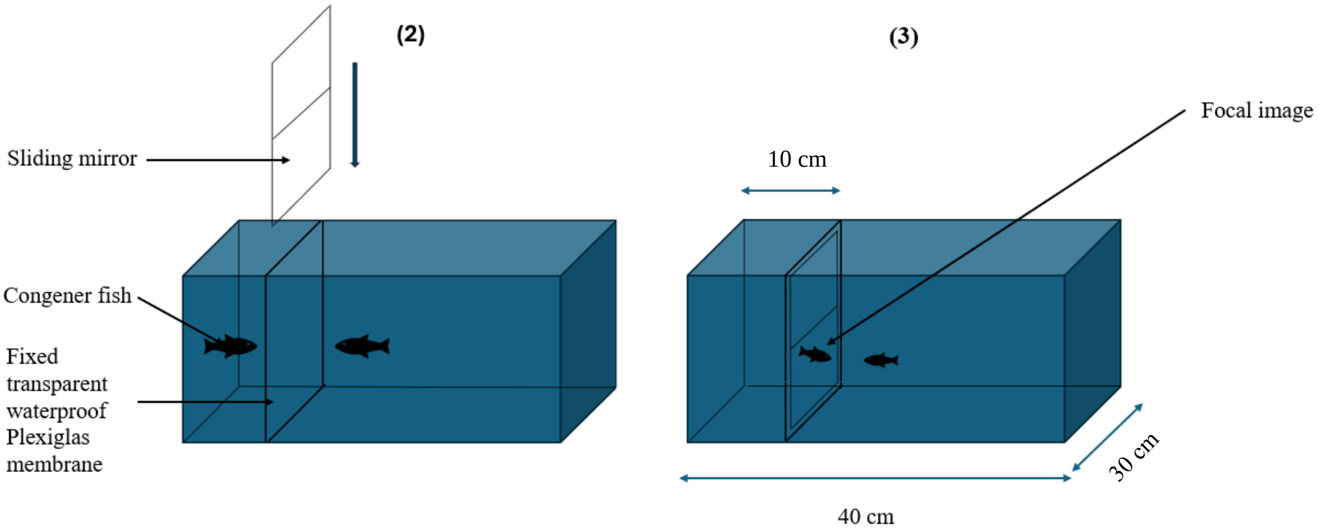


Figure 7: *Experimental design of the arenas: (1) Overview of the arenas (2) Congener Test setup (3) Mirror Test setup*

Tests' procedure

The tagged fish from each bucket were transported in groups of four to the behaviour analysis room, each tank undergoing three rounds of tests. Upon arrival, each fish was individually introduced into an arena where it underwent the mirror or Congener Test according to the schedule. The timeframe was then set at 20 minutes, including 5 minutes of acclimation in a box offering enclosure, quiet and weak luminosity to reduce stress and standardize testing conditions. After each repetition, tagged fish and congeners were anesthetized to be weighed and measured.

Tracking, data extraction and pretreatment

The arenas were virtually divided into 4 zones (Figure 8), including a "contact" zone of 1.5 cm sufficient to contain the entire fish body and quantify the time spent in contact with a mirror (Mirror Test) or membrane (Congener Test). Other zones were defined, ranging from close to far away, including the "Interest" zone (8.5 cm), "Middle" zone (10 cm), and opposite to the Interest Zone (10 cm). The perimeter of the arenas was calibrated to allow precise measurement of the total distance covered by the subjects (Appendix B, Figure 1).

In accordance with previous studies (Beukeboom, 2023; Cattelan et al., 2017; Sloman & Baron, 2010), time spent in the "Contact" and "Interest" zones was taken as the principal metrics for sociability. Those proxies were studied separately to be investigated in the statistical analysis. Profiles used are listed in the figure 9 below. Center point was used to track presence in intermediate zones (s) and to calculate the total distance moved (cm), velocity (cm/s), absolute mean of angular velocity (°/s). Additionally, nose point was used to track the presence of the fish in the Contact Zone (s). Data other than "time spent" served as exploratory insights and a backup option for analysis and not as primary factors for the statistical models.

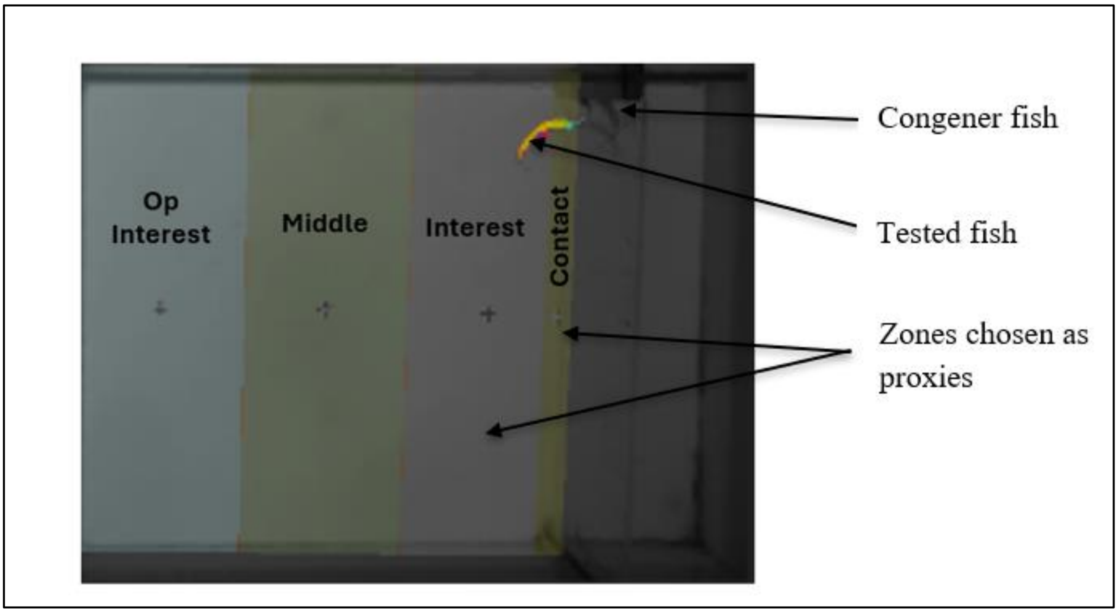


__Figure 8:__ Example of live tracking with the zoning inside of the arena

Distance moved	Distance moved of the Center-point
Velocity	Velocity for the Center-point
In zone	For Center-point and Nose-point: when point is in zone for the zones Arena, middle, op interest and interest
Angular velocity	The absolute angular velocity based on the heading of the Center-point
In zone 2	When Nose-point is in Contact

Figure 9: *Analysis profiles setup taken in Ethovision XT15*

Tracking started once the acclimation boxes are lifted (after 5 minutes) and automatically stopped after 15 minutes of recording. Detection settings were verified prior to tracking. Nose point appeared in blue and center point in red (Figure 8). If the fish was not detected, the subject would be all in black and the detection settings were set to reverification. Tracking also permitted to individually visualize the heatmaps (Appendix B, Figure 2) and trajectories of the sample (Appendix B, Figure 3), it could help us identify the general zonal preference of the fish as a preliminary visual investigation.

Fish measurements: Growth follow-up

Individual measurements: Fish measurements for the tagged fish and congeners were conducted at the end of each repetition to allow a 7-day recovery period from testing and anaesthesia. Fish were anesthetized using a solution of phenoxyethanol at a concentration of 0.3 g/l. Subsequently, they were measured (total length; cm), weighed (g) and then placed in a recovery bucket (oxygenated tank) before being returned to the tanks. Tanks that underwent fish measurements were not fed during all day.

Fish measurements for every tagged fish and congener were done to monitor individual Specific Growth Rate[5] (SGR) and to correlate it to the behaviours observed during the analysis. Differences observed between congener and tagged fish in terms of total length and weight were recorded to analyse potential size influence on social behaviour.

Tank level: Fish measurements at the tank level were conducted, independently from the experimentation period, in order to characterize the environment of each fish in terms of general growth performance. Mortality percentage and SGRs were also calculated at the tank level.

[5] **SGR (%) = ln (Wf) − ln (Wi) / t ×100** [Wf = final weight of the organism (in grams) / Wi = initial weight of the organism (in grams) / t = duration of the experiment or growth period (in days)].

The calendar for both types of fish measurements is shown on the figure 10.

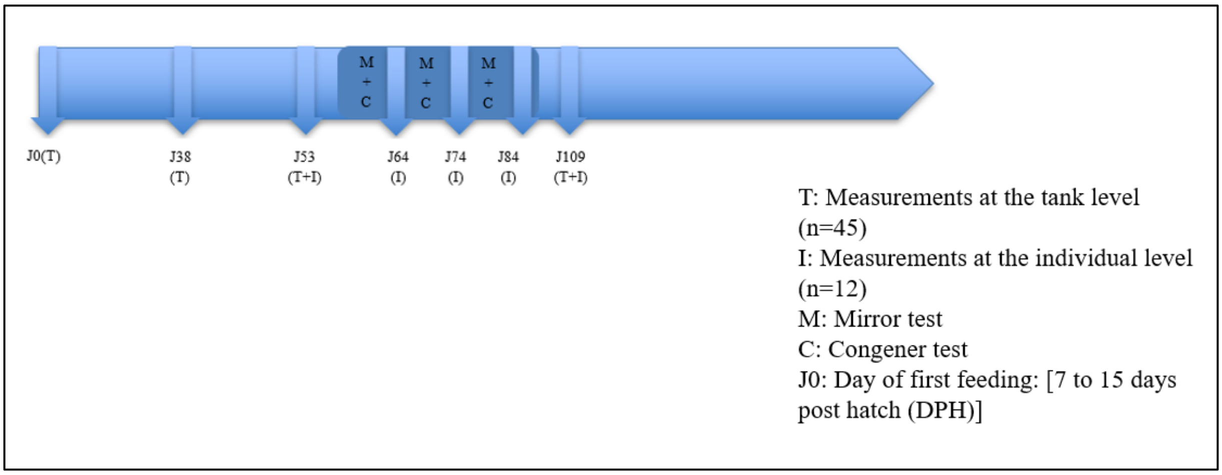


Figure 10: *Calendar for fish measurements at individual and tank levels*

Statistical approach

Statistical analyses were conducted using RStudio version 4.2.2. Data subsets for Mirror (Mir) and Congener (Con) tests were created from the original dataset using the `filter()` function from the `dplyr` package. These subsets contained observations related to different tests ('M' for Mirror and 'C' for Congener). Variables for time spent in contact or Interest Zones were added, and the `Test` column was removed. The subsets were merged into a comprehensive dataset featuring, the time spent in Contact and Interest Zones for the Mirror Test (MirC - MirI), Congener Test (ConC - ConI), the length difference between the congener and the tested fish (LdiffCon.z), fish length (Length.z), and Specific Growth Rate (SGR.z). All the variables that have ".z" were scaled to be included as fixed factors.

Bayesian regression models bf_mir (Equation 1) and bf_con (Equation 2) were fitted using the `brm ()` function from the `brms` package (Bürkner, 2017) then merged into a multivariate model to investigate the response variables `MirC` and `ConC` (Equation 3). These models included fixed effects for `Length.z`, `SGR.z`, `LdiffCon.z`, and the categorical variable `Rep`, aiming to elucidate the influence of fish length, growth rate, and test repetitions on time spent in the zone. Random effects were specified to account for hierarchical dependencies, with `(1 | Family:Tank)` capturing variations between Family and Tank combinations, and `(1 | p | ID)` modeling individual-specific variation nested within ID. The random-effects structure included individual fish identity (ID) as a grouping variable, allowing us to calculate the repeatability of the personality estimates as the ratio of the among-individual variance and the sum of the among-individual and residual-level variances (Equation 4).

The models were fitted using three Markov chains (`chains = 3`) and two CPU cores (`cores = 2`) for parallel computation. A warm-up phase of 2000 iterations (`warmup = 2000`) was followed by 6000 total iterations (`iter = 6000`) to sample from the posterior distribution. Control parameters (`adapt_delta = 0.999`, `max_treedepth = 50`) were set to enhance the accuracy and efficiency of the sampling process (Equation 3). Posterior sampling and summarization of model results were performed using functions from the `bayestestR` and `rstan` packages (Carpenter et al., 2017) , providing estimates, credible intervals, and diagnostics for fixed effects coefficients and random effects variance components.

Equation 1: *Bf_mir formula for the Mirror Test*

bf_mir= bf (MirC ~ Length.z +SGR.z + Rep + (1|Family:Bucket) + (1|p|ID), family=gaussian())

Equation 2: *Bf_con formula for the Congener Test*

bf_con= bf (ConC ~ Length.z + SGR.z + LdiffCon.z + Rep + (1|Family:Bucket)+ (1|p|ID), family=gaussian())

Equation 3: *the fit multivariate model that takes into account both tests*

fit = brm (bf_mir+bf_con, data = dataset, chains = 3, cores = 2, warmup=2000, iter = 6000, seed = 1234, control = list (adapt_ delta = 0.999, max_treedepth = 50))

Equation 4: *The repeatability formula*

Repeatability = Variance Among / (Variance Among + Variance Within)

Results

The fit summary

The model with the response variable: Time spent in the Contact Zone

The results from the Bayesian model (Appendix C, Table 1) revealed detailed insights into the estimated parameters and their associated uncertainties. The coefficient plot (Figure 11) gave more details about the significance (Credible Intervals) of each fixed factor counted in this model.

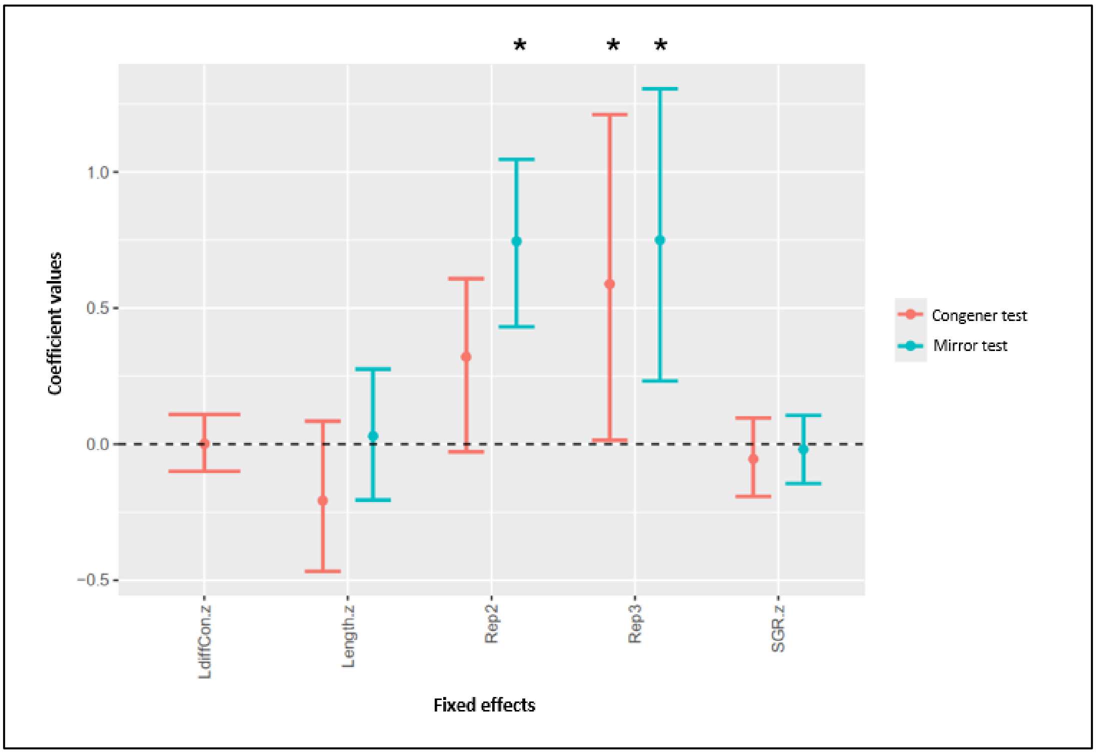


Figure 11: *Coefficients values of the fixed effects for the model ran with the "Contact Zone" as a response variable.*

LdiffCon.z (Scaled length difference between tested fish and congener): The credible intervals for Congener Test overlapped with zero. This implied that there was no statistically significant effect of the length difference between the tested fish and the congener on the time spent in the Contact Zone for either the congener. For the Mirror Test, the length difference was automatically attributed a null value.

Length.z (Scaled length of the fish): The credible intervals for both Congener Test and Mirror Test included zero. This implied that the length of the fish did not significantly influence the time spent in the Contact Zone for both congener and Mirror Tests.

SGR.z (Scaled specific Growth Rate): The credible intervals for both Congener Test and Mirror Test overlapped with zero. This indicated that the specific growth rate did not have a statistically significant effect on the time spent in the Contact Zone for either congener or Mirror Tests.

Rep (Repetitions): The credible intervals for most repetitions did not overlap with zero. For Rep 2 (Mirror Test) and Rep 3 (both tests), the bars were on the positive side, indicating a significant effect. For the Rep 2 of the Congener Test, there was a slight overlap with 0 for the credible intervals that suggested that this repetition was not statistically of significant effect on the time spent in the Contact Zone for the Congener Test.

The model with the response variable: Time spent in the Interest Zone

The summary of the model ran with Time spent in the Interest Zone as a response variable gave insights about the factors used (Appendix C, Table 2). In the provided coefficient plot (Figure 12), certain predictors' 95% credible intervals (error bars) overlapped with zero, indicating that the effect of these predictors on the response variable was not statistically distinguishable. Other fixed parameters had significant effect on the time spent in the Interest Zone.

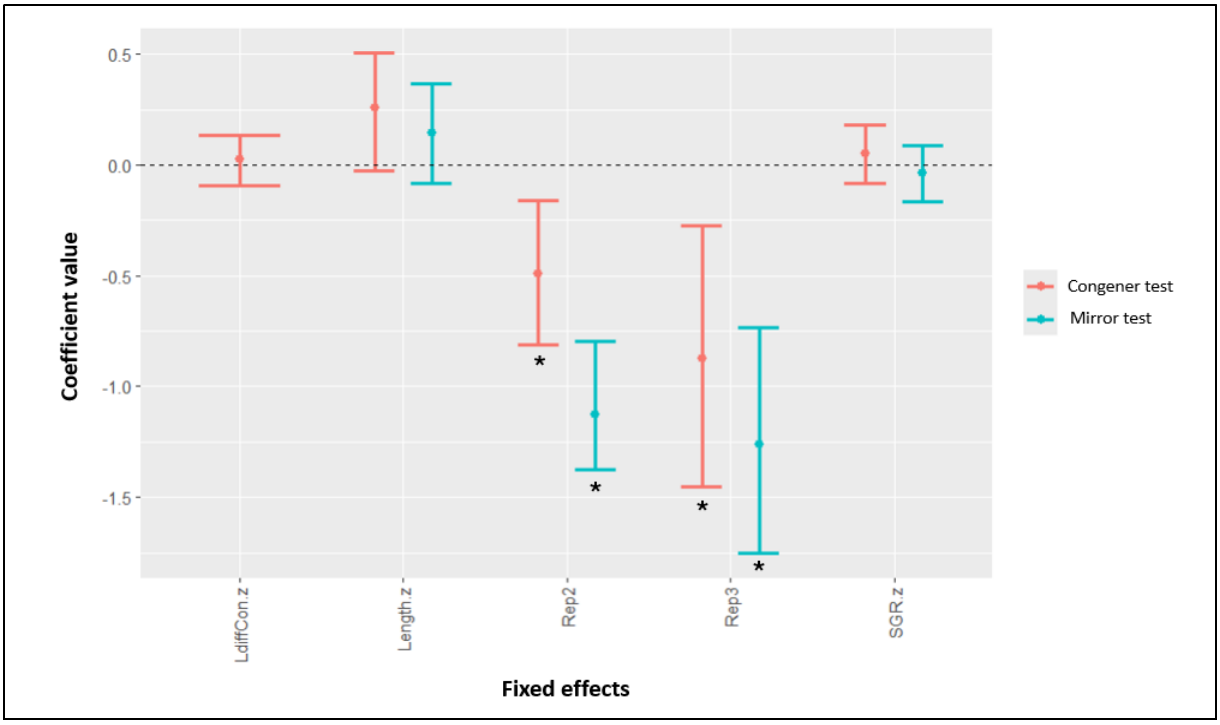


Figure 12: *Coefficients values of the fixed effects for the model ran with the "Interest Zone" as a response variable.*

LdiffCon.z (Scaled length difference between tested fish and congener): The credible intervals overlapped with zero, suggesting there was no statistically significant effect of the length difference between the tested fish and the congener on the time spent in the Interest Zone during the Congener Test. For the Mirror Test, the length difference was automatically attributed a null value.

Length.z (Scaled length of the fish): The credible intervals for both tests overlapped with zero. This indicated that fish length did not significantly affect the time spent in the Interest Zone for both the Mirror Test and the Congener Test.

SGR.z (Scaled specific Growth Rate): The credible intervals for both tests included zero, indicating that the specific growth rate (SGR) did not significantly affect the time spent in the Contact Zone for both tests.

Rep (Repetitions): The analysis revealed significant effects of repetitions on the time spent in the Interest Zone for both groups. For the Mirror Test, MirI_Rep2 and MirI_Rep3 had credible intervals that did not include zero, indicating a negative impact of these repetitions on the time spent in the Interest Zone. Similarly, for the Congener Test, ConI_Rep2 and ConI_Rep3 also had credible intervals that excluded zero, suggesting negative effects of these repetitions on the time spent in the Interest Zone for the Congener Test.

Among/Withing variances and repeatability

The model with the response variable: Time spent in the Contact Zone

The table 5 presents Bayesian estimates of variances and repeatability coefficients for experiments conducted under Mirror Test and Congener Test conditions taking the time spent in the Contact Zone as a proxy. For the Mirror Test condition, Va Mirror was estimated at 0.18 (95% credible interval: 0.06 to 0.32), indicating variability due to differences among individuals in terms of times spent in front of the mirror. Vw Mirror was estimated at 0.7 (credible interval: 0.58 to 0.86), representing variability within individuals over repeated measurements in the Mirror Test. Rep Mirror (repeatability) was estimated at 0.2 (credible interval: 0.07 to 0.33). Similarly, for the Congener Test condition, Va Congener was estimated at 0.27 (credible interval: 0.15 to 0.43), Vw Congener at 0.59 (credible interval: 0.49 to 0.73), and Rep Congener had a value of 0.31 (credible interval: 0.18 to 0.44).

Table 5: *Variances and repeatability estimates for the "Contact Zone" model*

R Estimates	Median	lower_CI	Upper_CI	Pmcmc
V_a Mirror	0,18	0,06	0,32	1
V_w Mirror	0,7	0,58	0,86	1
Rep Mirror	0,2	0,07	0,33	1
V_a Congener	0,27	0,15	0,43	1
V_w Congener	0,59	0,49	0,73	1
Rep Congener	0,31	0,18	0,44	1

The model with the response variable: Time spent in the Interest Zone

For the Interest Zone (Table 6) as a response variable, in the Mirror Test condition, variance among individuals (V_a Mirror) was estimated with a median of 0.2 (95% credible interval: 0.09 to 0.34), indicating substantial variability due to individual differences among individuals. Variance within individuals over repeated measurements (V_w Mirror) in the Mirror Test showed a median estimate of 0.58 (credible interval: 0.48 to 0.71), suggesting significant variability across repeated measurements within individuals. Repeatability within the mirror (Rep Mirror) test was also estimated at 0.26 (credible interval: 0.12 to 0.39). Moving to the Congener Test condition, variance among individuals (V_a Congener) was estimated at 0.15 (credible interval: 0.03 to 0.29), demonstrating variability due to individual differences within this group. Variance within individuals over repeated measurements in the Congener Test (V_w Congener) was estimated at 0.73 (credible interval: 0.6 to 0.9). Repeatability (Rep Congener) within the Congener Test was calculated based on the variances found and equaled 0.17 (credible interval: 0.03 to 0.31).

Table 6: *Variances and repeatability estimates for the "Interest Zone" model*

R	Median	lower_CI	Upper_CI	Pmcmc
V_a Mirror	0,2	0,09	0,34	1
V_w Mirror	0,58	0,48	0,71	1
Rep Mirror	0,26	0,12	0,39	1
V_a Congener	0,15	0,03	0,29	1
V_w Congener	0,73	0,6	0,9	1
Rep Congener	0,17	0,03	0,31	1

Overall, the results (Table 5 and 6) showed comparable repeatabilities (0.19/0.2 – 0.26/0.17) for the two response variables taken as proxies for the model ran in the two test conditions. The only difference that could be distinguished was the Variance within individuals observed at the Mirror Test (0.83 for the Mirror Test and 0.7 for the Congener Test). This indicates that, for Mirror testing, the sample showed more variabilities within individuals across repeated measurements when using the Interest Zone as a proxy rather than the Contact Zone.

Correlation between the Mirror Test and the Congener Test

Time spent in the Contact Zone as a response variable

The table provides Bayesian analysis results (Table 7) for the covariance (Cov) between the two tests. The median estimate of Cov was 0.1, with a 95% credible interval ranging from 0.01 to 0.2. This interval indicated uncertainty around the exact strength of the relationship, but the high posterior probability (Pmcmc = 0.99) supports a positive covariance between the variables or conditions. This suggested that changes in one variable are highly likely to co-vary positively with changes in the other variable by a correlation value of 0.1 (Figure 13).

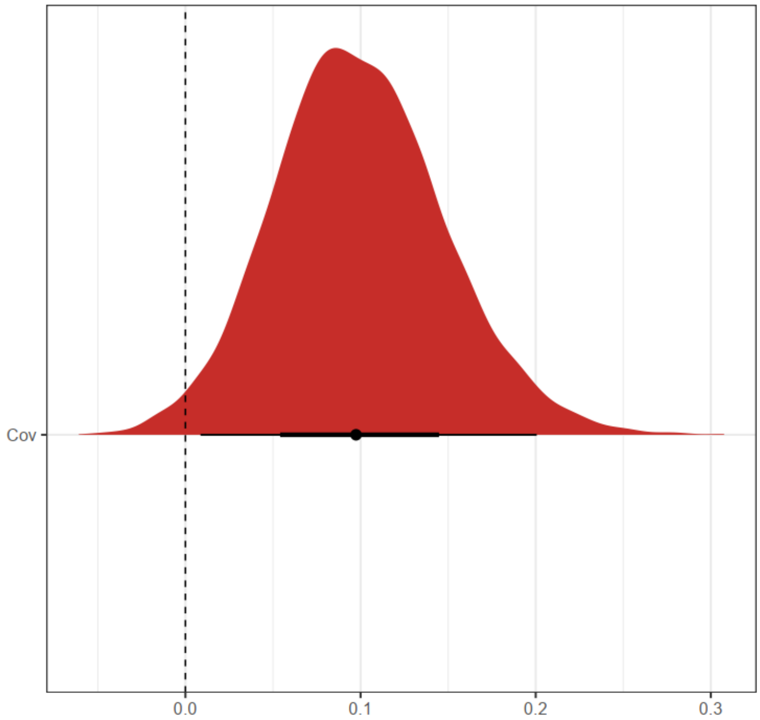


Figure 13: *Correlation of the Mirror Test and Congener test for a "Contact Zone" proxy*

Table 7: *Correlation between the Mirror Test and the Congener Test according to the time spent in the Contact Zone*

Delta	Median	lower_CI	Upper_CI	Pmcmc
Cov	0,1	0,01	0,2	0,99

Time spent in the Interest Zone as a response variable

The table presents Bayesian analysis results (Table 8) for the covariance (Cov) between variables or conditions studied. The median estimate of Cov was 0.07, with a 95% credible interval ranging from -0.01 to 0.17. This interval overlapped with 0, implying that changes in one variable do not statistically tend to co-vary with changes in the other. (Figure 14).

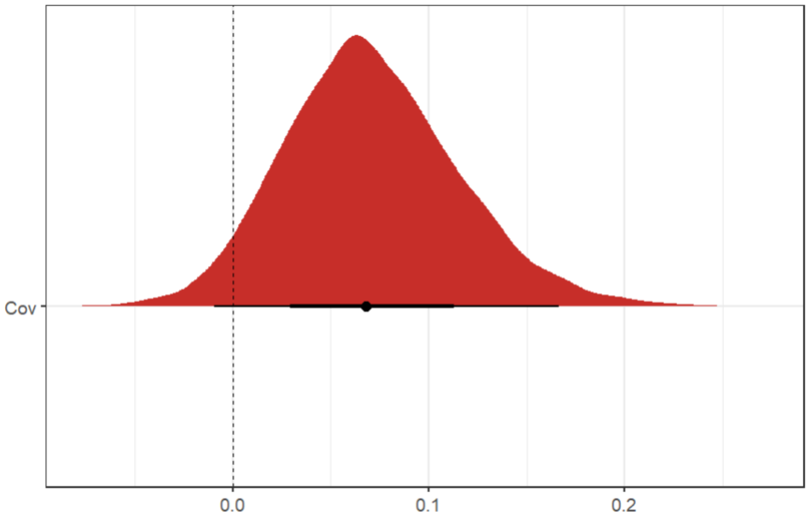


Figure 14: *Correlation of the Mirror Test and the Congener test for an "Interest Zone" proxy*

Table 8: *Correlation between the Mirror Test and the Congener Test according to the time spent in the Interest Zone*

Delta	Median	lower_CI	Upper_CI	Pmcmc
Cov	0,07	-0,01	0,17	0,96

Correlation between the Interest Zone and the Contact Zone

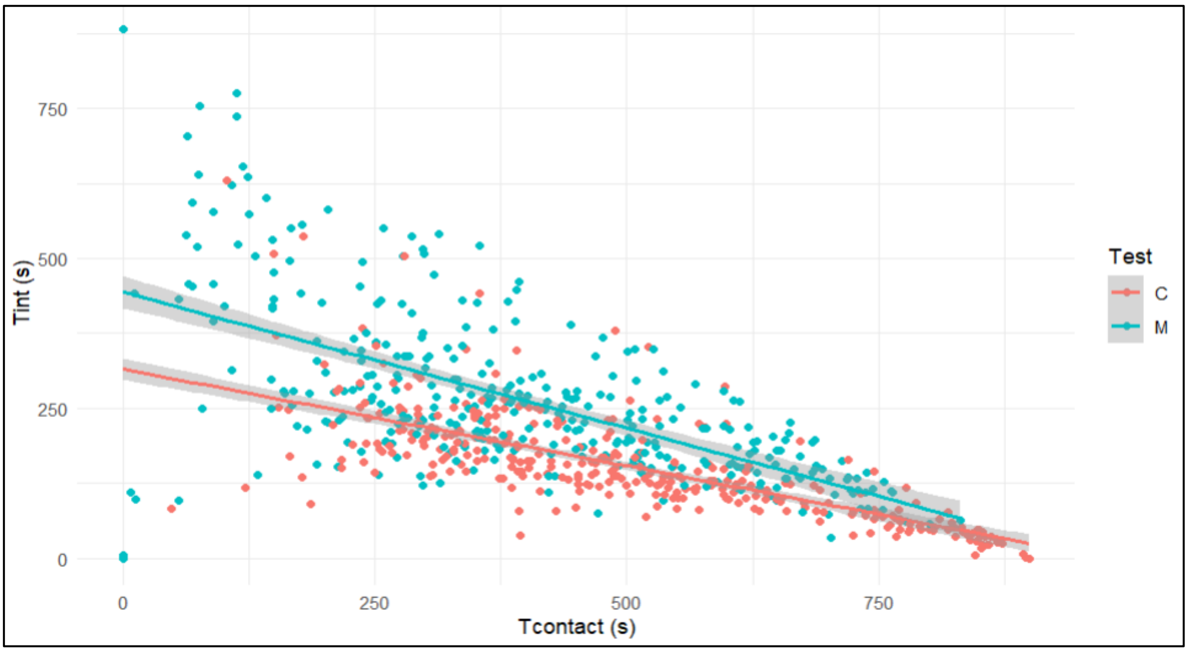


Figure 15: *Correlation between the time spent in the Contact Zone and the Interest Zone for both tests separated*

A correlation graph (Figure 15) illustrating the distribution of the sample under the two response variables for the two tests showed that the two variables studied (Time spent in Contact and Interest Zones) tend to be highly correlated. Furthermore, we plotted the medians for the distribution of these two variables under the two conditions and deduced that the correlation observed in the previous graph tend to be less explicative.

Distribution of the variables and Student paired test

Distribution and medians

This box plot compared the distribution (Figure 16) of the variable "Time spent in the Contact Zone" between two test conditions, Congener (C) and Mirror (M). The median of Tcontact (481.71 s) for Congener Test was bigger than that of the Mirror Test (376.68 s). The difference in medians levels and IQRs suggested differences in time spent in the Contact Zone between the Congener and Mirror Tests.

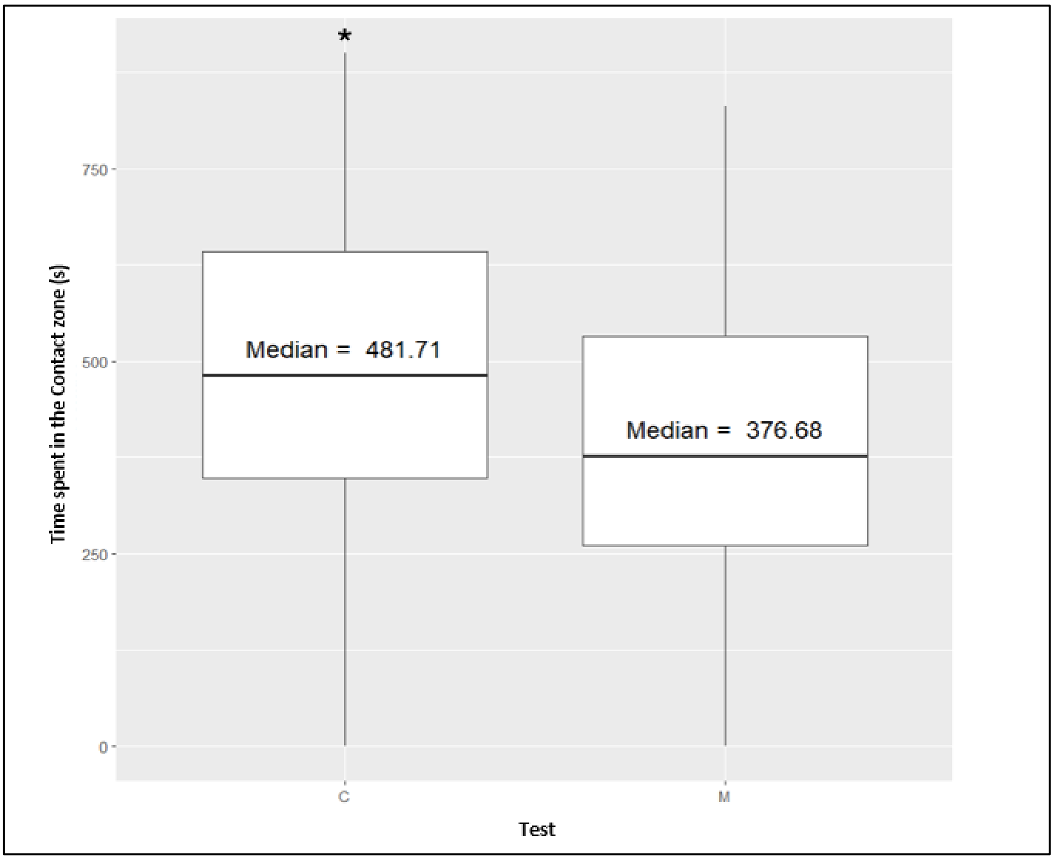


Figure 16: *Distribution of the time spent in the Contact Zone (s) for both tests: Medians and IQRs*

The distribution of the variable "Time spent in the Interest Zone" was also compared between two test conditions, Congener (C) and Mirror (M) (Figure 17). The median for the Congener Test (144.29 s) was lower than that of the Mirror Test (233.76 s). The difference in medians levels and IQRs suggested differences in time spent in the Interest Zone between the Congener and Mirror Tests.

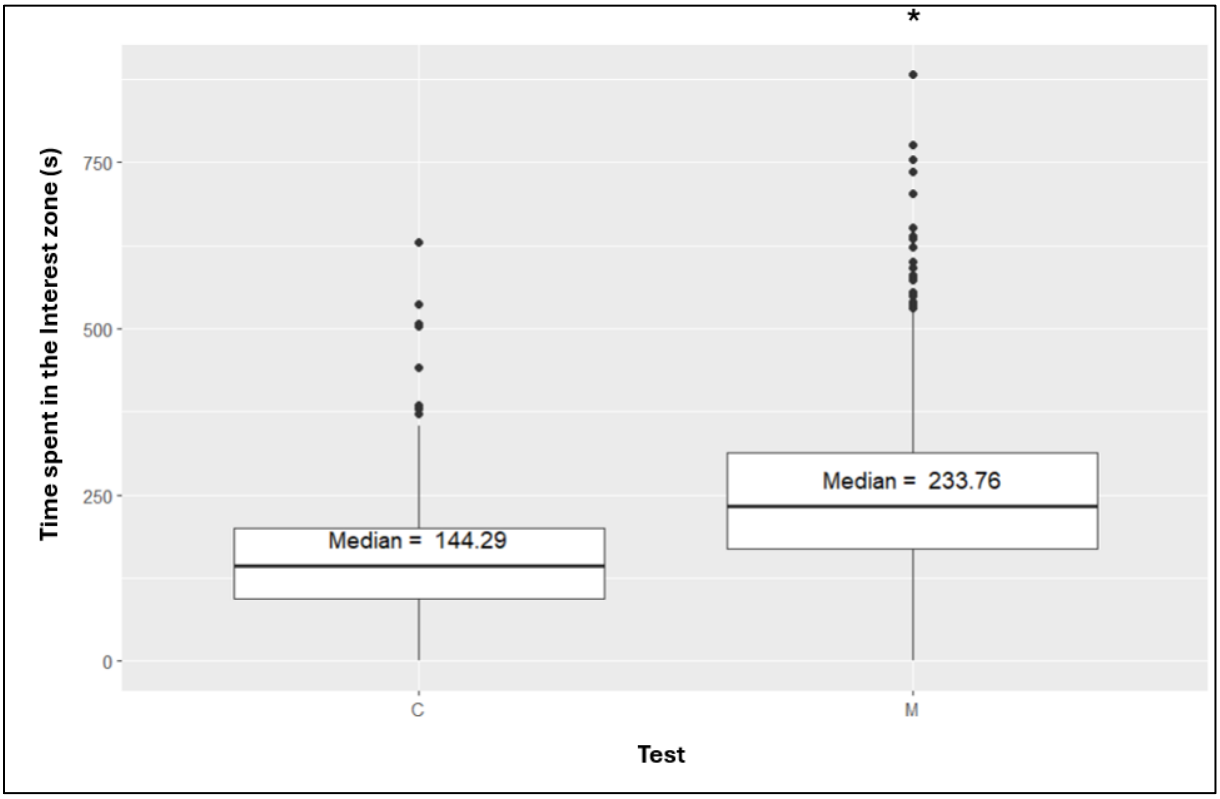


Figure 17: *Distribution of the time spent in the Interest Zone (s) for both tests: Medians and IQRs*

Pair-wise student test

A paired t-test was performed to compare the time spent in the Contact Zone between the Mirror Test and the Congener Test distributed under the categorical variable "test". The results indicated a significant difference between the two conditions. The t-statistic was -7.87 with 332 degrees of freedom, and the p-value was 5.21e-14, demonstrating a highly significant result. The 95% confidence interval for the mean difference in time spent ranged from -133.36 to -80.00, and the mean difference was estimated at -106.68. This indicates that, on average, the time spent in the Contact Zone was significantly higher during the Congener Test compared to the Mirror Test.

A paired t-test was also conducted to compare the time spent in the Interest Zone between the Mirror Test and the Congener Test. The analysis revealed a significant difference between the two conditions. The t-statistic was 13.95 with 332 degrees of freedom, and the p-value was less than 2.2e−16, indicating a highly significant difference. The 95% confidence interval for the mean difference in time spent ranged from 96.25 to 127.85, with a mean difference of 112.05. This result suggests that, on average, the time spent in the Interest Zone was significantly higher during the Mirror Test compared to the Congener Test.

Discussion

The study aimed to evaluate the validity of the Mirror Test as an alternative to the Congener Test for assessing sociability in juvenile Arctic charr using video tracking techniques. Additionally, we sought to enhance the testing methodology by determining which zones of the test arena provided more significant insights into the species' social traits. Lastly, the potential link between the Specific Growth Rate (SGR) and behavioural traits was investigated to assess the relevance of developing a selective breeding program based on sociability or aggressiveness. Hereafter, the findings, limitations and perspectives of this research are discussed.

The validity of the Mirror Test for both Zones

The Contact Zone

The variable “Time spent in the Contact Zone” measured in Congener and Mirror tests showed repeatability and correlation. Consequently, we could say that the Mirror test indeed measures a repeatable trait reflecting personality in juvenile Arctic charr. This demonstrates repeatability for the Mirror test for first time in this species. In previous studies, tests for traits like boldness for aquaculture strains (Philip et al., 2022), exploration (Beukeboom, 2023) and sociability (Horta-Lacueva et al., 2020) for wild species have been demonstrated for Arctic charr. Moreover, the correlation between the Mirror test and the Congener test for the Contact Zone suggests that the Mirror test could potentially replace the congener test as a tool to measure a personality trait such as sociability. However, the weak covariance found leads us to question what kind of behaviour the mirror exactly measures in this zone and if the congener and the focal image gauge different stimuli that cause the fish to display distinct behaviours in both tests. Although we eliminated olfactive cues and tried to homogenize the behavioural stimuli with acclimation time and proximate size selection, we still believe that a live stimulus was more understandable and perceivable to the fish as tested fish tend to spend 10% of time more in the Contact Zone for the Congener Test compared to a Mirror Test. A mirror does not probably induce the exact same response as a real opponent because a real conspecific typically initiates moves that elicit reactions from the fish. In contrast, a mirror image does not generate engaging stimuli unless the fish itself initiates movement. If the fish remains stationary or shows little interest, the mirror will not prompt any new behaviour; instead, the fish is essentially reacting to its own reflection. This lack of dynamic interaction could lead to increased time spent in areas other than the Contact Zone, as the fish may freeze or display less movement in response to the mirror. Consequently, the fish might exhibit less activity or engagement in the Contact Zone where responses to the stimuli are most pronounced (Elwood et al., 2014; Van Dyk & Evans, 2008).

This difference between fake and real stimuli could also trigger genetic factors (transcription factors) that result into behaviours other than social or aggressive, as highlighted by Li and colleagues (2018). Regular mirrors elicit higher immediate early gene (IEG[6]) expression in the basolateral amygdala (Dm) and hippocampus (Dl), suggesting heightened alertness or fear due to the novel and confusing nature of the reflection. In contrast, lower IEG expression (egr-1 and c-Fos) in the preoptic area (POA) indicates that mirrors do not stimulate the neurobiological pathways needed for appropriate social or aggressive behaviours due to the lack of genuine threat or social interaction cues. While regular mirrors provide both morphological and behavioural cues, the information is inaccurate as the reflection does not match typical social or threat postures seen with live opponents.

Furthermore, we noticed an effect of the second and third repetition on the time spent in the Contact Zone for the Mirror Test and the effect of the second repetition on the same response variable for the Congener Test. Those effects were all positive indicating that with each repetition, fish became accustomed to entering the Contact Zone and interacting more with the opponent. This can be explained by the social learning (Odling-Smee & Braithwaite, 2003) acquired through repetitions, which helped the fish stress less and socialize more.

[6] **egr-1 (Early Growth Response 1)** and **c-Fos** are both immediate early genes (IEGs) that play crucial roles in cellular responses to various stimuli, including stress, growth factors, and neural activity. Odling-Smee and Braithwaite, 2003

The Interest Zone

The variable "Time spent in the Interest Zone" was repeatable but no significant correlation was found between the Mirror Test and the Congener Test. This means that this variable is not relevant and therefore cannot be used in a Mirror Test to measure sociability. We also noticed that fish spent approximately 10% more of the total test time on average in the Interest Zone during the Mirror Test. These findings are consistent with previous observations: in the Mirror Test, fish often either remain stationary or freeze within the Interest Zone or occasionally move to the “Middle” and “OpInterest” Zones, avoiding visual contact with the mirror as it causes more fear and confusion than social or aggressive cues. In contrast, during the Congener Test, fish spent less time in the Interest Zone, likely moving towards direct contact with the live stimulus. Repetitions of the tests significantly reduced the time spent in the Interest Zone for both methods, indicating that fish became habituated to the presence of the congener and the focal image over successive trials.

Potential behaviours displayed

Our findings indicate that the Contact Zone is a more reliable indicator of sociability, as it shows higher and more significant correlation and repeatability between tests and resulted in more time spent by the fish than in the Interest Zone. However, the weak covariance between the Congener Test and the Mirror Test might be due to the lack of social display, and other behaviours may have interfered with sociability in different proportions during one of the tests. To precisely identify the behaviours exhibited in the Contact Zone, we collaborated with a bachelor’s student in applied biology to manually score individual behaviours using an ethogram, which we couldn’t achieve with the video tracking technique. During her project, Kuhn (2024) observed the same behaviours we did but only using the last repetition and a different, specifically placed camera (Appendix D, Figure 1). Her analysis revealed that both tests exhibited social and aggressive behaviours, with avoidance also being observed. Her results also showed a correlation in terms of the nature of exhibited behaviours between the Mirror Test and the Congener Test.

Table 9: *Percentage of each proportion of behaviour by category (Sociability, Aggressiveness, Avoidance) and Zone (Contact, Interest). Category "test" is not used because the tests were found correlating. From Kuhn, 2024*

Zone	Sociability	Aggressiveness	Avoidance
Contact	24.5 %	20 %	0%
Interest	5 %	0.5 %	50 %

The data indicated that the Contact Zone elicits both aggressive (20%) and social (24.5%) behaviours, while the Interest Zone largely excluded aggressive behaviours (0.5%) and elicited a small proportion of sociability (5%). This again supports the notion that the Interest Zone is not relevant as a measure of sociability or aggressiveness, as it accounts for only 5.5% of the total behaviour related to these traits. Additionally, avoidance behaviours, which constituted 50% of the total timeframe, predominantly occurred in the Interest Zone. This suggests that the Interest Zone is more indicative of fear and avoidance behaviours (such as freezing and swimming away) rather than sociability. Moreover, her findings suggested that the timing during the test influenced the types of behaviours displayed. In other words, the fish exhibited different proportions of behaviours depending on the time interval studied during the 15-minute test. This insight could help improve behaviour detection during automatic tracking, as the software could analyze each interval separately to identify specific or predominantly displayed traits.

The combination of these findings suggests that while the "Time spent in the Contact Zone" exhibits a correlation between the Mirror Test and the Congener Test for the video tracking technique, it measures both sociability and aggressiveness in similar proportions. We therefore tend to finally confirm, in the case of juvenile Arctic charr, that the Mirror Test couldn't be a useful technique to measure sociability exclusively. This test, using the Contact Zone, measures both aggressiveness and sociability traits which could indicate that they are linked to each other and may form one axis. It could also measure a third component, which is avoidance, if a larger zone, including the Interest Zone, is used. Further studies on how behaviours of aggressiveness, sociability and avoidance interact should be studied for Arctic charr as they might be forming confounding axes.

However, there is clear potential to improve this test. Based on the positive impact of learning process on the time spent in the Contact Zone and consequently sociability, we recommend increasing the number of repetitions to improve the test's repeatability. The first repetition could be excluded, as it likely represents an acclimation phase and may not accurately reflect true sociability. Additionally, given the influence of time intervals on the proportions of behaviours observed, we suggest analyzing video recordings during automated tracking by focusing on intervals that highlight sociability traits over aggressiveness. According to Kuhn (2024), the most suitable interval seemed to be the first 5 minutes as it featured more dominant proportions of sociability. With these improvements, the time spent in the Contact Zone could serve as a reliable indicator of sociability in the Mirror Test.

Behavioural links with growth

Sociability or aggressiveness did not appear to influence growth rates in juvenile Arctic charr. Although the study's duration may not have fully captured growth performance, the results suggest that, given adequate food and care, fish meet their biological needs irrespective of social behaviours. In aquaculture settings, fish are generally not driven to aggressive behaviours for food, as agonistic interactions typically occur under conditions of insufficient feeding. For Arctic charr, which feeds both in the water column and at the bottom (Alanärä & Brännäs, 1996) and for which feed intake is more influenced by mobility rather than agonistic behaviour (Benhaïm et al., 2003), aggression and sociability did not seem to impact food intake or specific growth rate (SGR), since food was always available and the species can tolerate high stocking densities (Brown et al., 1992). This aligns with observations that Arctic charr naturally swims in shoals, which minimizes agonistic interactions (Baardvik & Jobling, 1990).

The social nature of the species helps avoid unnecessary energy loss from fighting over food resources or space. Therefore, we can conclude that the aggressive or social behaviour of the juvenile Arctic charr does not significantly impact its growth, as it obtains what it needs to compensate for any minimal energy loss. These findings contradict with some research as for example for juvenile coho salmon aggression was found to be negatively correlated with growth (Vøllestad & Quinn, 2003). However, as mentioned in the introduction, this species is an atypical salmonid that thrives in high stocking densities and non-size-sorted groups (Baardvik & Jobling, 1990), provided that food is readily available and housing conditions are adequate. This characteristic could simplify selection processes. There is an opportunity to develop selection programs aimed at ethical considerations by selecting for less aggressive and more social fish as sociability and aggressivity were found not to impact growth. In fact, producing fish raised in a more welfare-friendly environment that promotes positive, nature-based well-being could give a more sustainable aspect of the aquaculture industry and serve as a marketing advantage, attracting consumer interest and satisfaction. However, the logistics of these decisions need further investigation to determine if profitability across the entire aquaculture value chain can be effectively combined with improved welfare practices.

Conclusion

Aquaculture focuses on providing the necessary care for housed species to ensure efficient and rapid growth, which in turn guarantees productivity. With increasing pressure from social and scientific communities, more attention has been given to fish welfare in aquaculture, addressing ethical concerns while also promoting economic benefits. Fish personality can be studied in behavioural ecology to identify traits that could be exploited to reduce stress and enhance welfare in aquaculture. In our case, we tried to assess the reliability of a Mirror Test to measure sociability in juvenile Arctic charr. We also investigated possible links between behavioural traits found and growth to see the usefulness of genetically selecting fish based on personality traits.

Our findings led us to conclude that the Mirror Test can be a tool for measuring sociability in juvenile Arctic charr. However, sociability cannot be exclusively measured with a Mirror Test as it also captures elements of aggressiveness and avoidance. Moreover, only the Contact Zone, an area of 1.5 cm of width that displays direct contact with the stimuli, showed repeatability for both Congener and Mirror tests, with a weak correlation between them in this zone. There was no correlation between the tests for the Interest Zone, an area of 8.5 cm of width not including the direct contact with the stimuli. Further investigation revealed that this zone is more indicative of confusion and fear avoidance. Overall, the Mirror Tests reflected a mixture of aggressiveness and sociability in similar proportions in the Contact Zone, with avoidance and fear dominating in the Interest Zone. We suspect that these traits are closely linked and may belong to the same behavioural axis.

To improve the accuracy of distinguishing between sociability and aggressiveness during the Mirror Test, it is essential to develop new tools. One promising approach involves analysing video footage in the Contact Zone based on specific time intervals, as the timing of the test significantly influences the behaviours exhibited. The first 5 minutes seem to give the best sociability dominance over aggressiveness in the Contact Zone, thus could be the best option to go with.

Additionally, increasing the number of repetitions could enhance the test's repeatability and strengthen its connection to personality traits. This would allow for extended observation of social interactions in the Contact Zone across multiple repetitions. Importantly, the initial repetition might serve as an acclimation phase and, therefore, could be excluded from the analysis. However, this approach would require longer recovery periods and greater attention to the welfare of the tested fish.

Our findings on growth indicated that sociability and aggressiveness did not influence the Specific Growth Rate. This suggests a potential approach for selection programs based on behaviour: we can select to improve welfare since it does not harm productivity. This approach could provide the aquaculture industry with a more humane aspect, enhancing its acceptability as a sustainable production field.

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Appendixes

Appendix A: Behavioural links and SCS association with fitness

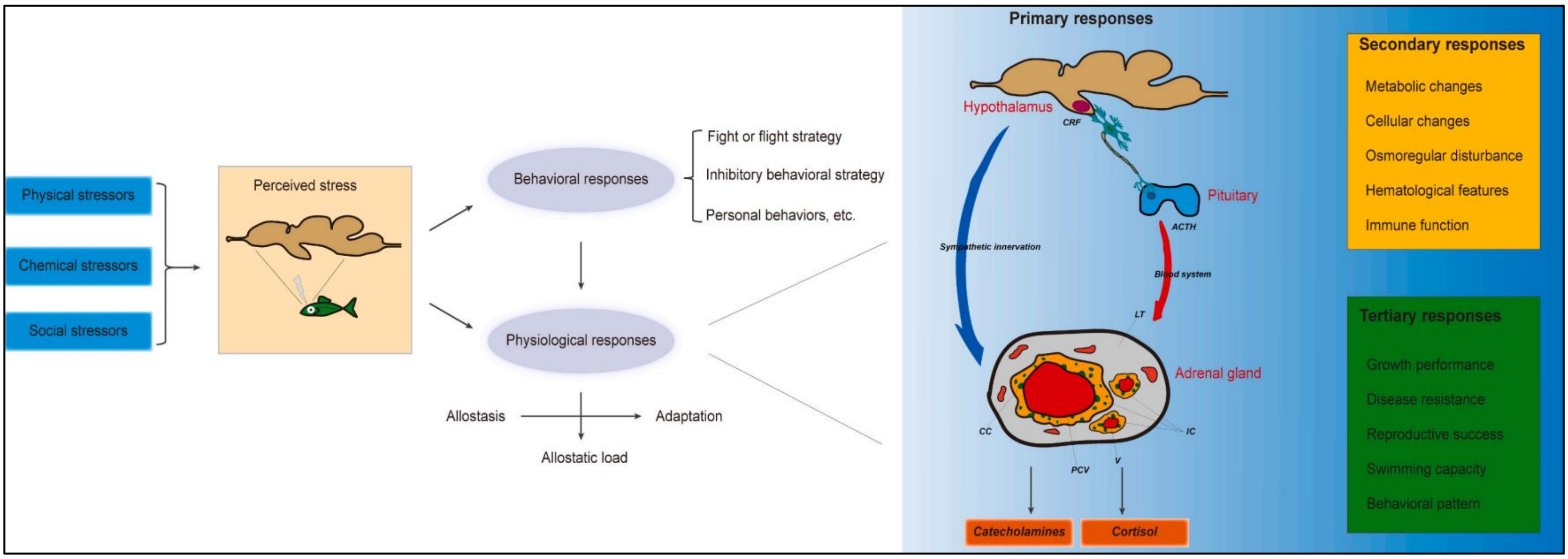

***Figure 1**: Schematic Overview of Key Behavioural and Neuroendocrine Components of the Stress Response in Teleost Fish: ACTH (Adrenocorticotropic Hormone), CC (Chromaffin Cells), CRH (Corticotropin-Releasing Hormone), IC (Interrenal Cells), LT (Lymphomyeloid Tissue), PCV (Postcardinal Vein), V (Small Blood Vessels). Tertiary responses represent the fish fitness functions. By Zhang et al. (2023)*

Appendix B: Tracking settings and data recording

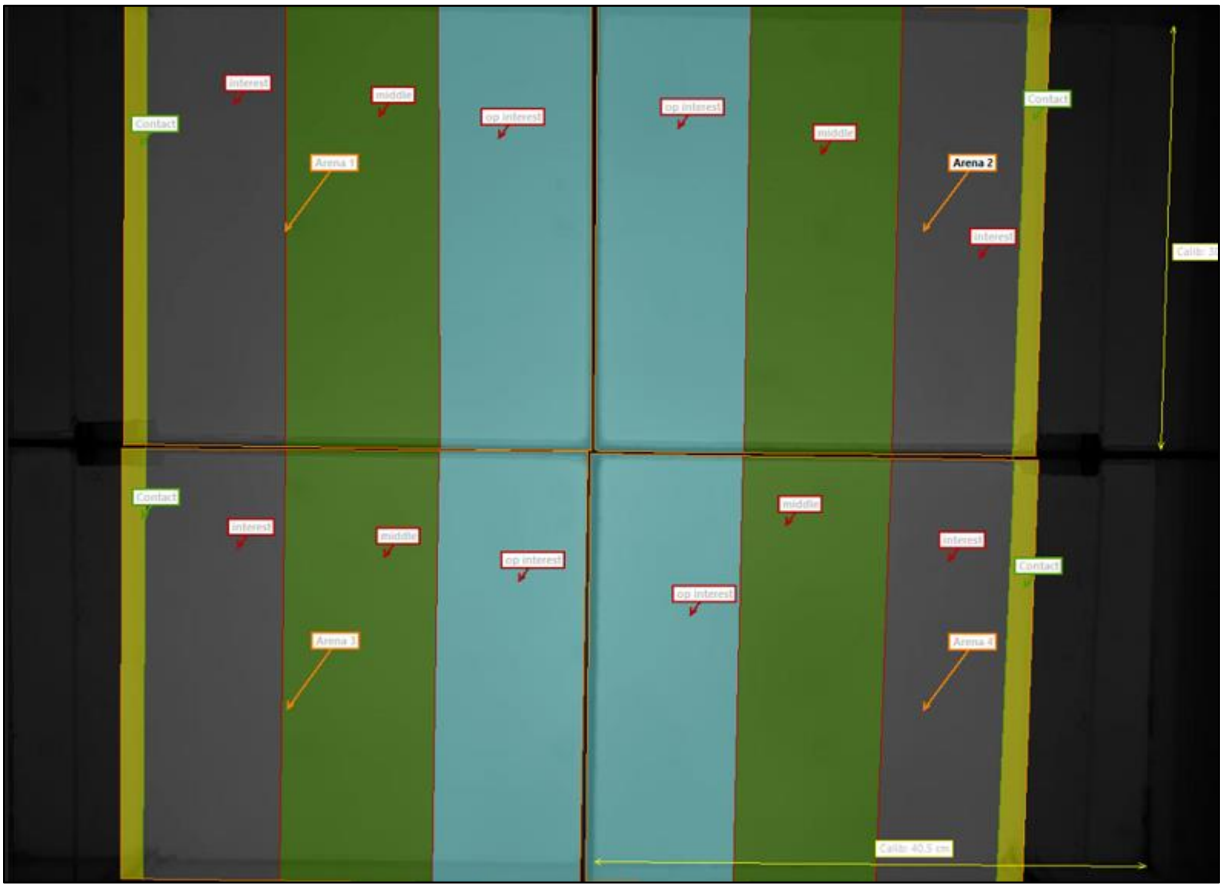

Figure 1: *Arena settings and calibration. Contact zone is 1.5 cm of width and each of the other zones are of 8.5 cm width in real life.*

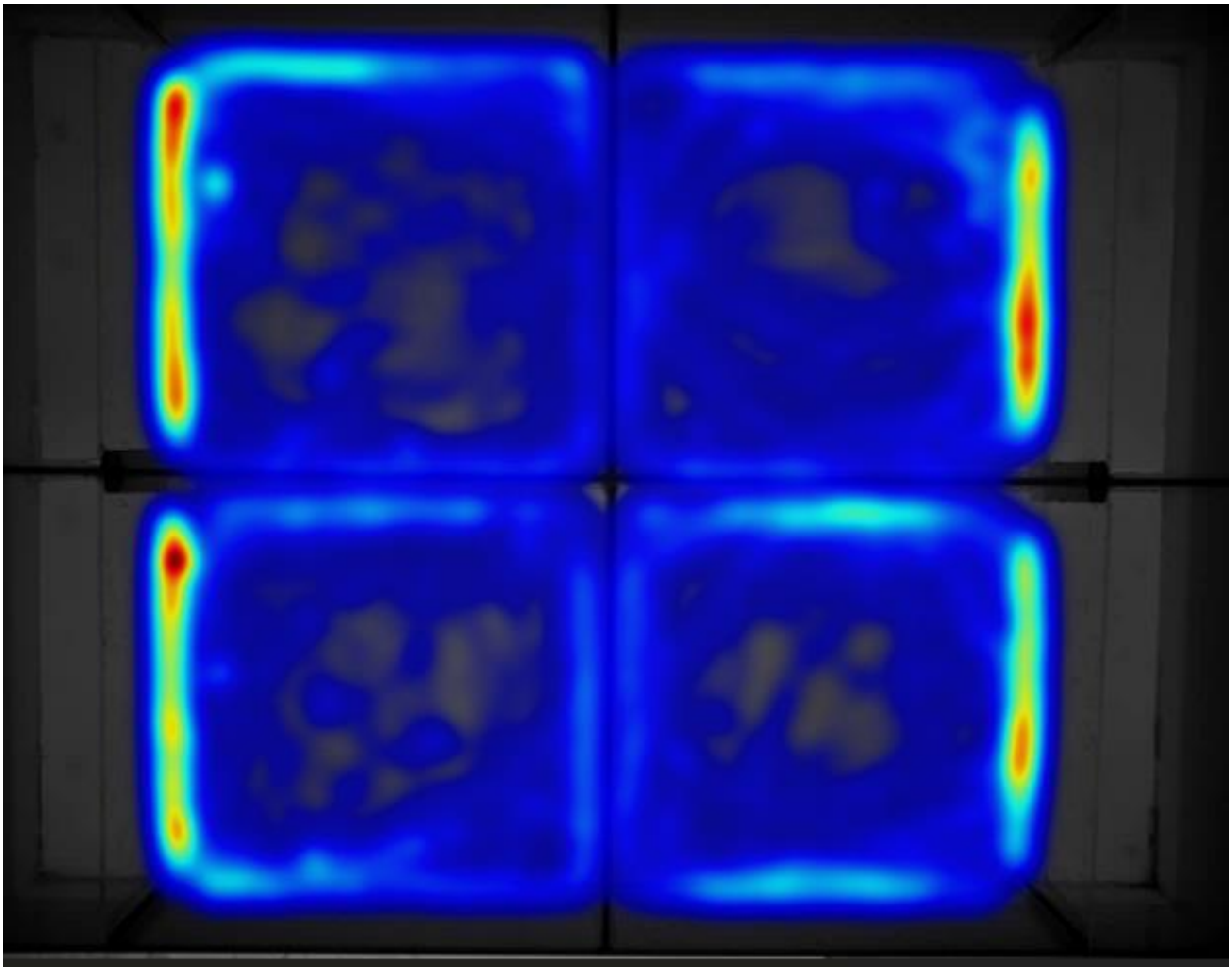

Figure 2: *A heatmap of the spatial preferences of the fish during a random trial. Red areas indicate where the fish spent most of the time, while lighter colours represent regions where the fish spent progressively less time. This figure is descriptive and demonstrates that we can visually discern the spatial preferences of the fish.*

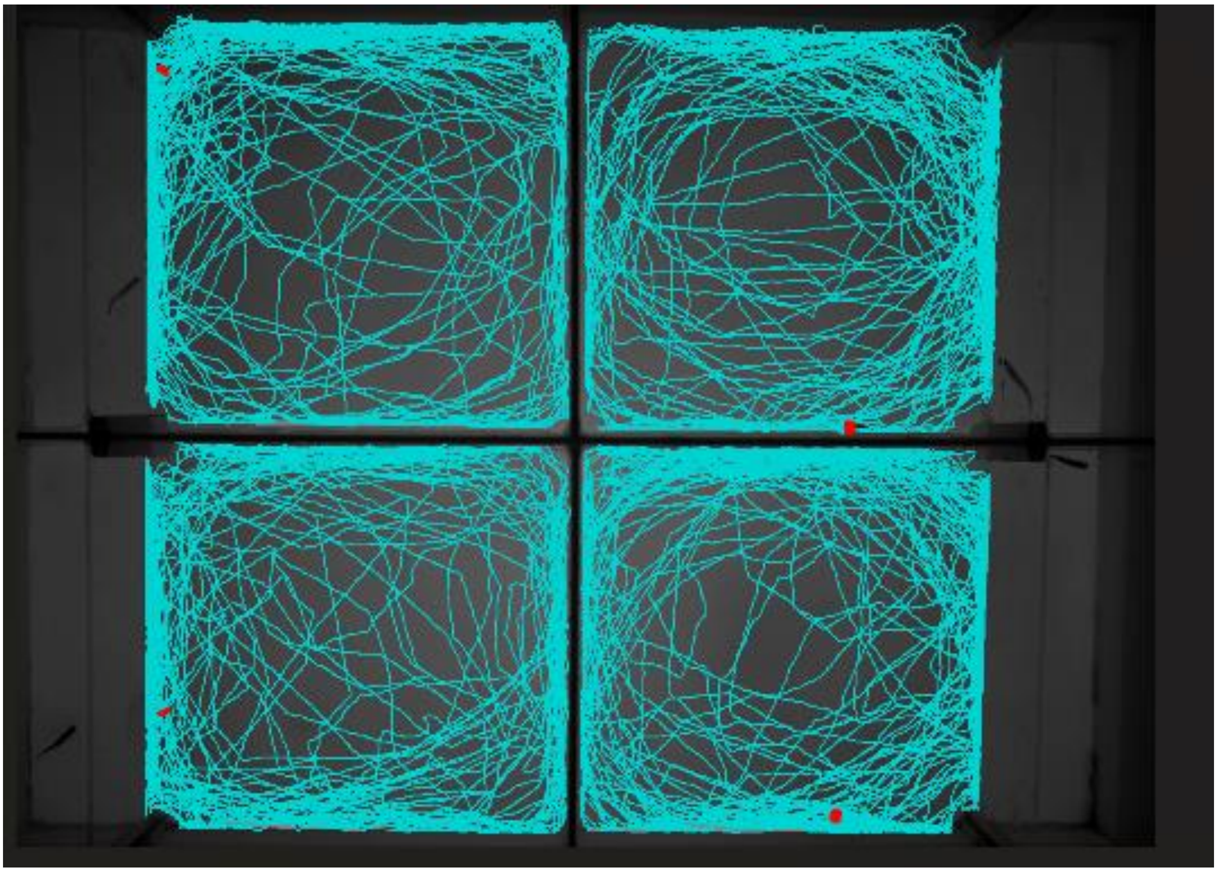

Figure 3: *Individual trajectory of a random sample showing how fish can exploit the space given and what are the more/less occupied zones.*

Appendix C: Fit summaries

***Table 1:** Summary of the fit (Time spent in the Contact Zone as a response variable). Estimates of the fixed variables and credible intervals.*

Predictor	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
MirC_Intercept	0,07	0,16	-0,26	0,39	1	5607	6358
ConC_Intercept	-0,17	0,19	-0,54	0,22	1	4555	5755
MirC_Length.z	0,17	0,13	-0,09	0,42	1	4866	6570
MirC_SGR.z	-0,06	0,07	-0,20	0,08	1	7481	8147
MirC_Rep2	-0,07	0,17	-0,41	0,27	1	6593	8564
MirC_Rep3	-0,17	0,29	-0,75	0,41	1	4978	6886
ConC_Length.z	-0,16	0,14	-0,43	0,12	1	4083	6475
ConC_SGR.z	-0,04	0,08	-0,19	0,11	1	6790	8694
ConC_LdiffCon.z	0,02	0,05	-0,08	0,12	1	11993	9367
ConC_Rep2	0,14	0,16	-0,17	0,46	1	5264	7074
ConC_Rep3	0,42	0,30	-0,17	1,01	1	4188	6249

***Table 2:** Summary of the fit (Time spent in the Interest Zone as a response variable). Estimates of the fixed variables and credible intervals.*

Predictor	Estimate	Est.Error	l_95_CI	u_95_CI	Rhat	Bulk_ESS	Tail_ESS
MirI_Intercept	0,75	0,15	0,46	1,04	1	4476	6670
ConI_Intercept	0,42	0,19	0,04	0,79	1	4711	6584
MirI_Length.z	0,14	0,12	-0,1	0,36	1	4076	6744
MirI_SGR.z	-0,04	0,06	-0,17	0,09	1	6157	8590
MirI_Rep2	-1,1	0,15	-1,39	-0,8	1	5228	7654
MirI_Rep3	-1,24	0,26	-1,75	-0,73	1	4192	6356
ConI_Length.z	0,24	0,14	-0,03	0,5	1	4762	6842
ConI_SGR.z	0,05	0,07	-0,08	0,18	1	8725	9329
ConI_LdiffCon.z	0,02	0,06	-0,09	0,14	1	13567	9410
ConI_Rep2	-0,48	0,17	-0,8	-0,15	1	5659	7826
ConI_Rep3	-0,85	0,3	-1,43	-0,25	1	4666	7088

Predictor: Name of the predictor variable. Estimate: Estimated coefficient value. Est.Error: Standard error of the coefficient estimate. l-95% CI: Lower bound of the 95% credible interval. u-95% CI: Upper bound of the 95% credible interval. Hat: Gelman-Rubin statistic (convergence diagnostic). Bulk_ESS: Effective sample size for the coefficient. Tail_ESS: Effective sample size for the coefficient (tail).

<u>Appendix D: Side project configurations</u>

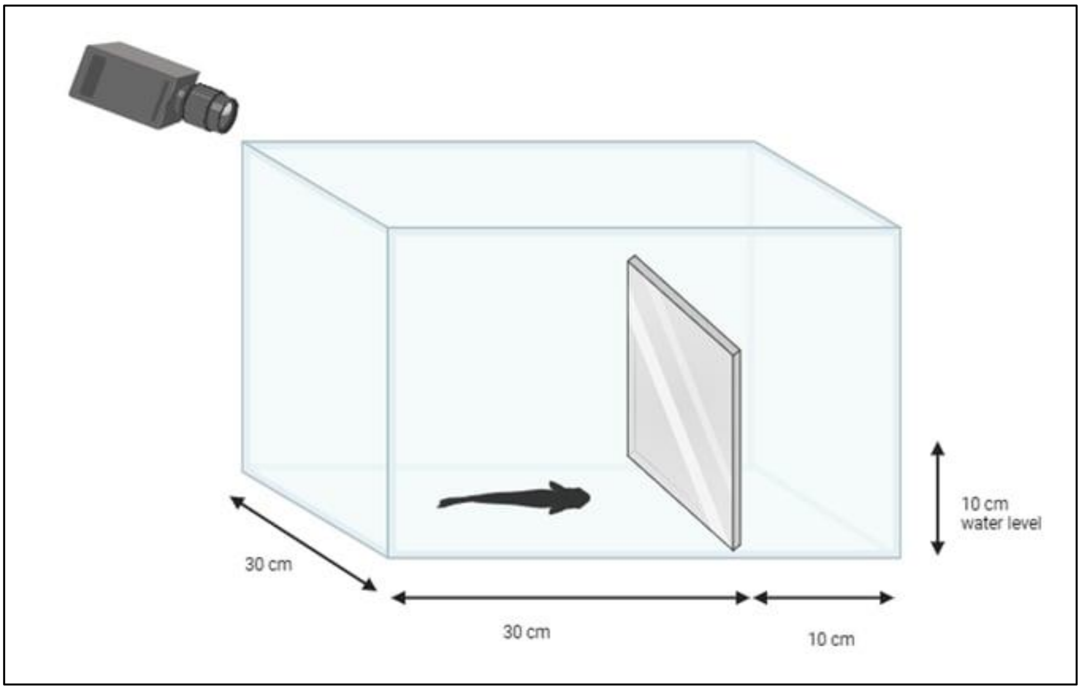

Figure 1: *Experimental design of the manual scoring technique. From Kuhn, 2024*

	Diplôme : Ingénieur agronome Spécialité : Sciences Halieutiques et Aquacoles Spécialisation / option : AQUA Enseignant référent : Bastien Sadoul

Auteur(s) : Chedi Hebaieb Date de naissance : 04/05/1998	Organisme d'accueil: Department of Aquaculture and Fish Biology, Hólar University Adresse : Hólar, 551, Iceland Maître de stage : David Benhaim
Nb pages : 61 Annexe(s) : 6	
Année de soutenance : 2024	

Titre français :

"Évaluation de la sociabilité chez des juvéniles d'Omble chevalier (*Salvelinus alpinus*) à l'aide du test du miroir pour des applications en aquaculture"

Titre anglais:

"Assessing sociability in Juvenile Arctic charr (*Salvelinus alpinus*) using the mirror test for aquaculture applications"

Résumé:

Ces dernières années, le bien-être des poissons en aquaculture a suscité un intérêt croissant, notamment par l'évaluation des traits de personnalité, ce qui permet d'améliorer le bien-être par la sélection de caractéristiques comportementales. L'omble chevalier (*Salvelinus alpinus*), une espèce valorisée dans l'aquaculture nordique, a été l'objet de cette étude, qui vise à évaluer la sociabilité, un trait pour lequel il n'existe pas de test standardisé. Nous avons examiné la fiabilité du test du miroir pour mesurer la sociabilité des jeunes ombles, en le comparant à un test utilisant des congénères vivants.

Nous avons affiné notre méthode en analysant deux zones : la zone de contact (1.5 cm de largeur, contact direct avec le signal visuel) et la zone d'intérêt (8.5 cm de largeur, sans contact direct). Les résultats ont montré que les deux zones présentaient une répétabilité, mais seule la zone de contact corrélait entre les tests. Toutefois, le test du miroir ne mesure pas uniquement la sociabilité ; il reflète aussi l'agressivité (zone de contact) et l'évitement (zone d'intérêt).

Enfin, aucun lien n'a été trouvé entre les comportements observés et le Taux de Croissance Spécifique (TCS), suggérant qu'il est possible de sélectionner des individus avec une agressivité réduite et une sociabilité accrue sans compromettre la productivité. Ces résultats ouvrent des perspectives prometteuses pour le bien-être des poissons en aquaculture.

Abstract:

In recent years, fish welfare in aquaculture has gained significant attention from both social and scientific communities. Assessing personality traits in fish has become a growing area of interest, as it offers a way to enhance welfare through the selection of specific behavioral characteristics. Arctic charr, a species increasingly valued in Nordic aquaculture, was the focus of this study. We specifically targeted sociability, for which no standardized test currently exists for this species. The study aimed to evaluate the reliability of the Mirror Test in measuring sociability in juvenile Arctic charr (*Salvelinus alpinus*) by comparing it to the more complex Congener Test, where live conspecifics are used. Additionally, we sought to refine this method by comparing two zones: the Contact Zone (1.5 cm in width, directly in contact with the visual cue) and the Interest Zone (8.5 cm in width, not in direct contact with the visual cue). We also examined whether the Specific Growth Rate (SGR) correlates with behaviors observed in the Mirror Test. Our findings indicated that both the Contact and Interest Zones showed repeatability, with only the Contact Zone correlating between tests. However, the Mirror Test does not exclusively measure sociability, as it also reflects aggressiveness (Contact Zone) and avoidance (Interest Zone). Finally, observed behaviors did not appear to influence Specific Growth Rate, suggesting the potential for selecting reduced aggressiveness and enhanced sociability without impacting productivity.

Mots-clés: Sociability – Mirror test – Arctic charr – Growth – Contact Zone - Welfare
Key Words: Sociabilité – Test miroir – l'Omble chevalier – Croissance - Zone de Contact – Bien être

* *Elément qui permet d'enregistrer les notices auteurs dans le catalogue des bibliothèques universitaires*