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### The missing link

Berlinghieri, Flavia

DOI:  
[10.33612/diss.929760767](https://doi.org/10.33612/diss.929760767)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2024

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Berlinghieri, F. (2024). *The missing link: Exploring laterality and personality development in two fishes*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.  
<https://doi.org/10.33612/diss.929760767>

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## Chapter 2

# The effect of light during embryonic development on laterality and exploration in Western Rainbowfish

*F. Berlinghieri*<sup>1,2</sup>, N. Jansen<sup>1</sup>, B. Riedstra<sup>1</sup>, C. Brown<sup>2</sup>, TGG. Groothuis<sup>1</sup>

1: Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands;

2: School of Natural Sciences, Macquarie University, Sydney, Australia



**“Laterality, 29(1), 1-18”**

**Abstract:**

Several factors affect the development of lateralization such as hormones and light exposure during early development. Laterality also often correlates with other behavioural traits such as aggression, boldness and activity. To examine whether there is a common mechanism underlying the development of laterality and these other behaviours, we manipulated laterality by exposing embryos of the Western rainbow fish (*Melatotaenia australis*) to light or continuous darkness during early development and determined whether a shift in laterality was associated with a change in behaviour in a novel environment test at two different ages. In accordance with the existing literature, exposing eggs to darkness led to offspring that displayed significantly less lateralized behaviour in the mirror test two weeks after hatching than offspring from eggs exposed to light. Interestingly, the effects of rearing condition were lost by 3 months of age. These data suggest that exposure to light can influence laterality very early in development, but such bias can be overwritten by developmental processes post-hatch. Moreover, our manipulation of laterality apparently had no influence on exploration suggesting independent causal mechanisms. The experimental manipulation of light exposure during development could be a useful tool for enhancing individuals with a specific laterality and behavioural traits to aid future research into the causes and consequences of laterality.

Keywords: Laterality, Behaviour, Fish, Light, Development, Mechanism

## Introduction

The control and processing of many cognitive functions are often unevenly distributed over the two brain hemispheres. Because motor control is predominantly crossed over, the right hemisphere controls the left side of the body and the left hemisphere the right side, this often manifests itself as behavioural side biases, resulting in differences in behavioural side preferences between individuals from the same species (Bisazza & Brown, 2011a). Cerebral lateralization and behavioural laterality are present in many vertebrate and invertebrate species (Vallortigara & Rogers, 2005a). It has been suggested that lateralization is the consequence of an evolutionary process whereby the processing power of the brain is enhanced by dividing the processing of different types of information over the two hemispheres thereby increasing cognitive capacity and facilitates multitasking (Magat & Brown, 2009; Rogers, 2000; Vallortigara & Rogers, 2005a).

Like many traits, laterality is influenced by both genetic and environmental influences (Bisazza et al., 2000; Brown, Western, et al., 2007). Laterality is partly heritable (Bisazza et al., 2000; Brown, Burgess, et al., 2007; Ocklenburg et al., 2016; Wiper, 2017), so potentially this could lead to the artificial selection of lateralized and nonlateralized lines ((Bisazza et al., 2000) However, the genetic variance is not enough to explain the phenotypic variance of laterality which suggests that environment, early experience and parental effects might also shape laterality (Brown, Western, et al., 2007; Budaev & Andrew, 2009; Dale Broder & Angeloni, 2014; Freire et al., 2006).

Variation in traits between individuals of the same species can often be explained through different experiences during development (A.-L. Bibost et al., 2013; Schaafsma & Grootuis, 2012). Exposure to light during the embryonic phase may represent an important non-genetic factor influencing behavioural and neural development, including laterality (Andrew et al., 2009; Dadda & Bisazza, 2012; Rogers, 1990). Light exposure during early embryonic development affects laterality in both avian (Rogers, 1990) and fish species (Andrew et al., 2008a, 2009; Dadda & Bisazza, 2012) (Budaev & Andrew, 2009). The influence of light on laterality was first established in chicks, where the asymmetrical position of the embryo in the egg differentially affects light induced growth of the projections of the eyes to the contralateral hemisphere (Rogers, 1990). In fish species, light is suggested to be essential for the development of laterality during a sensitive period in embryonic development (Andrew et al., 2009). Despite the fact that

fish eggs and the developing embryos are usually quite transparent, light can influence the development of lateralisation very early in ontogeny in both live-bearing (Dadda & Bisazza, 2012) as well as egg-laying species (Andrew et al., 2009).

When quantifying laterality, researchers commonly focus on two aspects: strength and direction. Strength indicates how far from parity an individual deviates irrespective of the direction of bias, whereas the direction indicates if the individual is right or left lateralized. It has been proposed that natural selection might act at the level of the strength of laterality rather than the direction of cognitive asymmetry in some contexts since the cognitive benefits are realised irrespective of direction (Collins, 1991). However, in situations where coordinating within a group is essential for survival, selection should act in a frequency-dependent manner across the entire population in which case laterality in a certain direction may be favoured (Billiard et al., 2005; Brown & Braithwaite, 2005; Vallortigara & Rogers, 2005b).

As emotions, cognition and motor behaviour are lateralised, personality traits such as boldness and aggression may be expected to correlate with either the direction or strength of laterality. Indeed, in several vertebrates, variation in laterality has been associated with multiple behavioural traits, including boldness, activity and aggression (Brown & Bibost, 2014; Byrnes et al., 2016). Studies also indicate that fear, for example, is mostly a right hemisphere phenomenon in both humans (Murphy et al., 2003) and other animals (Deng & Rogers, 2002; Vallortigara & Rogers, 2005a). In order to explore these correlations more closely, we can use our knowledge of the effects of light during early embryonic development to manipulate laterality (Andrew et al., 2009; Dadda & Bisazza, 2012; Rogers, 1990) and determine if other behavioural traits also shift. To date, only a single study investigated the effects of light on laterality and personality traits in fish and found that rearing fish in the dark reduced laterality in response to a predator model and also reduced boldness (Andrew et al., 2009). The subjects were tested just 7 days post fertilisation (i.e. 3 - 4 days post hatch) thus the long term effects remain unknown. It might be the case that early exposure to light shapes laterality soon after hatching, but this may be eroded by interactions with the environment post-hatch or other developmental processes (Brown, Western, et al., 2007; Quaresmini et al., 2014).

Here we tested the influence of early experience of light and dark on the development of laterality and exploration trait of western rainbowfish (*Melanotaenia australis*) at two different ages. Rainbowfish have been used repeatedly and successfully in laterality and

personality research (see method section). To determine the longevity of the influence of embryonic light exposure on behavioural development we examined laterality and exploration shortly after hatching (ca 2 weeks of age) and again when the fish approached sexual maturity (ca 3 months of age). Based on previous studies, we hypothesized that offspring reared in the dark would be less lateralized, as measured through viewing their reflection in a mirror, and they would also be less active when tested soon after hatching. Moreover, we expected the influence of the embryonic rearing manipulation to decline over time, to be superseded by individual experience. That is, we hypothesized that there would be no influence of rearing environment on laterality or activity when fish were tested close to sexual maturity. Regarding laterality and exploration, we expected to find dark-reared offspring to spend more time close to the walls than light reared offspring.

## Methods

### *Study Animals*

In this study we used the eggs and offspring of Western Rainbowfish (*Melanotaenia australis*). Rainbowfish can live solitary but are usually found together with conspecifics in small dynamic shoals. Males more brightly coloured and typically larger than females (Brown & Culum, 2000; Young, 2010). After finding a suitable mating partner, eggs are externally fertilized and deposited in the vegetation which serves as a shelter for the fry until they are large enough to fend for themselves (there is no parental care). Hatching time is temperature dependent but usually takes around 7 days post fertilization. Most rainbowfish reach sexual maturity in approximately 90 days (Humphrey et al., 2003).

Rainbowfish have previously been used as a model species to study laterality (A. L. Bibost & Brown, 2013a, 2014; Brown & Bibost, 2014) social learning ((Brown & Warburton, 1999), cognition (Brown, 2001), animal personality (Colléter & Brown, 2011), stress responses (Zuberi et al., 2011, 2014), anti-predator behaviour (Brown, 2003; Brown & Warburton, 1997), and the effects of the rearing environment during development on behavior (A. L. Bibost & Brown, 2013b; Kydd & Brown, 2009). They are a preferred species for such studies, since they are easy to rear in captivity and breed throughout the year under laboratory conditions (Reid & Holdway, 1995).

### *Experimental Design*

To determine the effect of light exposure during embryonic development on laterality and its association with exploration behaviour in rainbowfish, eggs from 16 pairs were reared in either of two light regimes, normal light or darkness (see below), and the resulting offspring were tested for laterality and exploration in a rotational mirror test and a novel environment test respectively (see below). We tested the fish at two ages: c. 2 weeks of age and then c. 3 months of age. After the first test the subjects were returned to the home tanks. Since we did not tag the fish, as they were too small, we could not track individuals through to the second test. Thus, here we consider the population level effects of the treatment rather than following individuals over time.

### *Breeding*

Sixteen parent pairs were housed in eight different tanks (120x40x40cm), each with a divider in the middle. Water temperature was  $26 \pm 1^\circ\text{C}$  with 12 hours light and 12 hours dark (provided by a fluorescent tube light mounted overhead). Tanks were all connected to a recirculating system with water parameters controlled weekly and air bubbles provided constantly. Tanks were enriched with plastic plants and PVC tubes to provide shelter. Fish were fed twice daily. Each pair was provided with a spawning mop. Spawning mops were made of approximately 30 strings of green acrylic wool attached to suction cups and placed at one end of the tank (Oulton et al., 2013). These mops were essential for this process since rainbowfish may consume their own eggs and the mop made this more difficult for them while at the same time facilitating egg collection. The spawning mops were checked for eggs throughout the day.

### *Housing and light and dark exposure treatment*

For eggs reared in the Light treatment, mops were transferred to a plastic container and placed in an aquarium under 12/12h light conditions. In this treatment the aquaria were lit from an overhead aquarium light as well as the lights in the aquarium facility. The containers had small holes in them to provide waterflow and a single air stone providing air bubbles. To minimize light exposure, eggs for the Dark treatment group were only collected shortly after the onset of light in the aquarium facility or whenever the process of egg laying was witnessed. Directly after collecting the mops, they were transferred to

containers in a dark tank. This dark tank was covered with layers of black plastic and covered with a lid which allowed almost no light to penetrate to ensure the eggs on the inside of that tank were developing in darkness ( $<0.01$  lux). The eggs developing in darkness were only exposed to a minimal amount of light during maintenance (lasting less than 2 minutes once per day) or whenever a new container with eggs was introduced.

Rainbowfish eggs hatch after approximately seven days (Oulten et al., 2013), at this time the newly hatched larvae from both treatments were transferred to a larger container situated at the surface of the tank and were exposed to a normal light regime (12:12). These containers were also provided with a small sponge filter to provide extra filtration and aeration. The young larvae were fed with 10ml cultured paramecium three to five times daily. When the larvae reached the age of approximately two weeks the cultured paramecium was replaced with decapsulated, newly hatched brine shrimp. Offspring at the age of 2 months old were moved to bigger tanks (40x40x40cm), keeping their original group. These housing tanks were similar to those housing their parents and were all connected to a recirculating system with water parameters controlled weekly and air bubbles provided constantly. Tanks were enriched with plastic plants and PVC tubes to provide shelter. Fish were fed twice daily (artemia and flakes). Water temperature was  $26 \pm 1^\circ\text{C}$  with 12 hours light and 12 hours dark.

### *Behavioural tests*

We performed two tests: A rotational mirror test and rotational novel object test to determine laterality and a novel environment test to determine exploration behaviour. To test whether there was a lasting effect of the treatment, groups of fish were tested at 10 to 17 days of age (young) and at 42 to 60 days of age (mature). We had 8 pairs of parents, each clutch of eggs from each pair was divided for the light treatment and dark treatment. Each offspring group consisted of 4 to 6 individuals. For the dark treatment, we tested 20 fish at the younger age and 24 from the mature age group. For the light treatment, we tested 20 fish at younger age and 23 at the mature age. Within each age group, fish were tested on both the rotational mirror test, novel object test and the novel environment test.

Many animals have laterally placed eyes with non- or small overlapping optic fields and little interhemispheric communication. The optic fields of many species of fish (and birds) are directly linked to the contralateral hemisphere (Miletto Petrazzini et al., 2020), which makes them perfect candidates for studies on the asymmetric use of cognitive functions. Because of these characteristics, the laterality of many fish species can be established



through simple tests ((Bisazza & Brown, 2011b). For social fish species, mirror tests are utilized to establish laterality where the mirror image of the subject takes the place of a conspecific (Brown & Bibost, 2014; Irving & Brown, 2013a). Since the link between sociality and laterality has already been established in fish species (Reddon & Balshine, 2010), this test can be used to determine laterality by observing which eye is preferred for social interactions.

The tests were recorded using an overhead camera and recordings were analysed using BORIS software (Friard & Gamba, 2016).

#### *Laterality: Rotational Mirror and novel object Test*

To establish the strength of laterality for the offspring in both treatments, a mirror test was performed based on a previous experiment by Schaafsma & Groothuis(2012)). The test started with an acclimatization period which involved catching fish with a petri dish and placing it in a small plastic container (20x10x8) within a circular compartment. The compartment contained a smaller black cylinder in the middle and a camera mounted overhead approximately 15 cm above it (Fig 1). For the second part of this test the cylinder in the middle was replaced with another cylinder with a reflective surface with a novel object within (not visible to the fish). The last part of the test, we removed the cylinder exposing the novel object to the fish. For the first age group this was a piece of curled up piece of silver wire and a small yellow hairclip for the second age group. Each part of the test lasted 5 mins. We scored which eye the fish used to look at the mirror and at the novel object, based on their body orientation every second for 10 minutes, we then calculated laterality index and strength laterality. We expect to find more lateralized individuals from the light raised group compared to the dark. We did not have expectation regarding the direction of laterality.

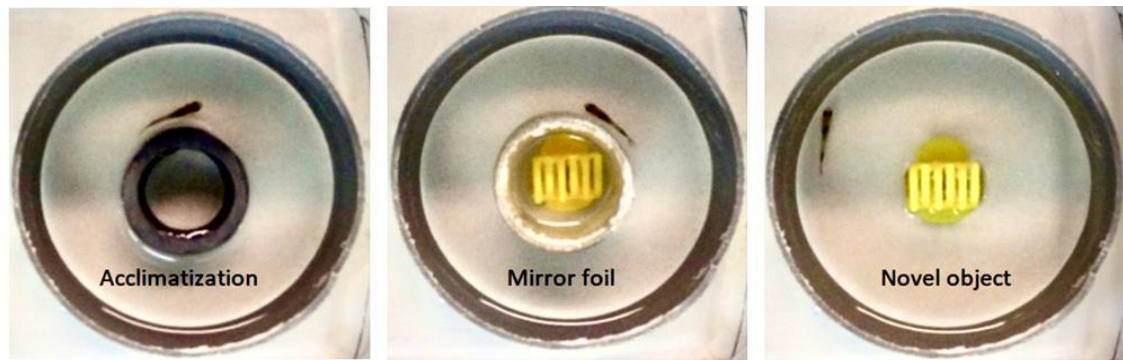


Fig 1. Graphic representation of the laterality test. Acclimatization, mirror test with mirror foil in the tube and novel object. Second age group representation with yellow hair clip, while for the first age group there was a silver wire.

#### *Novel environment test*

To measure exploration behaviour and activity, individual animals were subjected to a novel environment test (Mazue et al., 2015; Dingemans et al., 2007). A subject was placed in a plastic arena (20x10x8 cm) that contained five small stones, one was placed in the middle of the container and the remaining four were used to mark a square in the centre that equated to half the surface of the container (Fig 2.). The behaviour of the fish was recorded for 10 minutes by a camera mounted approximately 15 cm above the arena starting directly after introducing the fish to the novel environment. We scored the time spent in the inner part of the arena as delimited by the stones (Fig 2). Activity was measured as the time spent active/swimming during the test and was measured manually observing the video with the software BORIS.

Based on a previous study Andrew et al., (2009) we expected to find dark-reared offspring to spend more time close to the walls than light reared offspring. Thigmotaxis is more common in shy fish and is often used as an indicator of anxiety (Maximino et al., 2010).

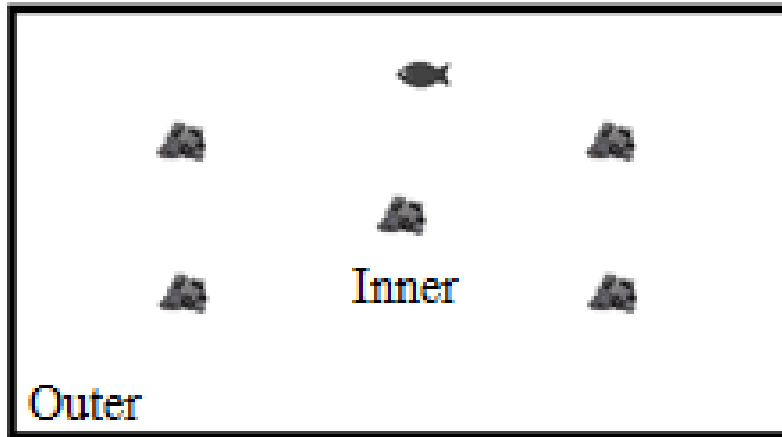


Fig2. Novel environment test with inner and outer part indicated.

## Data Analysis

We compared the laterality between the treatments but also within each treatment at each of the two different age classes. We tested the distribution of all data for normality using the Shapiro–Wilk test and used Mann-Whitney U Test for the tests because the data were not normally distributed, using SPSS 26.0. We calculated the laterality index and the strength of laterality using the formulas:

$$Laterality\ Index = \frac{Left\ eye - Right\ eye}{Left\ eye + Right\ eye} \quad Strength\ of\ laterality = \left| \frac{Left\ eye - Right\ eye}{Left\ eye + Right\ eye} \right|$$

Here we do not report the results for the laterality index because all of them were not significant and we had no specific hypotheses about how the treatment might affect laterality direction.

For the novel environment test we analysed the time spent in the inner section of the arena and the time spent active between the treatment groups and within the population for the different age classes.

## Results

### - Laterality - Mirror test

Young offspring from the light treatment (n= 20) were more lateralized than dark-reared (n=20) offspring (Mann-Whitney U Test U = 280.0, p=0.030) confirming that the dark treatment was effective in lowering the strength of laterality in the offspring. There was

no effect of rearing conditions when the fish were mature (dark n=24, light n=23) (Mann-Whitney U Test  $U = 292.0$ ,  $p=0.733$ ) Fig 3.

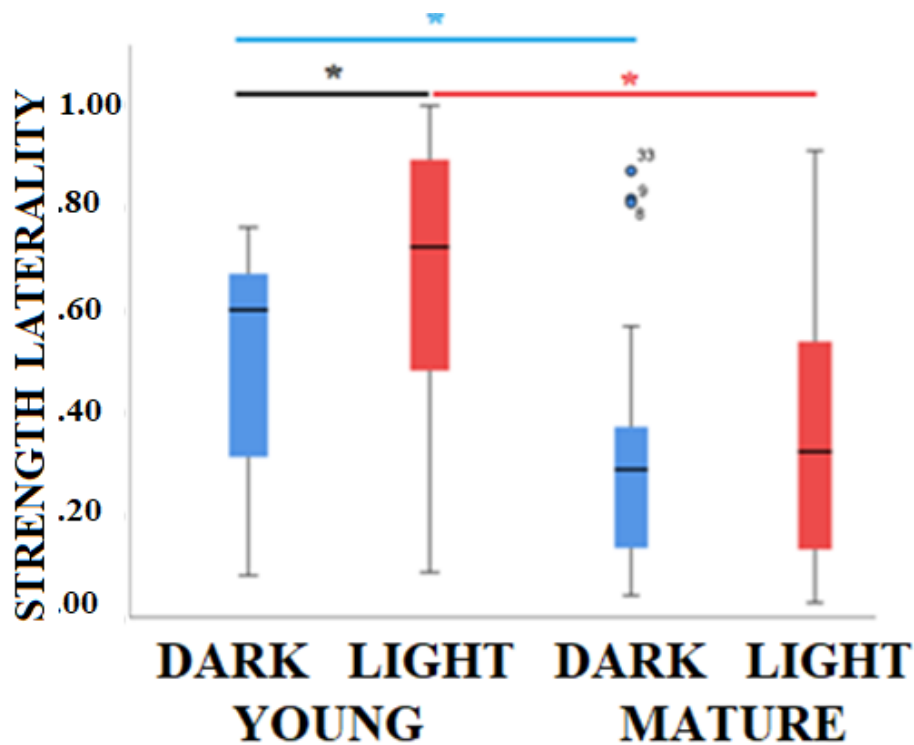


Fig3. Medians, quartile 1 (25%) and quartile 3 (75%) in the boxplots of the strength of laterality in the mirror test for offspring of the dark (blue) and light (red) treatment when they were young and mature. Asterisks indicate statistically significant differences.

- Age effect of Laterality in the mirror test

Both fish reared in the light (n=20) condition as well as in the dark (n=20) condition were more lateralized when younger (Light: Mann-Whitney U Test  $U = 90.000$ ,  $p=0.001$ ; Dark  $U = 169.000$ ,  $P=0.025$ : Fig. 1). This confirms that the strength of laterality decreases during ontogeny.

- Laterality- Novel object

There was no effect of treatment when the fish were young (light n=20; dark n=20) (Mann-Whitney U Test  $U = 256.0$ ,  $p=0.134$ ) or mature (dark n=24, light n=23) (Mann-Whitney U Test  $U = 249.0$ ,  $p=0.566$ ). However, light-reared fish were more lateralized when young compared to mature (Mann-Whitney U Test  $U = 52.0$ ,  $p=0.001$ ), whereas this

did not reach significance in dark-reared fish (Mann-Whitney U Test  $U = 169.0$ ,  $p = 0.094$ ) see Fig 4.

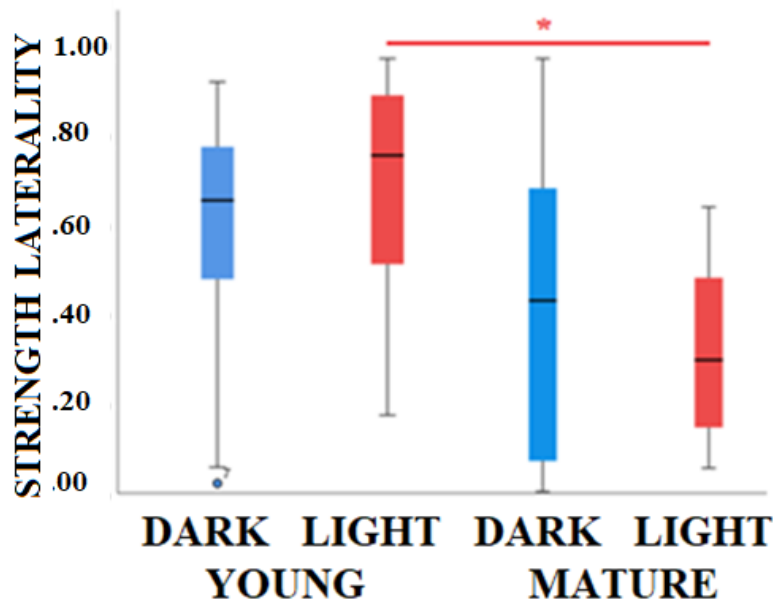


Fig 4. Medians, quartile 1 (25%) and quartile 3 (75%) of the strength of laterality in the novel object test of fish reared in the dark (blue) and light (red) tested when young and mature and their equivalent significant results (\*).

- Novel environment test

Exploration

There was no effect of treatment when the fish were young (light  $n = 24$ ; dark  $n = 24$ ) in the time spent in the inner part of the test arena (Mann-Whitney U Test  $U = 337.5$ ,  $p = 0.307$ ;) nor when they were mature (light  $n = 27$ ; dark  $n = 20$ ) (Mann-Whitney U Test  $U = 209.5$ ,  $p = 0.472$ , : Fig 5). Young dark reared fish spent more time in the inner part compared to mature fish (Mann-Whitney U Test  $U = 114.0$ ,  $p = 0.005$ ). Whereas there was no difference between young and mature fish reared in the light (Mann-Whitney U Test  $U = 248.0$ ,  $p = 0.409$ ).

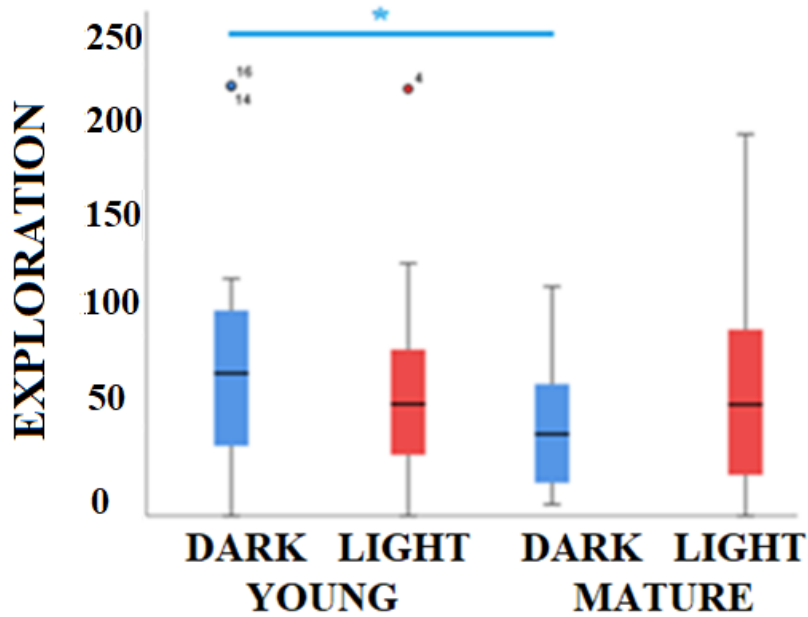


Fig 5. Medians, quartile 1 (25%) and quartile 3 (75%) of exploration for the dark (blue) and light (red) groups when they were young and old. Exploration is time spent (s) in the inner zone.

#### -Activity

There was no effect of treatment on the time spent active when the fish were young (light  $n=24$ ; dark  $n=24$ ) (Mann-Whitney U Test  $U = 53.0$ ,  $p=0.470$ ), nor when they were mature (light  $n=27$ ; dark  $n=20$ ) (Mann-Whitney U Test  $U = 259.0$ ,  $p=0.654$ ; Fig 6).

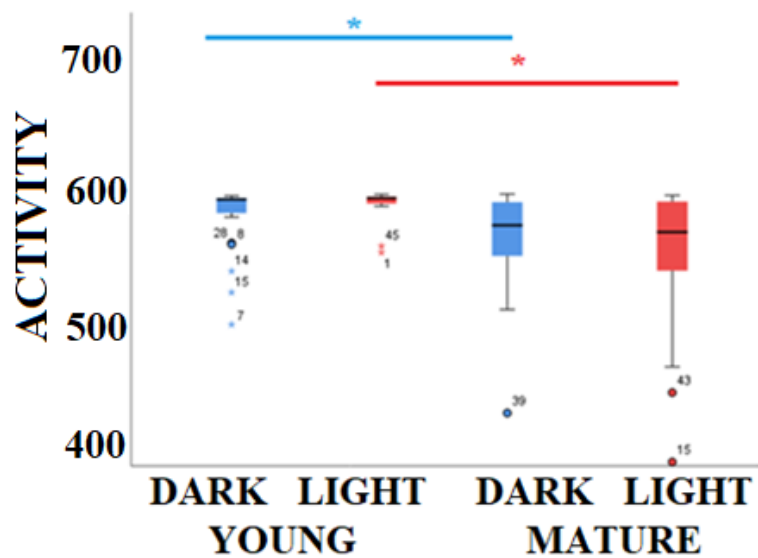


Fig 6. Medians, quartile 1 (25%) and quartile 3 (75%) of activity the dark (blue) and light (red) groups when they were young and mature. , Activity is time (s) spent swimming

Young fish within the dark treatment were more active than mature fish reared in the dark (Mann-Whitney U Test  $U = 148.0$ ,  $p = 0.046$ ). Similarly, young fish reared in the light were more active than mature fish reared in the light (Mann-Whitney U Test  $U = 146.5$ ;  $p = 0.004$ )

Fig 6.

## Discussion

Here we set out to determine if manipulating exposure to light during embryogenesis affects laterality in young fish and if this is maintained until sexual maturity. Further, we examined if manipulating laterality also caused shifts in other behavioural traits (exploration and activity). The mirror test revealed that fish reared under standard conditions (light) were more lateralized than those reared entirely in the dark. The effect of rearing environment on laterality was only evident in the young fish (tested at ca 2 weeks of age) but was lost when tested at ca 3 month of age. Within treatment groups, we found that fish were more lateralized when young, both in the light and dark group, confirming that personal experience or development can influence laterality throughout ontogeny. We found no differences in laterality between light and dark treatment groups while viewing a novel object. Young fish were more lateralized in the light treatment, but no such differences were evident in the dark-reared fish. Contrary to our expectations, there was no corresponding shift in either exploration (as measured by the time spent in the inner part of a novel arena) nor activity as tested in a novel arena. Young fish were more active than older fish irrespective of their rearing conditions. Fish reared in the dark spent significantly more time in the inner part when young but there was no difference between age groups in the light-reared treatment. These results show that they are probably indirectly correlated because they change over time. However, the limitation of the experiment was about the impossibility to tag the fish and to have individual data over the two repetitions, so we only have results at the population level.

The mirror test revealed a significant difference between rainbowfish that were reared under different lighting regimes during embryogenesis. Rearing under normal conditions, where embryos had considerable exposure to light, positively influenced the development

of lateralization. Those reared in darkness were less strongly lateralized than those reared in the light when tested at a young age. This result is in accordance with the previous research on chicks (Rogers, 1990), zebrafish (Andrew et al., 2008b) and a live-bearing Poeciliid (Dadda & Bisazza, 2012). For example, chicken embryo is naturally oriented in a way that during the later stages of incubation, after day 17 of incubation precisely, the left eye is occluded by the wing and body while the right eye can receive the light input through the eggshell and membranes (Rogers, 1990). In the zebrafish, light exposure early in development is needed to generate lateralization (Andrew et al., 2009). In a live bearing goldbelly topminnow, fish from the high-light treatment were significantly more lateralized in both visual and motor experiments, whereas fish exposed to low light intensities were not lateralized. In both chickens and zebrafish, the main consequence of light was the alignment of the laterality of the individuals in the same direction (Dadda & Bisazza, 2012) but here we have not found a difference between the groups in the laterality index.

Previous experiments examining the impacts of light on laterality in fishes examined reasonably young individuals. Subjects were tested at 7 and 10 days of age for zebrafish (Budaev & Andrew, 2009) and poeciliids respectively (Dadda & Bisazza, 2012). Here we also found an impact on the strength of laterality in the young rainbowfish in the mirror test, but the rearing effect was lost by 3 months of age when the fish reached sexual maturity. In the novel object laterality test, the young light exposed fish were also more strongly lateralized than mature fish. Although several studies provide evidence of an effect of light during early embryonic development on laterality across multiple species, none have investigated a possible effect on older individuals. There are no studies referring to long term effect of eggs exposed to dark during their development. It is interesting to note, however, that asymmetry in chicks generated by light and as measured on the pebble-grain task is no longer present after 3 weeks of age, and this is also the case for asymmetry in the thalamofugal visual projections (R. Andrew, 1991). Because these tests used individuals that were all still in a highly developmental stage, the result of the present study provides reason to believe that the effect of the treatment may be neutralized over time. That is, as the larvae age the difference between treatments should become smaller. To our knowledge, no one has yet examined the longevity of light manipulation of laterality in any animal.

Exposure to light and the timing of exposure are suggested to be essential to activate asymmetric gene expression in the habenula of fish (Budaev & Andrew, 2009), which in



turn results in asymmetric development of the habenula and generates eye preferences during visual tasks. This process of lateralization starts when light reaches undifferentiated cells in photosensitive regions (Kuan et al., 2007) during a sensitive period in the embryonic stage (Budaev & Andrew, 2009). Given the lower strength laterality score of dark developed larvae in the present study, this mechanism is likely to be acting in the same way in rainbowfish. The theory behind this mechanism was based on a similar mechanism in chicks where light exposure was considered essential to develop laterality (Vallortigara et al., 1999). De Borsetti et al., (2011) suggested that the absence of light suppresses habenular development. In that study, the formation of the habenular nuclei was examined in zebrafish embryos that develop in darkness and found no difference in gene expression in both group developing zebrafish but rather a temporary accumulation of habenular precursor cells. This indicates that prolonged darkness during embryonic development slows down neurogenesis. Indeed, a research on zebrafish suggests that light can change visual acuity, but in fact it appears that it is exposure to constant light that causes problems rather than constant darkness (Bilotta, 2000). However, our casual observations suggest that rearing in the dark had no obvious impact on the behaviour of the fish, which feed and move normally .

Several studies have found that light exposure can influence personality traits. In a wild bird population, for example, individuals with high exposure to light during prenatal development had a shorter duration of tonic immobility than those with low-light exposure (Ruiz-Raya & Velando, 2022). Budaev & Andrew, (2009) found in zebrafish, eggs reared under dark condition, the larvae were shyer and had a reduced behavioural laterality in response to a predator model. Similarly, Budaev & Andrew,(2009) found that zebrafish reared in the dark spent less time in the centre of the novel arena (exploration).

In the present study, we found that rearing environment (dark and light) did not influence the amount of time fish spent active (Activity). However, activity levels were significantly higher in young fish compared to mature fish irrespective of their rearing conditions. While more explorative young fish rather than mature only in the dark reared fish. The fact that we manipulated laterality at the young age group but found no corresponding change in these behavioural traits suggests that they are likely not always linked by common underlying mechanisms. Our results suggest that correlations between laterality and personality traits likely occur independently, even if was not possible to test it. Indeed, reviewing the literature it has been demonstrated that laterality and boldness are linked in guppies and rainbowfish(Brown & Bibost, 2014; Irving & Brown, 2013b).

In conclusion, we confirmed that exposure to light during embryogenesis influenced the development of lateralization in rainbowfish. Indeed, dark reared fish were less lateralized than fish reared under standard conditions at 7-10 days of age. Interestingly, laterality shifted over time and by the time the fish were mature the impact of rearing exposure to light had been erased. We found no corresponding shifts in activity in the novel environment test suggesting no common underlying mechanisms shape these traits. Future experiments should follow the development of laterality in young fish in much closer detail to pinpoint when the impacts of early light exposure on laterality are lost. Clearly more work is needed to investigate the underlying mechanisms of laterality and its link with personality traits.

#### **Data availability statement**

Raw data were generated at University of Groningen. Derived data supporting the findings of this study are available from the corresponding author F.B on request.

#### **Ethical notes**

Housing and testing of behaviours were in adherence to the project permit from the Instantie voor Dierenwelzijn (IvD light protocol, University of Groningen, the Netherlands).

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