Freie Universität Berlin

Bachelor-Thesis

The effects of size-selective harvesting on hooking-vulnerability and hook-avoidance learning in adult zebrafish (*Danio rerio*).

By Jonas Peter Häusler

Student ID: 5308878

First examiner: Prof. Dr. Robert Arlinghaus

Second examiner: Prof Dr. Jonathan Jeschke

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Declaration of Independence

5308878
Student ID

B.Sc. Biology
Degree Program

Häusler,
Last Name

Jonas, Peter
First Name(s)

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Abstract

Size-selective mortality (either positive or negative) in fish stocks can bring about evolutionary changes in life-history traits as well as behavioural traits such as collective risk-taking and food finding and induce cognitive changes like decision-making and learning-speed. However, evolutionary changes in behaviour and avoidance-learning of fishing gears like hooks are relevant in the context of recreational fisheries. Yet, how collective behavioural changes can impact mortality outcomes in fishing is underexplored and lacks empirical studies. In this study, I investigated the impact of size-selective mortality on hook-avoidance learning and memory in adult zebrafish (*Danio rerio*) derived from three selection lines; that were generated by intensive removal of the large or small or random sized fish for five consecutive generations. My objectives were (1) to test if zebrafish selected for either large or small body size differed in hook-avoidance learning behaviour, (2) to examine if the zebrafish selection lines differed in remembering the hooking experience, and (3) to determine if hook-recognition varies among selection lines in zebrafish. To that end, I tested inspection and hooking-vulnerability in fish groups among the three selections lines over a period of time and tested for memory after an interval. I found the small line fish to be significantly less vulnerable to fishing hooks compared to the control line, though the selection lines learned to avoid the fishing hook equally well. All lines retained the memory of hooking when retested after a time interval. Additionally, I found evidence for the ability to distinguish between a sharp hook and a sham hook in the large line fish compared to the control. My study provides support for the assumption that intensive size-selection alone can leave a cognitive legacy in evolving fish populations. Selection typical of most fisheries where the larger individuals are harvested (positive size selection), can lead to reduced vulnerability to passive gears like hooks, while in fisheries where the larger fish are spared (negative size selection), can lead to increased cognizance of hook.

Introduction

Fisheries-induced-evolution of life history and behaviour

Global fisheries exploit fish stocks significantly, often serving as the primary source of adult mortality (Jørgensen et al. 2007; Mertz and Myers 1989; Finney et al. 2006; Lopes et al. 2016). This selective removal of specific phenotypes by humans may induce evolutionary changes in fish populations if the trait that is selected for is heritable (Allendorf and Hard 2009). Excessive adult mortality reduces overall biomass and leads to demographic truncation of size and age distributions (Heino and Dieckmann 2008b). Behaviourally, it can cause changes in traits such as foraging behaviour, boldness, and activity levels (Pauli and Sih 2017; Claireaux et al. 2018; Sbragaglia et al. 2019). However, fisheries-induced evolution (FIE) specifically refers to heritable, rather than plastic, trait changes (Heino et al. 2015).

High and potentially selective mortality can act as an evolutionary force that heritably alters trait distributions within exploited fish stocks (Jørgensen et al. 2007; Heino et al. 2015; Law 2000). Selection pressures might be positive, harvesting the larger individuals, or negative, targeting smaller individuals (Heino et al. 2015). FIE often leads to heritable changes in life-history traits - including size and age at maturation, growth rate, and reproductive investment favouring current over future reproduction - not only through direct selection but also due to elevated mortality (Uusi-Heikkilä et al. 2015; Marty et al. 2014; Fenberg and Roy 2007; Dieckmann and Heino 2007; Heino and Dieckmann 2008b).

Behaviour can change directly through selection on behavioural traits, or indirectly via life history adaptations or energy allocation trade-offs. For example, increased and positive size selective mortality typical of most fisheries can lead to the evolution of fast life history that is characterized by early maturation at small size, high investment in current reproduction, slower adult growth, and shorter life expectancy. Behaviourally, fast life history is often associated with higher boldness, aggressiveness, exploratory behaviour and lower sociability (Realé et al. 2010). Conversely, slow life histories involve long lifespans, later maturation at larger sizes, greater investment in future reproduction, shy and less exploratory behaviour, and high sociability (Realé et al. 2013).

Experimental evidence supports FIE's capacity to alter behavioural traits. Positive size-selection has increased boldness in guppies (*Poecilia reticulata*) subjected to experimental harvesting (Diaz Pauli et al. 2023) and reduced explorative tendencies in juvenile zebrafish (*Danio rerio*) (Uusi-Heikkilä et al. 2015). Reduced willingness to forage evolved in Atlantic silversides (*Menidia menidia*) under selective pressures (Conover and Munch 2002; Walsh et al. 2006). Further, size-selective mortality altered recruitment and foraging ability in medaka (*Oryzias latipes*) (Evangelista et al. 2021). These studies illustrate how fisheries not only impact population structure but also drive heritable changes in behaviour and life-history, emphasizing the evolutionary consequences of selective harvesting.

Behavioural traits and its relation to cognition

When behavioural traits such as boldness and aggression are linked (correlated behaviours) they are referred to as behavioural syndromes (Sih et al. 2004). These behavioural syndromes help maintain variation within populations (Sih et al. 2004) and help distinguish individuals, for example along a *shy – bold* – continuum or a *reactive - and – proactive* continuum (Sih et al. 2004; Louison et al. 2019; Carere and Locurto 2011). Proactive individuals tend to be more aggressive, bold and active, whereas reactive ones rather shy, less aggressive and less active (slow) (Carere and Locurto 2011; Realé et al. 2010). Additionally, proactive individuals are expected to be less flexible and more rigid (Sih and Del Giudice 2012; Pintor et al. 2014). These different behavioural traits, which are expected to be consistent (Realé et al. 2010) can influence cognitive processes like decision-making: proactive individuals should make fast but less accurate decisions.

Size-selective harvesting influences both life-history and personality traits, and there is a close relationship between personality and cognitive functions (Sih and Del Giudice 2012). Thus, changes in behavioural traits like activity, exploration and boldness as a result of size-selective harvesting can lead to changes in cognitive abilities in fish (Louison et al. 2019; Roy et al. 2024; Hessenauer et al. 2016). Changes in cognition can either be a direct evolutionary response, e.g., when selection directly acts on cognition-based behaviours (e.g., lure recognition) (Hessenauer et al. 2016) or an indirect response to changes in behaviour of energy-expensive allocation pathways (Roy et al. 2024). The *energetic trade-off hypothesis* (Enberg et al. 2012) posits that allocation of energy to gonads (early reproduction) rather than expensive tissues such as the brain can alter behaviour and cognition (Roy et al. 2024; Aiello and Wheeler 1995). Behavioural traits may evolve indirectly as correlated responses to life-history traits (Biro and Post 2008; Heino et al. 2015). Some behaviours, like boldness, which make fish vulnerable to fishing gear, can be directly targeted, indirectly influencing growth and survival (Andersen et al. 2018; Klefoth et al. 2017). Roy et al. (2024) and Villa-Pouca et al. (2025) argue through the behavioural pathway, the cognitive abilities of an individual are affected because behavioural expose of fish to certain experiences may become the basis of learning.

Positive size selection through fisheries can favour shy phenotypes (Andersen et al. 2018; Diaz Pauli and Sih 2017). This behavioural type (associated with a reactive nature) may in turn lead to slow cognitive phenotypes. Thus, shy fish may not only learn about rewards more slowly, encounter rewards later, and generally make slower decisions, but also sample the local environment more intensively (Roy et al. 2024). For example, Hessenauer et al. (2016)

suggests, that individuals from fished populations avoid lures faster compared to individuals from unfished populations. Furthermore, Roy et al. (2023) reported that size-selective mortality induced cognitive changes, resulting in slower learning speed in the line selected for small body size (i.e., positive size-selection) that is typical of most fisheries. But how size selective mortality may affect angling vulnerability is less known.

Angling vulnerability

What makes fish vulnerable towards angling? All fishing is selective, including angling, for some or the other individual traits of individuals (Arlinghaus et al. 2017; Monk et al. 2021). This means that some individuals are more vulnerable to the gear compared to others. According to Lennox et al. (2017) the vulnerability to angling of an individual fish is dependent on the fish's internal state, its encounter with the fishing gear and the fishing gear itself (Lennox et al. 2017). Individuals are either vulnerable or invulnerable and can move between those states. The internal state, for example, includes factors like feeding motivation (i.e. a vulnerable fish is motivated to feed). The internal state is dependent on other factors like the abiotic and biotic environment. The encounter with the gear should theoretically be increased for more explorative/ more active individuals. The gear type could be selective in terms of gape size (i.e. vulnerable fish with limited gape size, that cannot fit the hook or the bait inside their mouth move to the invulnerable state). There are therefore a range of behavioural traits that make fish more or less vulnerable and in addition, fish might also learn to avoid future capture, e.g., in catch and -release fishing (Askey et al. 2006; Beukema 1970a, b; Hessenauer et al. 2016). In theory, boldness is one trait that is associated with increased vulnerability, by for example increasing foraging rate and thus encounters with bait or lures (Arlinghaus et al. 2017). Therefore, bolder individuals (i.e. proactive fish) are found to be caught more often (Lucas et al. 2023; Klefoth et al. 2017; Härkönen et al. 2014). However other studies did not identify boldness as key determinant of vulnerability to angling (Wilson et al. 2011). This vulnerability towards angling has shown to be heritable in exploited populations (Phillip et al. 2009) and to also affect learning. The cognitive ability of an individual to learn bait types can lead to acquired hook-avoidance: Juvenile red seabream (*Pagrus major*) exhibited hook avoidance after only one or two catches (Takahashi and Masuda 2021). In rainbow trout (*Oncorhynchus mykiss*) and pike (*Esox lucius*), catch rates dropped after intensive fishing pressure due to acquired hook avoidance (Askey et al. 2006; Beukema 1970a; Arlinghaus et al. 2017b; Lucas et al. 2023; Roser et al. 2024). Similar observations were reported for largemouth bass (*Micropterus salmoides*) (Hessenauer et al. 2016; Wegener et al. 2017), and carp (*Cyprinus carpio*) (Beukema 1970b; Czapla et al. 2023).

Importantly, some species such as carp can remember negative hooking experiences for more than six month and still show reduced vulnerability (Czapla et al. 2023) – which constitutes substantial cognitive performance. In carp, also social learning to avoid future capture was revealed, while other predatory species have not been found to show that behaviour, but still learn from private negative experience for example largemouth bass (Wegner et al. 2017). If evolutionary changes in fish populations affect their susceptibility to being caught and, in addition, the fishes' ability to learn from past experiences (e.g., in catch-and-release fishing or when evading a gear, Hessenauer et al. 2016), this would have significant implications for fisheries by affecting catchability (Alos et al. 2012; Arlinghaus et al. 2017; Sbrgagalia et al. 2021; Roy et al. 2024). Hessenauer et al. (2016) showed that fish from exploited populations showed faster hook avoidance learning and this is suggestive of evolutionary adaptation in cognitive ability. To conclude, the ability to avoid future capture by hook-and-line may be affected by private experiences or social learning in the context of catch-and-release fishing, which is common in recreational fisheries (Arlinghaus et al. 2007), but hook avoidance learning might also have a genetic basis and differ among population exposed to different historic fishing pressure.

Social species (such as zebrafish) group together to avoid predation, which is related to boldness and other traits (Krause and Ruxton 2002). Intensive size-selective harvesting in experimentally harvested zebrafish has been shown to alter shoaling behaviours (Sbragaglia et al. 2022). The study documented a decrease of individual vigilance in response to negative size-selection (i.e., harvesting the small individuals), linked to an increase in the attention to social as opposed to environmental cues, whereas the large-harvested line (i.e., positive size-selection) showed evidence for an increase of individual vigilance, paying less attention to social cues but more to environmental cues. An evolutionary increase of attention to social cues might be linked to increased ability for social learning in the context of hook avoidance or generally gear avoidance and vice versa for a decrease. Therefore, gear avoidance behaviour might be altered through fishing-induced evolutionary responses not only of cognition, but also through behavioural adaptation of behaviour that may increase the ability to learn from socially transmitted cues - “alarm cues” emitted by harmed fish (Brown et al. 2011). However, for actual cognitively learned lure-avoidance, according to Meekan et al. (2018), the targeted individuals need to survive the encounter. This typically happens in catch-and-release fishing. In the absence of experienced individuals, social learning may not occur (Meekan et al. 2018). Social learning, however, can still occur through the sensing of chemical alarm cues by conspecifics (Brown et al. 2011) as the cues spreads across the population and results in social learning (Meekan et al. 2018). According to that only few studies showed learned lure-avoidance (Beukema 1970; Askey et al. 2006; Lennox et al. 2015) and social learning was only reported by Czapla et al. (2023). Linking back to evolution, FIE might result in populations that are more sensitive to these cues and are thus quicker in learning to be captured by hook and line to avoid future capture (Hessenauer et al. 2016).

The need for size-selection experiments

The impact of size selection on behavioural traits and cognitive abilities in exploited fish stocks is hard to observe in the wild, and so there is a need for experimental studies. Walsh et al. (2006) showed lower consumption rates and longer latencies to forage in presence of a predator in the large-harvested (i.e., positive size selection) line. Diaz-Paul et al. (2023) reported the fish from the small line (i.e. positive size-selection) to become bolder, and Uusi-Heikkilä et al. (2015), Sbragaglia et al. (2021; 2022) and Roy and Arlinghaus (2022) reported the fish from the small line either to not differ from controls or become somewhat shyer. By contrast, zebrafish from the large line (i.e. negative size-selection) consistently developed bolder behaviours (Sbragaglia et al. 2021; Roy and Arlinghaus 2022). Furthermore, size-selective mortality has found effects on shoaling behaviour of zebrafish (Sbragaglia et al. 2022), where the small line evolved lower group cohesiveness and the large line increased group cohesiveness. Hower studies examining cognition are limited. For example, Roy et al. (2023) found that decision-making was altered by size-selective mortality in zebrafish. Specifically, the small line (i.e. positive size selection) showed slower associative learning abilities but made quicker decisions when tested for memory.

Because there are only a handful of studies that investigate the influence of size-selective mortality on altered cognition in the context of recreational fishing, there is a need for laboratory experiments like conducted in my thesis.

Study Hypotheses

***Experiment 1*: Foraging assay**

I first tested feeding motivation and behaviour towards a novel object (sham-hook with removed tip) across three trials in groups of zebrafish derived from the two selection lines (large line, small line) relative to controls. The large line represents negative size-selection and the small line positive size-selection. Behaviour was measured in terms of number of inspections of the sham hook and free food was available. As the small line showed shy behavioural tendencies and the large line was consistently bolder and took more risk to forage (Sbragaglia et al. 2021; Roy and Arlinghaus 2022), I expected/ hypothesized (H1) that the small line would have less feeding rate and would take more time (latency) to inspect the sham while the large line would have increased feeding rate and will take less time to inspect the sham, compared to the control.

***Experiment 2*: Test on readiness to ingest the sham hook**
Next, I tested the willingness of the fish to ingest the baited sham hook across three trials. This was measured again by number of inspections. Because of the boldness differences mentioned above I expected (H2) the large line to inspect the baited sham more often compared to the control and vice versa for the small line and that the large line would inspect earlier (i.e. decreased latency) and the small line later (i.e. increased latency) compared to the control.

***Experiment 3*: Hook-avoidance learning**
Subsequently, I tested vulnerability towards a real baited angling hook and change in vulnerability (i.e. learning) across five consecutive trials. Because boldness has proved to be a driver for angling vulnerability (Lucas et al. 2023; Klefoth et al. 2017; Härkönen et al. 2014), and behavioural experiments with these zebrafish have shown that the large line exhibited increased boldness compared to the control and vice versa for the small line (Sbragaglia et al. 2021; Roy and Arlinghaus 2022), I hypothesized (H3) that the large line would be more vulnerable (i.e. more actual hooking-events and more potential hooking-events) compared to the control and vice versa for the small line. Further, because the small line (i.e. reactive individuals showing rather shy tendencies) is expected to exhibit slower learning speed (Roy et al. 2023), I hypothesized (H4) that this line shows acquired hook-avoidance later compared to the control and because there was no difference found between the large and the control line (Roy et al. 2023) I expected similar outcomes for these.

***Experiment 4*: Hook-avoidance memory probe**
Finally, I tested memory retention to examine if the different lines exhibit hook avoidance after a resting period of 6 days. I used this time period, because avoidance behaviours in fear conditioning in zebrafish have shown to last for 7 days and the memory retention started to decline after 62 hours (Moreira et al. 2024). After this pause, I tested the lines for hook-avoidance in one memory trial. Due to decelerated learning speed in the small line (Roy et al. 2023) I expected (H5) this line to be more vulnerable (i.e. show more hooking-events) compared to the control, and as there were no significant differences in learning speed reported between the large and the control line (Roy et al. 2023) I expected similar vulnerability for these in the memory probe.

***Experiment 5*: Hook-recognition**
In another memory probe, I tested hook-recognition using a sham-hook instead of a real one. The idea was, if the fish inspected/ approached the sham more often compared to the real hook in the experiments before, they would recognise the hook. Because of the reasons mentioned above (i.e. regarding learning speed) I hypothesized (H6) that the large line and control would not differ, but that the small line would exhibit decreased recognition abilities.

***Experiment 6*: Adaption of feeding-behaviour**

In the last experiment, I repeated the design of experiment one for one trial, to investigate if the potentially acquired hook-avoidance would have led to altered feeding behaviour. Because of increased boldness in the large line (Sbragalia et al. 2021; Roy and Arlinghaus 2022) I expected (H7) that line to exhibit no altered behaviour and show similar feeding rates compared to the control, instead I expect the small line because of shyer tendencies (Sbragaglia et al 2021; Roy and Arlinghaus 2022) to feed less, because of increased weariness, compared to the control.

Methods

Selection lines

The parental generation of zebrafish in the lab was collected in the wild from West Bengal in India. Length-selective harvesting was conducted from generation F1 to F5 on the parental generation adapted to the laboratory and was then halted during subsequent generations (Uusi-Heikkilä et al. 2015) up until F19 size-selection (i.e. length-selection) was operating either positively on length (generating the small line, as in most commercial and recreational fisheries) or negatively on length (generating the large line as is common in recreational fisheries managed with a maximum-size limit). In the control line size-selection was random with respect to length (see Uusi-Heikkilä et al. 2015 for details). Selection always started when 50% of the random line fish matured. The per generation harvest-rate during selection was 75% of the respective line. In the small lines (indicating selection for small body length by removing the largest fishes), 75% of the largest (standard length) individuals were harvested, while in the large lines (indicating selection for large body length by removing the smallest fishes), 75% of the smallest individuals were harvested per generation. The selection in the control or random selection lines happened randomly. After five generations of size-selection, the individuals selected for spawning in the following generations (F6 onwards) were chosen randomly in all lines. The experimental fish belonged to three selection treatments, each with two replicates (six lines in total). These lines were identified as LS1 (i.e. large-selected, small, replicate 1), LS2 (small, replicate2), SS1 (i.e. small-selected large, replicate 1), SS2 (large, replicate 2), RS1 (i.e. random-selected, control, replicate 1) and RS2 (control replicate 2). For detailed information regarding the selection lines, please see Uusi-Heikkilä et al. (2015). The aforementioned lines are provided by the scientific group and had been produced without my involvement, before this investigation started.

Experimental fish

The fish used in my study originated from the F19 generation. Thus, selection stopped 14 generations ago.

To breed F19 generation, 150 fish from F18 stock population were chosen randomly. Three males and two females were grouped in acrylic boxes (5 L capacity), and the fish were allowed to spawn for 24h. A meshed platform in the boxes prevented egg cannibalism. On the following day, all eggs were distributed into glass beakers (500 mL capacity) at a density of 100eggs/ container. The beakers were stored in an incubator for seven days at 35 °C (beakers were cleaned daily, by changing 50% of the water and removing the dead eggs). Once the larvae started to swim, they were stocked in six (every replicate) round tanks (diameter: 79 cm, height: 135 cm, volume: 320-L, see Roy et al, 2023) at densities between approximately 600 and 1000 fish per tank with a water exchange rate of 10 L/ min. The water temperature at these tanks was maintained at 26 °C as well as a 12:12 Light:Dark cycle. Fish were fed twice a day ad libitum with commercial flake food.

For my study, ten fish (approximately 1.5 years old) from every replicate were chosen randomly (irrespective of the sex) and housed in pairs of two in five three-litre boxes (per replicate), i.e. 30 boxes in total and a water exchange rate of 10 L/ min. This was done to ensure controlled conditions for at least three months, to be able to compare results in a pretrial on behavioural differences (risk-taking behaviour) with former results of selection experiments conducted with these fish (Sbragaglia et al. 2021; Roy and Arlinghaus 2022), because I used fish from holding tanks where the holding conditions might have been different. Therefore, to ensure that the replicates behaviourally differed in directions previously reported, a pretrial was conducted. To that end, I used three fish per group. It was ensured that the individuals in the boxes were morphologically different (i.e. one smaller, one medium sized and one slightly bigger individual) to be able to identify them throughout the experiment. The water temperature was kept consistent at 26 °C, and a 12:12 Light:Dark cycle was maintained during holding. Oxygen concentration of the water was kept at 8.3 ± 0.3 mg/L. All boxes were maintained in the same system with a water exchange-rate of 10 litre/ minute. Fish were fed twice a day ad libitum with bloodworms. Due to increased aggression levels over time the fish were moved to different tanks, providing more space. The new housing tanks held 10.6 litres of system water. Other parameters were maintained like before. I used 30 groups in total (3 fish per group, 5 groups per replicate line) for the hook-avoidance learning assays.

Because the experimental fish originated from the housing tanks and thus did not experience the same holding conditions, the different density conditions in the housing tanks could account for behavioural divergences among the selection lines. To investigate if the experimental fish were behaviourally similar to the fish used in previous studies, a tank-diving test (pre-trial) was conducted that tested for boldness in groups of zebrafish (Sbragaglia et al. 2021 and Roy and Arlinghaus 2022). As the behavioural trend broadly matched expectations and past findings, I used the fish for my subsequent learning experiments. The procedure is explained and presented in the appendix including results (*Fig. s.1a, b* and *Fig. s. 2a, b*).

Hook avoidance learning assay

To test hook-avoidance learning, three main experiments were conducted, followed by memory-trials (*Fig. 1*). The trials were conducted between 9:00 am and 4:00 pm. Before every trial, the fish were starved for 24 hours, to ensure feeding motivation.

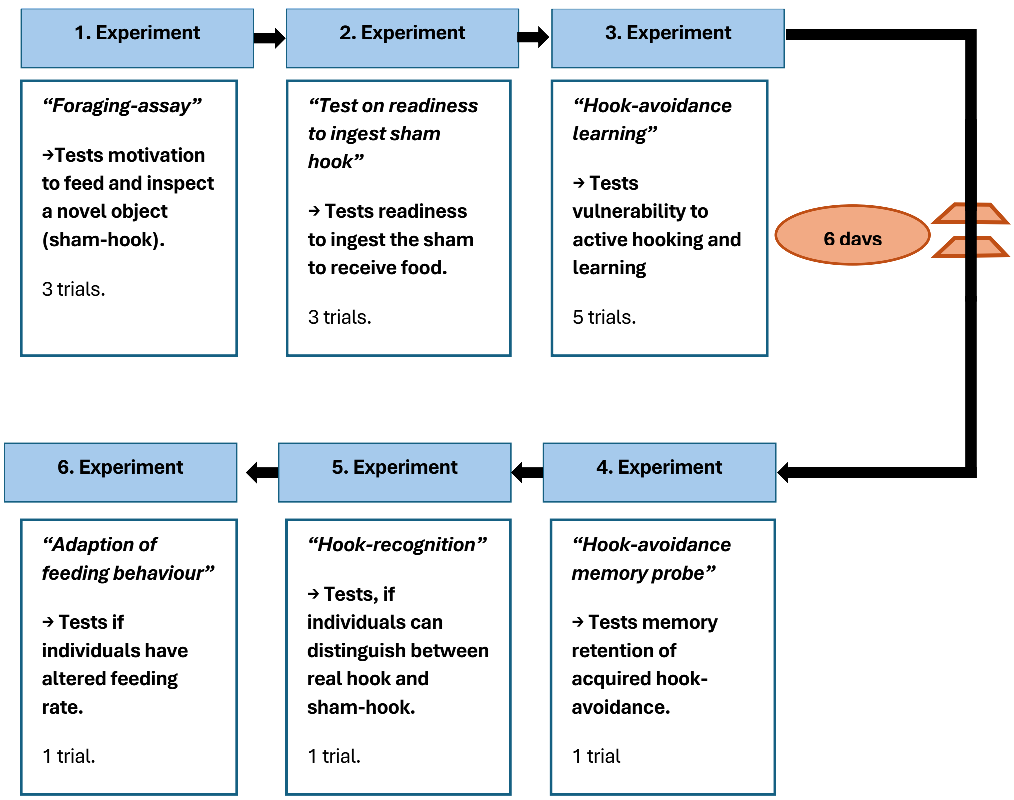


Figure 1. This figure shows the schematic flow of the experiments conducted to test hook avoidance learning and memory in the three selection lines of zebrafish.

Experiment 1 "Foraging assay"

The first experiment served the purpose to allow the fish to habituate to and explore the new environment and to test for general feeding motivation. The experimental setup is depicted in *Fig. 2a* and *2b*. At a time, a group of fish were placed in a transparent small cylinder in a round arena (46.4 cm inner diameter) and allowed to acclimate for two minutes. On the diametrically opposite end of the acclimation cylinder, I suspended a sham hook 1 to 2 cm above the ground of the arena. The sham hook consisted of a former fishing hook size 22 (brand: "Owner (perfection in hooks)") where the tip was removed. The hook was attached to a 42 cm monofilament fishing line (approximately 0.09 diameter) that was knotted onto a small "fishing rod" (58 cm plastic pipe). A few pieces of bloodworm (approximately 3 to 4) were added near the unbaited, plain sham hook (this experiment and experiment 6 are the only ones including free food). Through this approach feeding motivation in the presence of a novel object was tested. After the acclimation period of two minutes, the cylinder was removed, and the fish were allowed to explore the arena for five minutes. Fish behaviour was recorded using two Logitech B910 cameras connected to two computers. One camera was mounted 62 cm above the water level in the centre, and the other camera was placed 62 cm above the water level on the side of the arena, allowing two camera angles. The second camera was zoomed at 218% onto the sham hook. After the experiment the fish were netted and transferred back into their holding tanks. Three repeats of the trials in total were conducted, spread across three consecutive days, as fish had to be habituated to the new environment (Brown 2001).

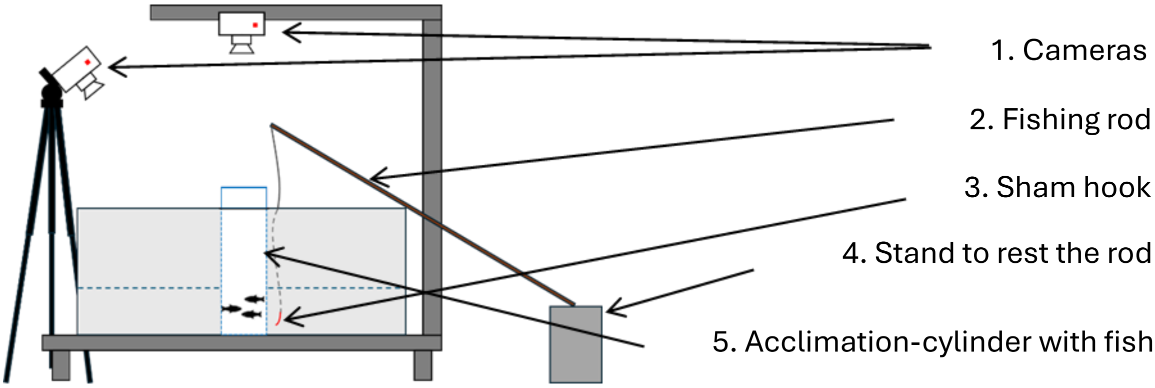


Figure 2a. Illustration of experimental set-up from the front.

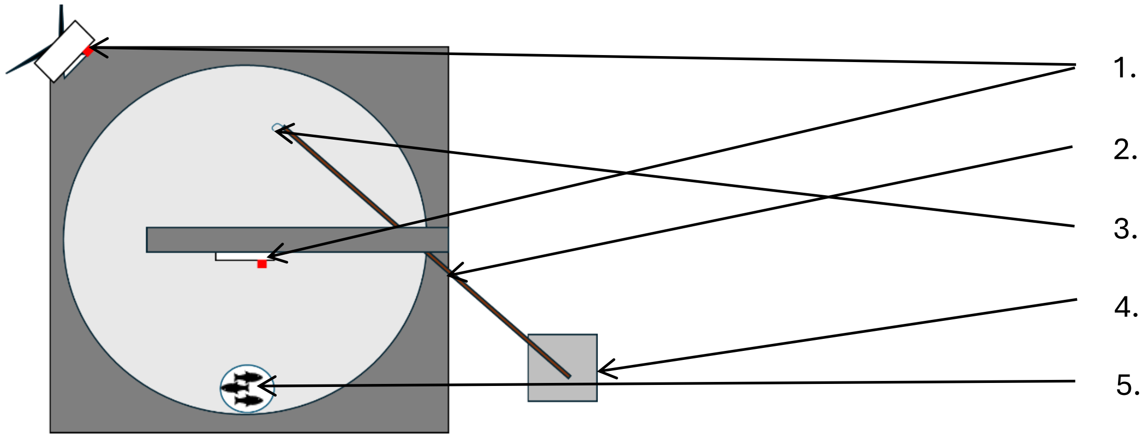


Figure 2b. Illustration of experimental set-up from above. Numbers 1-2 are explained in Fig. 2a.

Experiment 2 "Test on readiness to ingest (baited sham)"
The experimental setup for the "Baited sham" trials was the same as displayed in *Fig. 2a* and *2b*. Only, in this experiment, one bloodworm was baited onto the sham hook. Like in experiment 1, the group of fish were transferred into the cylinder within the arena. The individuals were allowed to acclimate for two minutes. After removing the cylinder, the behaviour was recorded for 10 minutes. Then, the group was transferred back to their holding tank. Three trials spread across six days were conducted. These trials, and the following "hooking-vulnerability" was split up into two batches run on two consecutive days, as handling times and preparation for trial was time-consuming.

Experiment 3 "Hook-avoidance learning"
The design of the third experiment also was congruent to the one displayed in *Fig. 2a* and *2b*. Instead of a sham hook, these trials incorporated a real micro-fishing hook (size 22, brand (Owner (Perfection in hooks)) (micro-fishing, Cooke et al. 2020). The group of fish was transferred into the cylinder and allowed to acclimate for 2 minutes. The baited hook was placed in the same position, where the former sham hook was positioned. After two minutes the cylinder was retrieved. The fish were allowed to move freely for 10 minutes (everything was recorded). I (an active angler) sat behind a curtain, holding the rod. The curtain was in a position that allowed avoidance of external disturbances but provided little view of the arena to the angler to see the hook. When I saw one fish ingesting the bait, I actively set the hook. Once a fish got hooked, it was pulled out of the water and held for 20 seconds in air, as increasing air-exposure time increases stress response in fish (Skrzynska et al. 2018; Flink et

al. 2024). This was done to ensure that a real negative experience for the individual in response to a fishing event was created. A net was placed below the fish to prevent it from falling back into the arena. After the air-exposure-time, the fish was placed in a petri-dish filled with system water, where the hook was removed using tweezers. After dehooking, the fish was put back into the arena immediately. The hook was baited again and placed in the same position as before (I had prepared several rods in advance to ensure that the time of a baited hook in the water was maximised). After the trial, the fish were transferred back into their housing tanks. Five trials spread across 10 days were conducted, to test for learning ability across trials, as this hooking-experience was meant to create a negative experience that might lead to avoidance behaviour. There were 5 groups left (out of 30) that had not gone through a single negative hooking experience because they did not get hooked. Therefore, I ran an additional 6th trial. The design of that trial followed that of experiment 3, only I placed the baited hook in front of a random individual to initiate a hooking event. In case of failure in that trial, I chose a random individual that was then hooked manually as in Czapla et al. (2023). To that end, I netted an individual of the group, placed it in a petri dish with system-water and set the baited hook in the front of the jaw using a tweezer. I then exposed the fish to air for 20 seconds (like in the trials before) and then removed the hook again.

Experiment 4 "Hook-avoidance memory probe"

After a pausing period of 6 days after the last trial, the fish were tested for hook avoidance memory. According to literature, zebrafish can retain aversive memory for up to 7 days, but an inflection point was observed at 62 hours after which zebrafish memory declined (Moreira et al 2024). This led to the decision to extent the pausing period not greatly and settle for testing memory of hooking avoidance after 6 days. For this experiment the exact same procedure of experiment 3 was conducted for a single trial spread across two days.

Experiment 5 "Hook-recognition memory"

This experiment followed the procedure of experiment 2 with one trial spread across two days. The aim was to test whether the fish would avoid the tackle when lacking a sharp hook tip, i.e., when it is non-threatening. That is, I wanted to test if the fish recognize the hook.

Experiment 6 "Adaption of feeding behaviour"

This experiment followed the procedure of experiment 1, with one trial in one day. I conducted this experiment to see if any acquired avoidance behaviour might lead to different foraging behaviour in presence of a "potentially dangerous" fishing gear, which is a sham hook.
At the end of every experiment-day the round tank was drained and cleaned, and the fish were fed.

Body length at the start of experiments

After these experiments were conducted I checked if the length of experimental fish differed across selection lines. Total length was measured using ruler. Individuals were wetted and placed onto a white smooth plastic platform with a fixed ruler. To that end, I compared body length among the lines using a linear mixed effects model where I fitted total length as response variable, Selection line as fixed effect and replicate as a random effect. This analysis showed there were no significant difference in body length among the selection lines (*Fig. 3*). Thus, the "small" and the "large" line did no longer differ in length, so that all possible differences were caused by population and not by intrinsic length differences among the selection lines. For reasons of simplification, the lines are still referred to as small, control and large, even size (i.e. total length) does not differ anymore. The lack of difference in length could have been caused by slightly different ages or by trait recovery in terms of body length from F5 to F19 (Van Dijk et al. 2024).

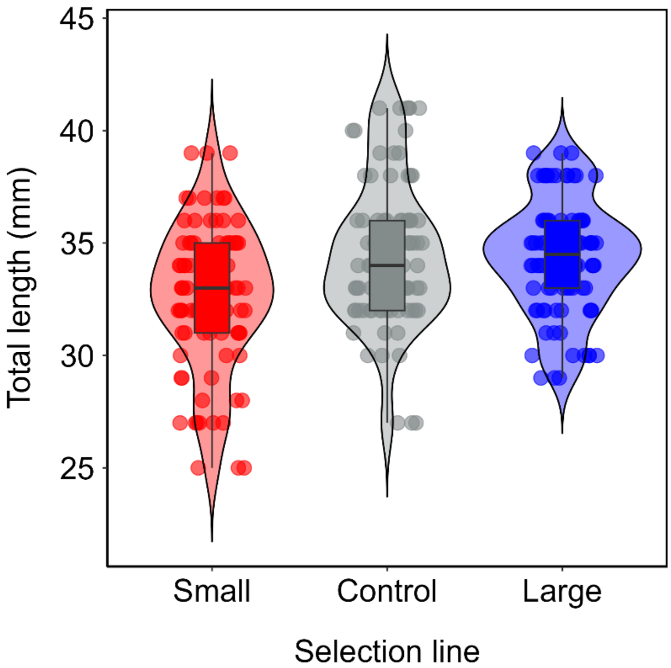


Figure 3. This violin plot shows the body-size distribution (i.e. total length) between the selection lines and indicates that there is no significant difference in body length between the lines (indicated by medians).

Video data analysis

Experiment 1: Foraging-assay

From the video recordings, I scored (1) the feeding rate was scored (i.e. how many worms were consumed by an individual with maximum of four worms), further (2) how many times an individual fish of each group inspected/ bit or chewed on the sham hook throughout the 5 minutes (i.e. no. of inspections) and (3) the latency (in seconds) to inspect the sham hook (i.e. latency until first inspection).

Experiment 2: Test on readiness to ingest (baited sham)

Again, I scored for experiment 2, (1) how many times an individual fish of each group inspected/ bit or chewed on the baited sham hook (i.e. no. of inspection) throughout the 10 minutes and (2), the latency to inspect the sham hook. Note that the first inspection almost always resulted in foraging-success and eating of the bloodworm because, it was easily detached off the sham hook lacking a hook tip.

Experiment 3: Hook-avoidance learning

Before any actual hooking event I scored (1) how many times each individual inspected the hook (i.e. touched it without ingesting deep enough (i.e. no. of inspections), (2) how many times did one individual ingest the hook and spit it out again or at least did not ingest the hook deep enough for the angler to set the hook (i.e. no. of potential hooking-events (PH)), (3) how many times an individual got actually hooked (i.e. no. of actual hooking-events(AH)).

The videos of experiment 4 were scored following the procedure of experiment 3, the videos of experiment 5 as in experiment 2 and the videos of experiment 6 as likewise done in experiment 1.

Statistical analysis

All statistical analysis was performed in R 4.1.0. I used the packages readxls, glmmTMB (Brooks et al. 2017), lmerTest (Kuznetsova et al. 2017), ggplot2 (Wickham 2011), plyr (Wickham 2020), rptR (Stoffel et al. 2017).

Foraging-assay

I compared the change of feeding rate (i.e. how man worms did an individual consume) across trials by running a generalised linear mixed effects model where I specified a Poisson error structure. feeding rate was used as the response variable, an interaction of selection line and trial as fixed effects, and group ID nested within selection line replicate as random effect. For example, to compare feeding rate across trials and among populations, I used the following formula:

*glmmTMB(Feeding_rate~Selction_line*Trial+(1|Replicate/GroupID), poisson, data=DATA)*

I used the same approach for comparing change of no. of inspections of the unbaited sham hook among selection lines. Then I compared the latency to inspect the sham hook using a linear mixed effects model. I used log-transformed latencies (to facilitate meeting the model assumptions) as the response variable and fixed and random effects were incorporated like explained above. Additionally, I calculated repeatability using feeding rate, no. of inspections and latency to inspect as response variables and trial as the fixed effect with a Poisson distributed error structure (Stoffel et al. 2017).

Test on readiness to ingest (baited sham)

The same approach explained above was used to analyse no. of inspections and latency to inspect the baited sham hook in experiment 2.

Hook-avoidance learning

I compared the no. of inspections, potential hooking-events (PH) and actual hooking-events (AH) among selection lines and across trials using mixed effects models with the same fixed and random effects structure as before. I also compared whether or not a hooking event happened among the selection lines by running a mixed model with a binomial error distribution.

Hook-avoidance memory probe

To investigate if fish among selection lines remember the hooking experience, I compared the no. of inspections, potential hooking-events and actual hooking-events during the memory probe trial with the last trial during hook-avoidance learning. In that, I ran a mixed effects model with fixed and random effects specified like before. I used the last trial for comparison because this represented the state where the fish potentially acquired the maximum avoidance behaviour. For comparing actual hooking events, I ran a model without interaction of selection line and trials because the model failed to run and generated an error when considering interaction.

Hook-recognition memory probe

To test if the fish among selection lines developed cognizance of the hook, I compared the no. of inspections made of the baited sham hook and the baited sharp hook during the memory trials with the inspections made during the last trials of test for ingesting a baited sham hook (expt 2) and test for hook-avoidance (expt 3). To achieve this, I ran a mixed effects model with two two-way interactions of hook-type (sham and hook) and selection line, and time (“before” the learning and during the "probe") and selection line, and a three-way interaction of hook-type, time and selection line as fixed effects.

Adaption of feeding-behaviour
Finally, I tested if the fish adapted their feeding behaviour compared to what was seen during the foraging assay in Experiment 1. To that end, I compared the feeding rates during the probe trial (experiment 6) with the feeding rate during the last trial of foraging assay using a generalised linear mixed effects model with similar fixed and random effects as before.

For every model in all experiments "*Body-size*" (i.e. total length) was included as a fixed effect. This did not change any significant effect. Because of that and due to limitations, I decided to exclude it from this thesis.

Results

All results of the experiments one to six are summarised in the table below (*Table 1*).

Experiment 1 Foraging assay

The number of inspections of the unbaited sham hook was found to be repeatable in fish among all selection lines, whereas feeding rate and latency to feed were not repeatable (except for feeding rate in the small line) (*Table 2*). I found no significant difference in feeding rate (i.e. number of bloodworms eaten per fish) across trials among the selection lines (*Fig 4a, Table 1*). The number of inspections of the unbaited sham hook in the initial foraging assay with hook-naïve fish was significantly low in the small line compared to the control (*Fig. 4a, Table1*). In addition, a significant interaction between the large line and trial showed that the large line inspected the unbaited sham hook less with increasing number of trials compared to the control ($z=-2.12$, $p=-0.03$) while the small line showed no change in inspections across trials (*Fig. 4b*). The small line fish took marginally less time to inspect the sham hook across trials compared to the control line ($t=-1.71$, $p=0.09$) (*Fig. 4c, Table 1*). This research suggests no support for my initial hypothesis H1 regarding the feeding rate but provides partial support for the assumptions regarding the latency to inspect the unbaited sham hook.

Experiment	Model	Parameter	Estimate	z-/ t-value	p-value
1. Motivation assay	Inspections	Intercept	1.556	4.280	1.87e-05
		Large	0.199	0.386	0.6997
		Small	-2.732	-4.222	***2.43e-05***
		Trial	-0.372	-4.692	***2.70e-06***
		Large:Trial	-0.242	-2.115	***0.034***
		Small:Trial	0.215	1.103	0.270
	Feedingrate	Intercept	0.293	1.101	0.271
		Large	0.285	0.784	0.433
		Small	-0.465	-1.028	0.304
		Trial	-0.105	-0.857	0.392
		Large:Trial	-0.101	-0.592	0.554
		Small:Trial	-0.148	-0.678	0.498
	Latency Inspection	Intercept	3.977	16.553	< 2e-16
		Large	0.488	1.438	0.153
		Small	1.438	4.232	***4.58e-05***
		Trial	0.292	3.237	***0.001***
		Large:Trial	-0.130	-1.016	0.311

		Small:Trial	-0.217	-1.707	**0.090**
2. Readiness to ingest the baited sham	Inspections	Intercept	1.440	6.451	1.11e-10
		Large	-0.224	-0.721	0.471
		Small	-1.353	-3.581	***<0.001***
		Trial	-0.329	-3.864	***<0.001***
		Large:Trial	0.240	2.115	***0.034***
		Small:Trial	0.222	1.474	0.140
	Latency Inspection	Intercept	3.824	7.669	3.34e-13
		Large	-0.112	-0.158	0.874
		Small	1.527	2.166	***0.031***
		Trial	0.137	0.600	0.552
		Large:Trial	0.124	0.383	0.702
		Small:Trial	0.043	0.134	0.894
3. Hook-avoidance learning	Inspections	Intercept	1.155	4.948	7.50e-07
		Large	0.246	0.768	0.442
		Small	-0.965	-2.518	***0.0118***
		Trial	-0.386	-6.984	***2.86e-12***
		Large:Trial	-0.001	-0.014	0.989
		Small:Trial	-0.035	-0.316	0.752
	Latency Inspection	Intercept	3.995	9.683	1.63e-05
		Large	-0.053	-0.090	0.930
		Small	1.612	2.764	***0.026***
		Trial	0.362	4.331	***1.84e-05***
		Large:Trial	-0.062	-0.530	0.596
		Small:Trial	-0.220	-1.865	**0.063**
	Potential Hooking (BH)	Intercept	0.272	1.022	0.307
		Large	0.355	1.025	0.305
		Small	-0.794	-1.779	**0.075**
		Trial	-0.392	-4.399	***1.09e-05***
		Large:Trial	0.060	0.537	0.592
		Small:Trial	0.036	0.236	0.814
	Actual hooking events	Intercept	-0.504	-0.926	0.355
		Large	-0.438	-0.621	0.534
		Small	-0.690	-0.811	0.417
		Trial	-0.836	-3.269	***0.001***
		Large:Trial	0.429	1.432	0.152
		Small:Trial	0.109	0.276	0.782
	Hookings (Binomial)	Intercept	-0.169	-0.257	0.797
		Large	-0.171	-0.196	0.845
		Small	-1.068	-1.055	0.291
		Trial	-0.936	-3.273	***<0.001***
		Large:Trial	0.339	0.978	0.328
		Small:Trial	0.215	0.502	0.615
4. Hook-avoidance memory probe	Inspections	Intercept	-1.823	-3.649	<0.001
		Large	1.092	1.657	**0.097**
		Small	-0.764	-0.930	0.352
		Trial:Probe	1.988	5.895	***3.74e-09***

		Large:Probe	-1.559	-3.752	***<0.001***
		Small:Probe	-1.177	-1.708	**0.088**
	Potential Hooking (BH)	Intercept	-2.227	-3.956	7.62e-05
		Large	0.663	0.929	0.353
		Small	-0.835	-0.878	0.380
		Trial:Probe	1.163	2.270	***0.023***
		Large:Probe	-0.588	-0.890	0.373
		Small:Probe	-1.163	-1.035	0.301
	Actual hooking events	Intercept	-5.097	-2.368	0.018
		Large	1.173	0.716	0.474
		Small	-19.649	-0.001	0.999
		Trial:Probe	-1.386	-1.240	0.215
5. Test for hook recognition	Inspection/ Hook recognition	Intercept	0.186	0.633	0.526
		Large	0.630	1.561	0.118
		Small	-0.225	-0.526	0.599
		PROBE	0.374	2.093	***0.036***
		Sharp Hook	-1.668	-4.837	***1.32e-06***
		Large:Probe	-0.336	-1.412	0.158
		Small:Probe	-0.884	-2.690	***0.007***
		Large:Sharp Hook	0.631	1.542	0.123
		Small:Sharp Hook	-0.501	-0.795	0.426
		Probe:Sharp Hook	1.614	4.231	***2.32e-05***
		Large:Probe:Sharp Hook	-1.223	-2.553	***0.011***
		Small:Probe:Sharp Hook	-0.293	-0.383	0.702
6. Adaption of feeding behaviour	Feedingrate LAST	Intercept	0.065	0.365	0.715
		Large	-0.065	-0.254	0.780
		Small	-0.827	-2.580	***0.001***
		TrialProbe	-0.330	-1.208	0.227
		Large:TrialProbe	-0.298	-0.723	0.470
		Small:TrialProbe	-1.210	-1.748	**0.081**

Table 1 summarises the all the results of experiments 1 to 6. First column represents the experiment, second the model, third the parameters/ effects. The fourth column shows the estimate and the fifth the z-value (for generalised linear mixed effects models) and t-value for (linear mixed effects models). The sixth column indicates the p-values, in which significant results are depicted in ***italics and bold print*** *and marginal results only in* **bold print**.

Response variable	Selection line	Confidance intervals	r-value	p-value
Feeding rate (EXP1)	Small	[0, 0.569]	0.295	***0.009***
		[0, 0.602]	0.233	***0.009***
	Control	[0, 0.107]	0	0.999
		[0, 0.082]	0	0.999
	Large	[0, 0.124]	0.003	0.483
		[0, 0.097]	0.002	0.483
Inspection (EXP1)	Small	[0, 0.793]	0.48	***0.001***
		[0, 0.581]	0.2	***0.001***
	Control	[0.057, 0.629]	0.404	***<0.001***
		[0.049, 0.632]	0.384	***<0.001***
	Large	[0.14, 0.768]	0.539	***2.13e-07***

		[0.11, 0.659]	0.436	***2.13e-07***
Latency (EXP1)	Small	[0, 0.184]	0.049	0.202
		[0, 0.171]	0.044	0.202
	Control	[0, 0.385]	0.204	***0.002***
		[0, 0.296]	0.149	***0.002***
	Large	[0.068, 0.555]	0.371	***8.52e-07***
		[0.051, 0.411]	0.27	***8.52e-07***
Inspection (EXP2)	Small	[0, 0.329]	0.117	0.08
		[0, 0.196]	0.051	0.08
	Control	[0, 0.142]	0.016	0.408
		[0, 0.112]	0.011	0.408
	Large	[0, 0.114]	0	0.5
		[0, 0.093]	0	0.5
Latency (EXP2)	Small	[0, 0.113]	0	0.5
		[0, 0.057]	0	0.5
	Control	[0, 0.105]	0	0.5
		[0, 0.009]	0	0.5
	Large	[0, 0.104]	0	0.999
		[0, 0.017]	0	0.999

*Table 2 summarises the repeatability outcomes of experiment 1 and 2. First column shows the response variable and experiment, second the selection line, third the confidence intervals, fifth the r-value and sixth the p-value. Significant results are depicted in **italics and bold print**, marginal results in* **bold print.**

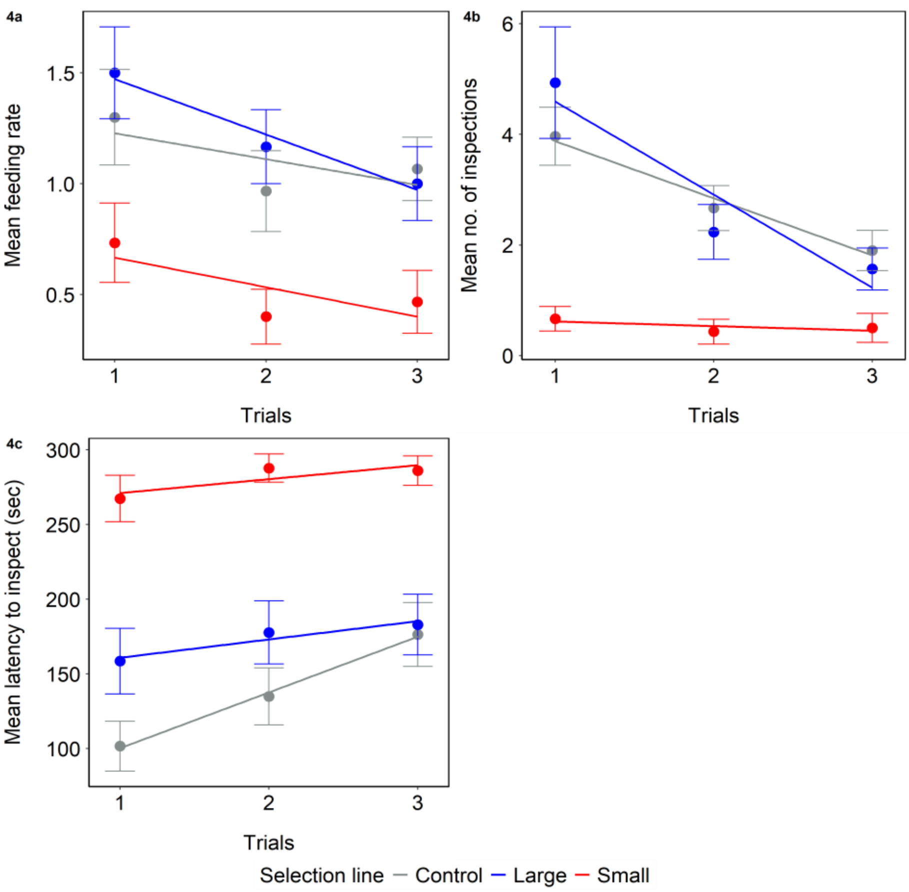


Figure 4 depicts the results of experiment 1 (Foraging-assay) with unbaited sham. (a) compares the mean feeding rate (± SE) across trials, (b) displays the mean latency (± SE) to inspect the unbaited sham in seconds against trials. (c) compares the mean no. of inspections (± SE) across trials. Crossing lines indicate significant effects.

Experiment 2 Test on readiness to ingest a baited sham hook

Number of inspections of the baited sham hook was not repeatable for the selection lines in experiment two, neither was the latency to inspect (except for control and large line) (*Table 2*). The small line made significantly less no. of inspections of the baited sham compared to the control (*Fig. 5a, Table 1*). large line fish maintained greater interest in the baited sham and inspected it more often over the course of trials compared to the control fish (z=2.12, p=0.03) (*Fig. 5a, Table 1*). This showed that the large line fish-maintained interest (compared to the control) in the sham, because this was baited as compared to experiment one (unbaited sham hook) where no. of inspections dropped across trials. The small line fish took significantly more time to inspect the sham compared to the control (t=2.17, p=0.03) (*Fig. 5b, Table 1*). Note that in this case inspections were on a baited sham (i.e. food-reward), and this research suggests partial support for H2, because expected differences were only observed statistically significant for the small line.

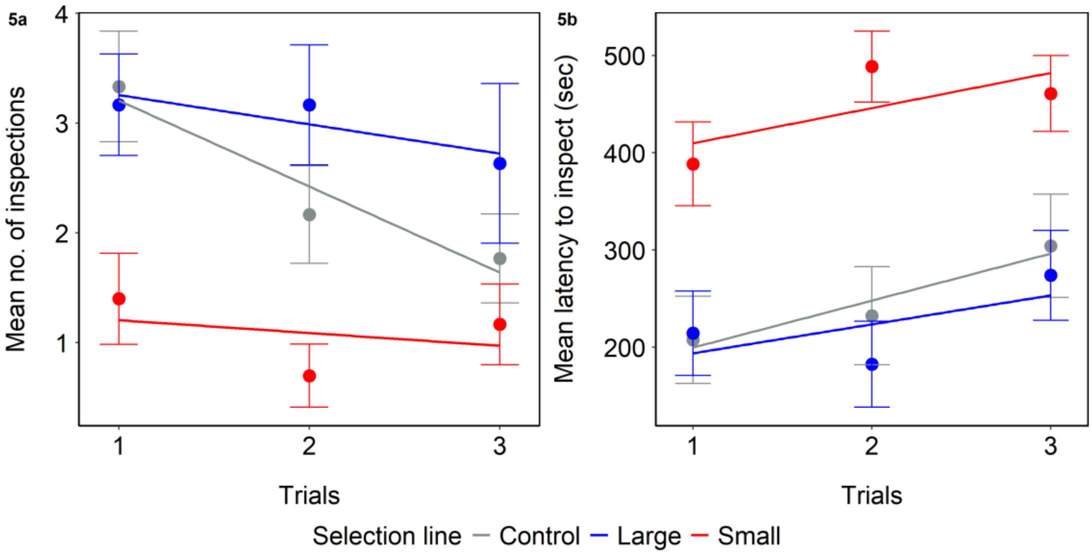


Figure 5 illustrates the main results of experiment two (Test on readiness to ingest a baited sham). (a) shows the mean number of inspections across trials for all lines (± SE). The small line significantly inspects less compared to the control, indicated by touching regression-lines. (b) plots mean latency until first inspection (± SE) against trials, reporting longer latencies for the small line (i.e. great distance between the lines). (d) illustrates the latencies against body size (positive interaction displayed by steep lines).

Experiment 3 Hook vulnerability and hook-avoidance learning

The small line inspected the baited sharp hook significantly less compared to the control (z= -2.52, p=0.01) (*Fig. 6a, Table 1*), while the large line fish did not differ significantly from the controls (*Fig. 6a, Table 1*). The small line fish took less time to inspect the baited hook compared to the control (t=2.77, p=0.03, *Fig. 6c*). The small line fish also took marginally less time to inspect the hook across trials compared to the control (t=-1.87, p=0.06) (*Fig. 6c, Table 1*) indicating improvement in inspection latency across trials. The number of potential hooking events in the small line fish were marginally less (z=-1.77, p=0.08) and did not differ in the large line compared to the control line (*Fig. 6b*, *Table 1*). I did not find a significant difference in the number of actual hooking events among selection lines (*Table 1*), but the hooking events declined significantly across trials indicating learning in all selection lines (z=-3.27, p=0.001) (*Fig. 6d, Table 1*). Vulnerability measured by no. of inspections PH and AH therefore suggested only partial support for my hypothesis H3. Because no interaction with trial was found to be significant, there was no support for my hypothesis H4.

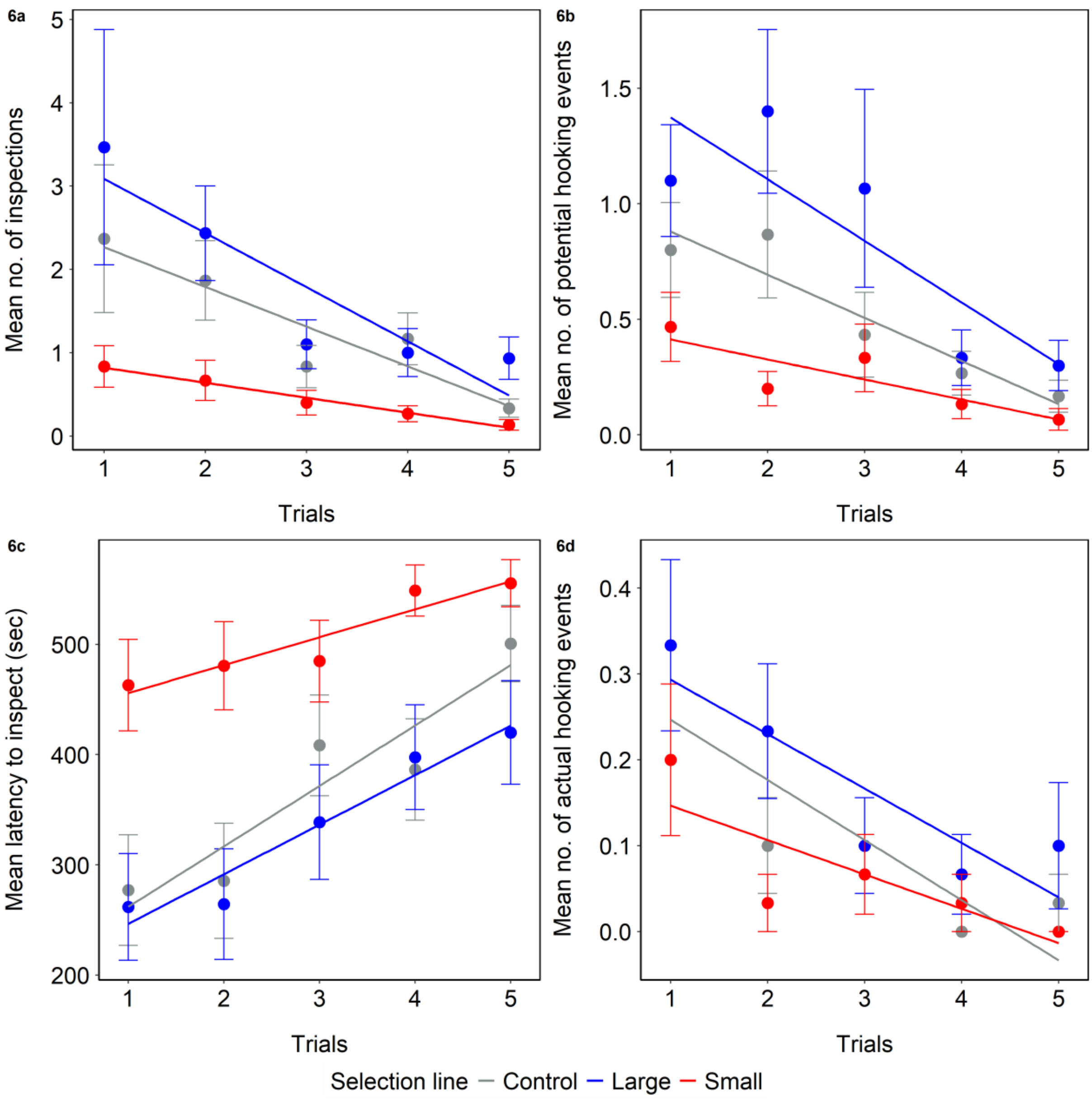
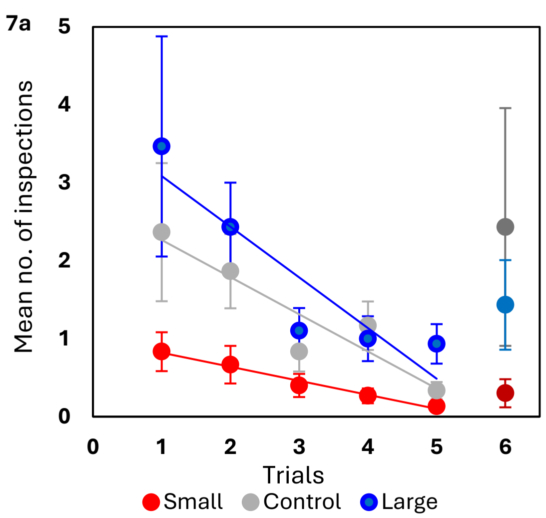
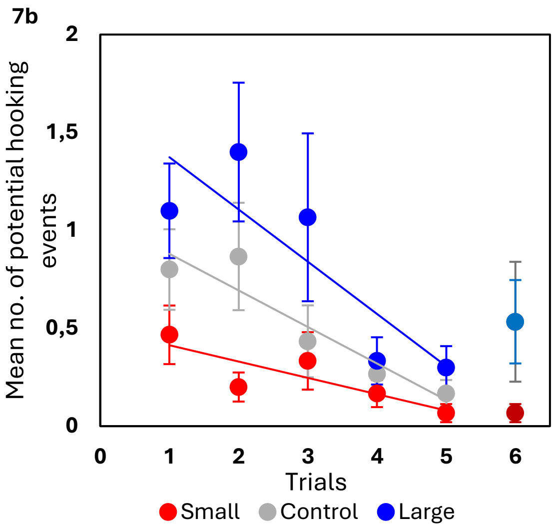


Figure 6 displays the main results of experiment 3 (Hook-avoidance learning). (a) illustrates the mean no. of inspections of the baited hook across trials (± SE), significant results show touching regression-lines. (b) illustrates the mean no. of potential hooking events (PH) (± SE) significant results show touching regression-lines. (c) shows the mean latency to inspect the baited hook against trials (± SE) and (d) plots the mean no. of actual hooking-events (AH) across trials (± SE).

Experiment 4 Hook-avoidance memory probe

The small line inspected the baited hook marginally less (z=-1.71, p=0.09) while the large line inspected the hook significantly less (z=-3.75, p<0.001) across trials (last trial of exp. 3 and probe) compared to the control line (*Fig. 7a, Table 1*). The number of potential hooking-events significantly increased in the probe (0=2.27, p=0.02) (*Fig. 7b fainted dots, Table 1*), meaning that all fish tended to ingest the hook more often but not deeply enough to get hooked and therefore successfully avoided getting hooked. The selection lines did not differ in actual hooking events during memory probe compared to the control (*Fig. 7c fainted dots, Table 1*) and this indicated retention of learned/acquired hook-avoidance. Therefore, these results provide no support for my initial hypothesis H5, as hook avoidance was equally distributed among the lines.





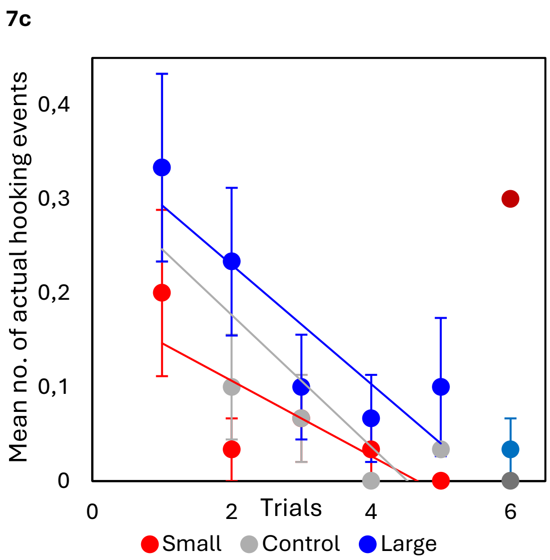


Figure 7 summarises the main results of experiment 4 (Hook-avoidance memory probe with baited hook). ***The fainter coloured dots show the mean (± SE) results of the Hook-avoidance memory probe (Trial 6).*** *Trial 1 to 5 show the main results of experiment 3 (Hook-avoidance learning) and trial 6 represents the Hook-avoidance memory probe. (a) displays the mean no. of inspections across trials (± SE), (b) shows mean (± SE) no. of PH across trials and (c) plots the mean (± SE) no. of AH against trials. The plots only for experiment four were generated using Excel Version 2509.*

Experiment 5 Hook-recognition

When tested for hook-recognition, the model showed significant two-way and three-way interactions. The small line made significantly less no. of inspections of the sham and the sharp hook during the memory probe trials than the habituation and learning trials, when compared to the controls (z=-2.69, p<0.01, *Fig. 8, Table 1*). This indicates significant avoidance of both sham and sharp hook in the small line fish after the hooking experience. The large line fish made less inspections of the sharp hook than the sham compared to the controls in the memory probe (z=-2.55, p=0.01, *Fig 8, Table 1*), which indicates that they developed a cognizance of the hook. These results provide no support for my hypothesis H6 but instead show greater hook recognition in the large line.

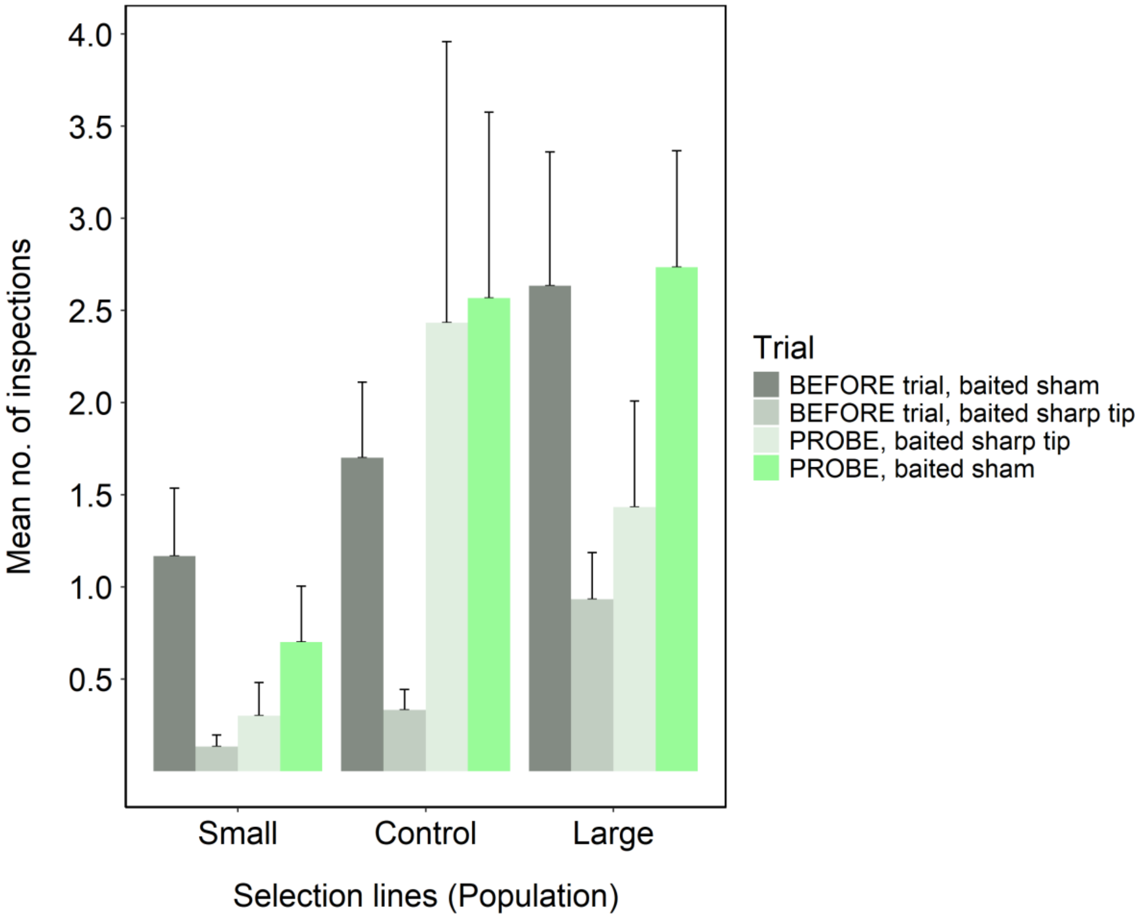


Figure 8 illustrates the main results from experiment 5 (Hook-recognition memory probe). The dark grey bar shows the mean no. of inspections (± SE) of the last trial of experiment 2 (with a baited sham hook), the grey bar displays the mean no. of inspections (± SE) of the last trial of experiment 3 (baited hook with sharp tip), the light grey bar represents the mean no. of inspections (± SE) of the probe of experiment 4 (baited hook with sharp tip) and the green bar depicts the mean no. of inspections (± SE) of the probe of experiment 5 (with a baited sham hook).

Experiment 6 Adaption of feeding behaviour

The small line had a significantly lower overall feeding rate compared to the control (z= -2.58, p<0.01) (*Fig. 9*). The feeding rate in the small line fish was also marginally less in the probe trial than during test for foraging (z=-1.75, p=0.08; *Fig 9*), which indicated that exposure to the hooking caused these fish to modify their feeding rate even further. By contrast, the large line fish did not differ in foraging rates before and after the hooking trials compared to the control, thus showing no adaptation of foraging rate in the large line. Considering these results, they suggest support for my hypothesis H7 of no altered feeding-behaviour in the large line compared to the control, but vice versa for the small line compared to the control.

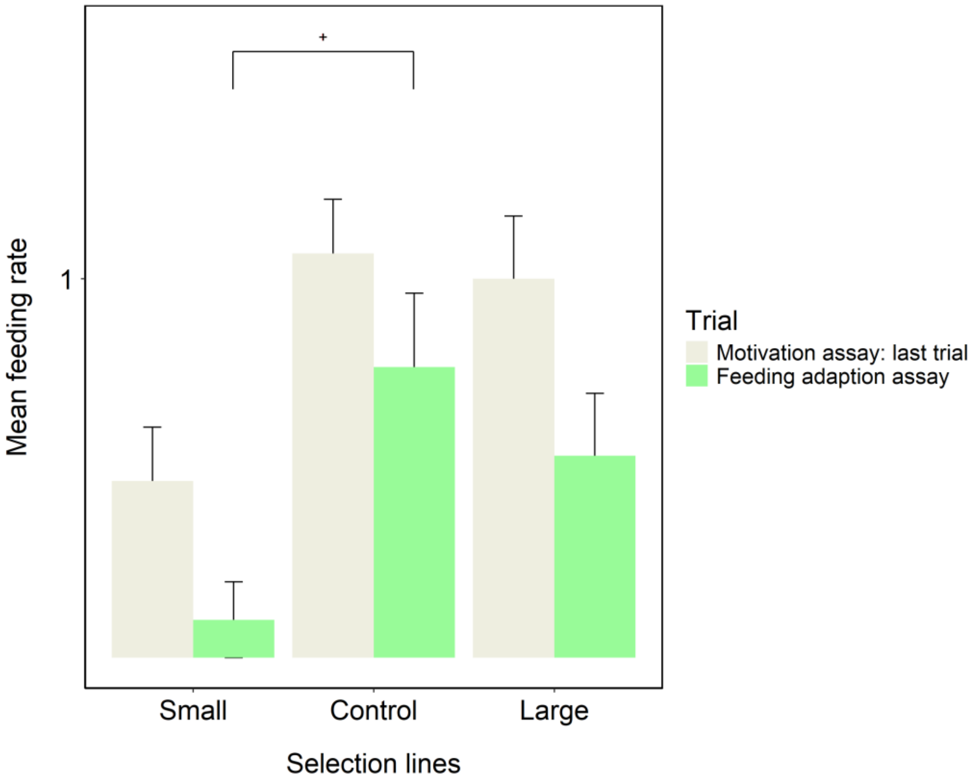


Figure 9 depicts the results of experiment six (adaption of feeding-behaviour in presence of an unbaited sham). It illustrates the mean feeding rate (± SE) comparing the last trial of the foraging-assay (exp. 1) with the feeding adaption probe (exp. 6). Marginal results are indicated with a "+".

Discussion

Overall, I saw that size-selective mortality in zebrafish leaves a legacy in cognitive abilities related to hooking vulnerability, avoidance and recognition. I found that fish under positive size-selection (small line) were shy to inspect shams and hooks and were significantly less vulnerable to hooking - when considering inspections, PH and AH - than the control line and these agreed with my hypotheses H1 to H3. However, the opposite for the large line was not observed. All selection lines showed similar hook avoidance learning thus rejecting H4, and all lines also retained the memory of hooking when tested after a time interval thus rejecting H5. The large line fish showed significant cognizance of the sharp hook compared to the control line thereby disproving H6 and indicating significantly higher cognitive ability in this context in these fish. Lastly, the small line fish adapted their feeding behaviour and fed less post hooking experiences thus confirming H7 and this could be attributed to their risk aversive behavioural tendencies.

The repeatability outcomes for experiment one and two found to be significant for all lines only for no. of inspections in experiment one. Other variables were not repeatable for all lines. A reason for that could be the fish may have not fed as intensive in the first trial compared to the last trial, because they had to habituate to the new environment. Mean latencies might differ due to the same reason: after two trials, the fish were habituated to the environment and therefore latencies differed.

There is a general trend that is observed throughout all experiments: shyer/ more cautious tendencies in the small line and bolder tendencies in the large line compared to the control. However, only the former was found to be significant. The small line is a less intensive forager and shows more aversion to novel objects (indicated by the no. of inspections on the unbaited sham hook). While the large line did not overall forage more than controls. No. of inspections on the unbaited sham (exp. 1 - Foraging-assay) are highly pronounced in the large line at the beginning but drastically drop across the three trials indicating that they lose interest. However, when the sham hook is baited (exp. 2 - Readiness to ingest the baited sham hook) interest was maintained across trials, suggesting they are more intensive foragers than controls, when there is a food-reward included. Explanation goes back to evolutionary adaptation of shyness (in small line) and boldness (in large line) (and was also found in Atlantic silverside Walsh et al. 2006) and in zebrafish (Sbragaglia et al. 2021; Roy and Arlinghaus 2022). I expected the large line to reveal greater foraging rate due to these boldness related behaviours. Sbragaglia et al. (2021) and Roy and Arlinghaus (2022) reported increased boldness in terms of increased risk-taking behaviour to feed on the water surface. In fact, this rather considers behaviour in presence of food but not actual food-intake. Hence, boldness and foraging rate are probably interrelated but one is not an indicator for the other, i.e. there is a difference between *foraging more due to boldness* (being close to the surface taking risks to forage) and the *actual foraging rate*. Thus, the large line is behaviourally bolder but not necessarily eating much more. Yet, they maintain more interest in a baited hook than the controls, consistent with more evidence that they eat more and would be more vulnerable to hooks. Whether a fish is vulnerable to getting hooked in the first place is determined by its encounter (Lennox et al. 2017). Fish that encounter a hook more often are more likely to getting hooked compared to fish that face less encounters. Encounters in my study can be described by no. of inspections. Consequently, results from my study suggest that the small line is less vulnerable overall, when judged by inspections and potential hooking events (i.e. possible hooking events, where I was too slow to set the hook, or the hook was not ingested deeply enough). Evolutionary outcomes of selection were also observed by Hessenauer et al. (2016), who showed that fish from exploited populations showed faster hook avoidance learning and this is suggestive of evolutionary adaption in cognitive ability. Further, Phillip et al. (2009) reported that angling-vulnerability is a heritable trait, and conclusively an evolutionary outcome. I therefore argue that the lower vulnerability is likely a response to shyness in the small line and relatedly accompanied by more aversion to novel objects and lower willingness to take food. This supports the model predictions regarding mortality outcomes of fish groups among the three lines that showed that the small line would have reduced vulnerability to fishing gears because of shyer tendencies and they are more vigilant to environmental cues compared to the control (Sbragaglia et al. 2022).

I then found strong hook avoidance learning in all selection lines. This disagrees with Hessenhauer, who showed that differently treated populations (fished and unfished) learn lure-avoidance with different velocities (Hessenauer et al. 2016). A reason for that could be the adaptive value of learning overall and the overall larger impact of plasticity on behaviours compared to genetically coded contributions (Cauchoix et al. 2020; Buchanan et al. 2013). This study suggests that zebrafish are not different from other species, where rapid hook avoidance was reported (Takahashi and Masuda 2021; Askey et al. 2006; Beukema 1970a, b; Arlinghaus et al. 2017b; Hessenauer et al. 2016; Wegener et al. 2017; Czapla et al. 2023). Moreira et al. 2025 who also investigated aversive memory in zebrafish reported a retention for up to seven days using individual zebrafish. An inflection point occurred at 62 h, where duration started to gradually decline. Kenny et al. (2017) reported the duration of fear conditioned memory was retained for 14 days. Yet, in that study, fish were not individually identified (i.e. individual activity levels were not taken into consideration) (Moreira et al. 2025). In my study, all lines were able to successfully avoid the hook in the hook-avoidance memory probe after six days. Considering this, my results indicate that size-selective mortality did not lead to altered hook avoidance learning between selection lines.

In the hook-recognition memory probe, the large line (significantly) inspected the baited sham hook significantly more than the sharp hook. This indicated that selection for large body size had a significant evolutionary impact on hook recognition ability in zebrafish. This result - that the large line zebrafish can distinguish between different hooks - is an outstanding finding in itself. This can be explained through the two pathways elaborated in Roy et al. 2024. Firstly, because of altered energy expenditure through increased harvest mortality, less energy is allocated to expensive brain tissue, which can alter cognitive abilities (Roy et al. 2024). Secondly, harvest mortality can alter behavioural styles (i.e. boldness) which in turn can affect cognition (Roy et al. 2024). The first argument explains why there could be altered cognitive abilities in the large line, i.e. increased investment in neuronal tissue. The second argument suggest, that due to increased boldness (i.e. behaviour-driven) in the large line, these fish inspect the sham hook more often. On the other hand, it appears that the hooking related cognitive functions were elevated most strongly in the small line because they inspected the hooks and shams significantly less during the memory probe than during the habituation - and hooking-trials, but this can be attributed to their shy behavioural tendencies. These fish were probably wary of inspecting anything that resembled the hook after hooking experiences. However, a further argument for altered cognition in the small line is provided by a reduction of feeding rate in the memory probe after negative hooking experiences. This indicates learning in that line. The fish are aware of the potentially harmful object and therefore feed less in presence of it. On the one hand, it allows the small line to avoid capture, but it may have fitness consequences. However, it remains complicated to identify whether changes are due to altered cognitive ability (i.e. evolutionary) or because of behavioural differences that are consistent (i.e. the small line could have lower inspections in the hook recognition memory probe and lower feeding rate in exp. 6 due to shy tendencies).

Limitations:

In my study I used five groups per replicate (30 groups and 90 fish in total). Increasing the number of groups per replicate could have resulted in significant effects for the large line that only showed a marginal qualitative trend in some cases in my study, although I was able to report numerous other significant effects.

Additionally, handling these fish was difficult due to their small body size (2.5cm to 4.1cm total length, in my experiments). The hooks I used were tiny (size 22) (see *Fig. s. 3* in the appendix) but proportionally big compared to the zebrafish. Actively setting the hook in this small species was difficult, which produced rather high number of potential hooking events, compared to actual hooking events (i.e. successfully hooking the fish). Smaller hooks could have led to more hooking-events since it is easier to ingest them, but probably at a cost of harming the fish more intensively. Future angling-experiments with zebrafish must consider this, especially because micro-fishing is an emerging form of recreational angling (Cooke et al. 2020).

The large line showed the same qualitative trend in my pre-trial (dive test) as reported by Sbragaglia et al. (2021) and Roy and Arlinghaus (2022), although this trend appeared to be less pronounced in one of the replicates of the large line (Large 1) (*Fig. s.2a and s.2b* in the appendix). Since size-selection was halted 14 generations ago, there could have been trait recovery as reported previously in a study conducted with Atlantic silverside that showed that stopping selection for a few generations can lead to partial trait-recovery (Conover et al. 2009).

Further, one could argue that inspections in the first experiment (without a bait) is hardly comparable to inspections in the second experiment (baited), because in the latter there is a food-reward incorporated. Thus, inspections of the unbaited sham (exp 1) might be related to curiosity compared to the intention to forage on the baited sham (exp2). Yet, because of the removed tip on the sham, the bait was easily ingested and foraged. Conclusively, the very first inspection of the baited sham, always resulted in successfully feeding on the worm/ bait and then other inspections followed (on the empty sham) can be compared to the ones in experiment one. In fact, this is not how it happens in normal fishing, where always a sharp hook is used to successfully capture the fish.

It is left to mention that these results cannot be easily transferred onto the wild, as they were conducted under controlled, laboratory conditions. However, the results presented in this thesis offer a foundation for further elaboration in future research.

Conclusion

My work provides evidence that size-selection has resulted in an evolutionarily alteration of not only boldness/ shyness and foraging but also has altered cognitive functions that reduce vulnerability to harvest in the small line, while not changing the ability of fish to learn from hooking experiences, considering the fact that selection for size (i.e. length) halted 14 generations ago. Thus, evolutionarily adapted fish populations exposed to large size selective harvest will get harder to be captured evolutionarily over time, which is accompanied by consequences for the well-being of recreational anglers or commercial long-lines. My work is among the first to clearly show that size-selection alone can leave a cognitive/ behavioural legacy in evolving fish populations, so that evolutionary adaptations not only alter life-histories and personality traits, but also cognitive abilities.

Ethics statement

This study was approved by State Office for Health and Social Affairs
Berlin (LaGeSo), Germany (approval number: G 0036/21).

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Appendix

Pre-trial Boldness assay

As the experimental fish originated from the housing tanks and were not raised under the same holding conditions the different density conditions in the housing tanks could account for behavioural divergences among the selection lines. To investigate if the experimental fish were behaviourally similar to the fish used in previous studies, a tank-diving test was conducted that tested for boldness in groups of zebrafish (Sbragaglia et al. 2021, Roy and Arlinghaus 2022). In this assay, I tested risk-taking tendency to feed on the surface after a simulated aerial predator attack (Sbragaglia et al. 2021, Roy and Arlinghaus 2022). The experiment was conducted with 64 fish per replicate line, i.e. 8 individuals per group and 8 groups per replicate line (384 fish in total) (see Sbragaglia et al. 2021, Roy and Arlinghaus 2022). These fish originated from the six round tanks, like my experimental fish for the angling experiment to see if density conditions changed risk-taking behaviour. Fish were netted from the big holding tanks and transferred in groups of eight fish per 5l housing box. The fish were allowed to acclimate for one day. The water temperature and further housing conditions were maintained as mentioned above. Experimental fish were starved for 24h before the experiment to ensure feeding motivation during the trials.

A rectangular glass tank (30x10x25 cm) with white walls at three sides was used was used for the assay (Sbragaglia et al. 2021, Roy and Arlinghaus 2022) (see Fig. 1a, 1b). A white curtain was installed to avoid disturbances. The tank was filled with system-water up to a level of 20 cm. A "surface-zone" was marked at the glass tank (4 cm from the water surface) (Roy and Arlinghaus 2022). The fish were transferred into the tank and allowed to acclimate for two minutes. Then, food was added to the surface, and the fish were allowed to feed for 30 seconds. Then a fake bird cut-out made from cardboard was suddenly released from above and hovered 10 cm above the surface for 15 seconds. The simulated predator was retrieved, and the fish were allowed to resume feeding again for 5 min (Roy and Arlinghaus 2022). Trials were recorded using one Logitech B910 camera that was connected to a computer. After the trials, I scored the latency to feed after the simulated aerial predator was retrieved, the number of transitions to the surface and the total time spent feeding on the surface after the retrieval. It was expected that bolder individuals would exhibit shorter latencies to feed, more transitions to the surface and a longer feeding period at the surface. Results from past experiments showed that the large line was significantly bolder compared to the control, whereas the small line was not significantly different to the control (Sbragaglia et al. 2021, Roy and Arlinghaus 2022). The results from my experiment qualitatively showed the same trend. The large line (especially Large 2) was bolder compared to the control. The other replicate Large 1 was not significantly different (as displayed in the figure 2a and 2b below). The small line exhibited less boldness compared to the control, which was not observed in the former experiments (Sbragaglia et al. 2021, Roy and Arlinghaus 2022). As the behavioural trend broadly matched expectations and past findings, I used the fish for my subsequent learning experiments.

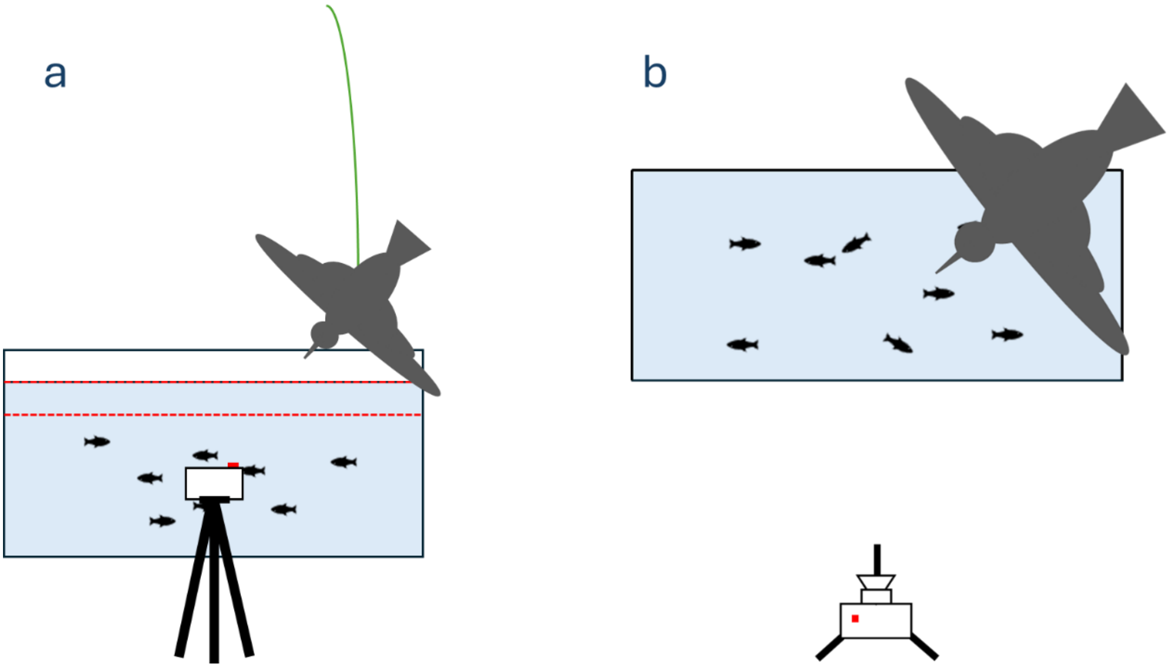

Figure s. 1 (a) illustrates the setup of the dive-test from the front and (b) from above.

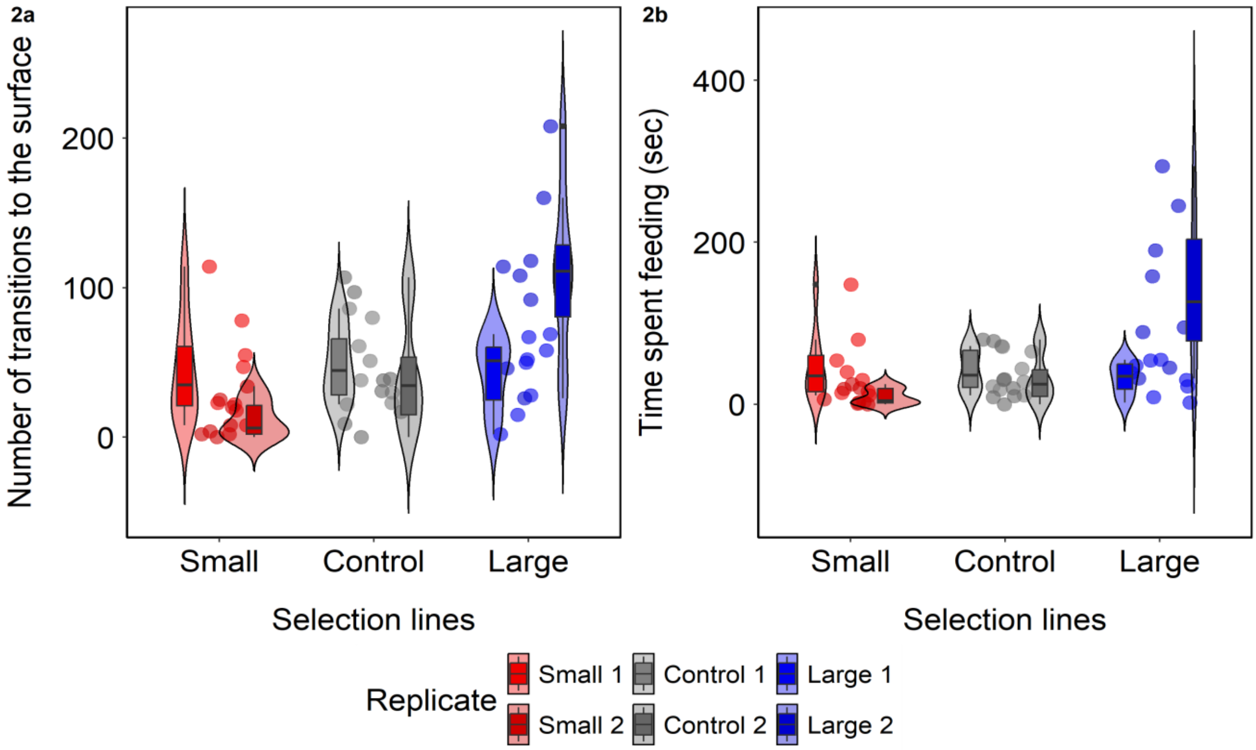

Figure s. 2 displays the main results of the boldness assay (pre-trial). (a) shows number of transitions to the surface (i.e. how often does an individual from each line enter the surface zone after predator release). Replicates are distinguished with lighter and darker colour (b) displays the time spent feeding (i.e. how much time does an individual from each line spent feeding on the surface after predator was released).

Figure s. 3 shows an image of the hooks I used, with a bloodworm baited (held using tweezers).