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# Cognition in captivity: Investigating learning, anxiety, and brain mass differences between captive and wild fathead minnow

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ABSTRACT

Captive animals are commonly used in laboratory research, however, captive and wild animals often differ, limiting the applicability of laboratory findings based on captive animals to the "real" world. A comprehensive understanding of how captive and wild animals differ is necessary to assess if captive animals are suitable substitutes for their wild counterparts, however, few studies have compared cognition between captive and wild animals. In this study, we compared aspects of cognition between captive and wild fathead minnow (*Pimephales promelas)* to investigate how life in captivity might impact traits essential to fitness in the wild. Specifically, we assessed aversive learning performance using an associative learning assay and a more complex reversal learning assay, and we also measured anxiety and brain mass. Performance in the associative learning task was negatively correlated with performance in the reversal learning task, suggesting a potential tradeoff. No differences were observed between captive and wild fish in associative learning performance, anxiety, or brain mass, but wild fish were three times more likely to complete the more complex reversal learning task. Aversive reversal learning, which appears to be dampened in captive fathead minnow, may be particularly relevant for appropriate anti-predator responses and fitness in challenging or fluctuating environments. We advocate for further research comparing wild and captive animal cognition and urge researchers to exercise caution when generalizing results from captive animals to wild populations.

## 1. Introduction

Humans hold animals in captivity for a variety of purposes, including food production in agriculture and aquaculture; conducting research studies to test medical treatments, genetic therapies, and product safety; and to learn more about the behaviour, physiology, anatomy, and genetics of animals (Salena et al., 2021; Bateson and Feenders, 2010). Compared to using wild animals, the use of captive animals can be more convenient (e.g., captive animals can be commercially purchased whereas wild animals must be caught), controlled (e.g., through the provisioning of specialized diets to promote growth), and ethical (e.g., common animal research ethical goals include reducing the animals taken from the wild; Soulsbury et al., 2020). Furthermore, when a species becomes rare in a particular location, conservation efforts sometimes include reintroducing them into the wild from captive-bred stocks, such as a zoos or hatcheries (Beck et al., 1994). However, these attempts have shown that captive animals often are morphologically, physiologically and behaviourally different to wild animals, necessitating careful planning and even retraining to the wild before re-introduction (Crates et al., 2023; Griffin et al., 2000; Beck et al., 1994). Reintroduced animals typically have extremely low survival and reproductive success rates (Jule et al., 2008; Beck et al., 1994), suggesting that living under captive conditions can alter traits and abilities required for survival in the wild. These findings highlight the need for careful consideration of whether captive animals are appropriate substitutes for their wild counterparts. Understanding how captive animals precisely differ from their wild counterparts will help us better assess the extent to which laboratory research findings can be applied to real-world contexts, and critically evaluate the effectiveness of conservation plans that include the reintroduction of captive-bred animals to the wild.

Previous studies have demonstrated several important phenotypic differences between captive and wild animals, including smaller brains (Burns et al., 2009; Guay and Iwaniuk, 2008; Marchetti and Nevitt,

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2003; Kihslinger et al., 2006) and diminished antipredator behaviour in captive animals (Jackson and Brown, 2011; Tang et al., 2017). These phenotypic differences can be attributed either to differences in environmental conditions experienced within an individual's lifetime (particularly during critical developmental periods; Burns et al., 2009) or to genetic changes that can persist across generations due to the relaxation of selective pressures in captivity (McDougall et al., 2006; Lynch & O'Hely, 2001). For example, the observed reduction in captive animals' brain size could be the result of a plastic trait if there is a within-lifetime response to a lack of environmental input necessary for proper brain development (Burns et al., 2009). Indeed, brain morphology is usually a somewhat plastic trait, and many previous studies have found that animals reared in impoverished or sterile conditions typical of captive environments (e.g., with decreased area for movement, low structural complexity, limited social stimulation) will have reduced brain size, fewer synapses per neuron, and less dendritic branching compared to individuals raised in enriched or ecologically normal conditions (Kihslinger and Nevitt, 2006; van Praag et al., 2000). Differences between captive and wild animals can also result from longer-term differences in selection pressures, such as predation intensity and food availability, being very different in captivity versus the wild. Natural selection is often weakened by the benign conditions of captivity, and hence in captivity we sometimes observe a wider range of possible phenotypes coexisting and traits which would be maladaptive in the wild can sometimes persist in captivity (Salena et al., 2021; Tave and Hutson, 2019; Lynch & O'Hely, 2001). Decreased brain size might result in reduced survival in the wild if smaller brains limit an individual's ability to respond to predation risk, but animals with small brains or other maladaptive traits might be able to survive in captivity due to benign conditions and high survival rates (Heath et al., 2003).

Brain size is often correlated with cognitive ability (Kotrschal and Kotrschal, 2020; Kotrschal et al., 2013, Buechel et al., 2018), so it is plausible that the reduction in brain mass observed in many captive animals could also be associated with impaired cognition. One brain region often smaller in captivity is the telencephalon, which is implicated in learning (Marchetti and Nevitt, 2003; Burns et al., 2009; López et al., 2000). Learning, defined as a neuronal representation of new information that facilitates change in behaviour (Dukas, 2004; Buechel et al., 2018; Shettleworth, 2009), is one cognitive ability which can affect fitness. For example, wild animals can sometimes increase their chances of survival if they learn about how predators behave, and high predation risk is hypothesized to have selected for the evolution of learning (Morand-Ferron, 2017; Griffin et al., 2000). Predation risk is usually relaxed in captivity (Geffroy et al., 2020), so learning about predation may not be as essential for captive animals. Furthermore, captive animals are often reared in environments with low spatial complexity (Mason et al., 2013), which may not provide adequate neural stimulation necessary for developing strong spatial learning abilities.

Few studies have directly compared the cognitive abilities of wild and captive animals (but see Benson-Amram et al., 2013; Vardi et al., 2020; Gajdon et al., 2004; Näslund, 2021). These previous studies found mixed results, some demonstrating superior cognitive performance in captive animals (e.g., innovative problem solving in hyena, *Crocuta crocuta,* and kea, *Nestor notabilis,* Benson-Amram & Holekamp, 2012; Gadjon et al., 2004*)* while others reported superior cognitive performance in wild animals (e.g., spatial reward learning in rainbow skink, *Lampropholis delicata*: Vardi et al., 2020). Results depended on the study species and specific task studied. To expand our understanding about the effects of captivity on cognition, in this study we compared learning performance in wild and captive fathead minnow (*Pimephales promelas*). We chose to test fathead minnow as they are easy to rear in the laboratory; they are a highly abundant fish species found throughout North American freshwaters; and because they are a common model species in ecotoxicological research that are used to inform environmental legislation aimed to protect water quality (Ankley and Villeneuve, 2006). In particular, we chose to investigate how captivity influences aversive learning, as this type of learning may be particularly relevant to the production of antipredator behavioural responses, which seem to be impaired in captive animals and could explain low survival rates of captive-born animals released to predator-abundant environments in the wild (Crates et al., 2023; Sahashi and Morita, 2022; Griffith et al., 1989; Jule et al., 2008). Using an aversive learning assay modified from one used by Lindeyer and Reader (2010), we studied two types of aversive learning: associative and reversal. In the associative stage, fish learned to escape an aversive stimulus (a trawl net) using one of two escape routes. In the reversal stage, the previous correct escape route association was reversed so that individuals had to "forget" and forge a new association (choose a different escape route). Reversal learning is a common paradigm used to assess cognitive flexibility and general cognitive function (Izquierdo et al., 2017) and is sometimes considered more cognitively demanding than associative learning, since it requires both inhibition of the previously learned response and the formation of a new response (Buechel et al., 2018; Brunet et al., 2023). Accordingly, previous studies have found that individuals typically require more trials to complete reversal learning tasks than simpler associative learning tasks (Brunet et al., 2023; Buechel et al., 2018). Although both types of learning as implemented in our study were associative and aversive, we use the term "associative learning" to describe the first phase of our learning assay where the fish learned an initial escape route, and "reversal learning" to describe the second phase of our assay where the exits/contingencies were reversed, similar to Bikker et al. (2024) and van den Heuvel et al. (2023).

We made a number of hypotheses and predictions regarding the differences in cognitive performance between wild and captive fathead minnow. First, we hypothesized that the benign and unenriched conditions typical of captive environments might not select for strong learning abilities or might not provide adequate neural stimulation required for the development of strong learning abilities. Based on this hypothesis, we predicted that captive fish would have inferior learning performance compared to wild fish. Second, we predicted that if captive fish had worse learning performance, then this might be because they have a smaller relative brain mass than do wild fish. This prediction was based on past studies showing that cognitive performance can be correlated with relative brain mass (Buechel et al., 2018; Kotrschal et al., 2013; Kotrschal and Kotrschal, 2020). However, several factors other than brain mass can also influence cognitive performance, and brain mass is not always predictive of cognitive performance. For example, Buechel et al. (2018) found that brain mass was predictive of cognitive performance in a more complex reversal learning task, but not in a simpler associative learning task. Cognition is also plastic and can be impacted by many factors other than brain mass, including gene expression, neurotransmitter activity, and stress (Lucon-Xiccato et al., 2022; Wang et al.,2025; Arechavala-Lopez et al., 2020). If these factors are affected by captivity, then living in captivity could affect cognition without necessarily involving effects at the level of brain size (De Russi et al., 2024; Brunet et al., 2023; Strand et al., 2010; Salvanes et al., 2013; Gatto et al., 2024). To investigate this possibility, we considered an alternative hypothesis where captive fish could be better learners compared to wild fish due to differences in anxiety and stress. Wild fish tested in captive environments can be more anxious (Wong et al., 2012), and anxiety has been shown to negatively impact learning (Mandler and Sarason, 1952; Darcet et al., 2014). Therefore, to consider this possibility, we compared the anxiety levels of captive and wild fish using a stress assay and predicted that wild fish would have higher anxiety. Finally, since reversal learning is regarded as more cognitively demanding than associative learning (Buechel et al., 2018), we predicted that both captive and wild fish would learn faster in associative learning compared to reversal learning. Furthermore, we explored whether a relationship exists between associative learning and reversal learning.

## 2. Materials and methods

### 2.1. Study animals and housing

We obtained captive fathead minnow (*Pimephales promelas)* from a commercial supplier (AquaTox Inc), where they had been bred in captivity for many generations (~30 years). We transported these captive month-old fish to McMaster University (Hamilton, Ontario, Canada) in December 2020, and in October 2021 when the captive fish had reached 11 months of age, we began the experiment. In late August 2021, we caught wild adult fathead minnow using a fyke net deployed for 24 h in Windemere Basin (Hamilton, Ontario, Canada) and transported these fish to McMaster in aerated coolers. The wild fish underwent a seven-week (52 day) acclimation period in the laboratory before the experiment commenced in October 2021. Although a transition from a natural environment to captivity can produce changes in relative brain size in adult fish within a few weeks (Park et al., 2012; Turschwell and White, 2016), a seven-week acclimation period was required in our study because our wild-caught fish were initially highly skittish (erratic swimming and excessive hiding) upon transport to the laboratory. We allowed the wild fish to acclimate to laboratory conditions over a 7-week period until all fish were demonstrating normal activity and feeding behaviour.

When we began the experiment, captive and wild caught fish were roughly similar in body mass and the sex ratios were also similar. Prior to starting experiments, we housed captive fish and wild fish separately in groups of ~15 fish. Each group of fish was placed in a 75 L housing tank outfitted with two static renewal filters (AquaClear and Marina brands) and two sponge filters, as well as two PVC pipes for shelter. We kept all housing tanks at room temperature (~20℃) and on a photoperiod of 13 h:11 h light:dark, and we fed the fish with commercial pellets (Corey Optimum feed, 0.7 gr) *ad libitum* six days per week. Following each block of 14-day aversive learning and anxiety experiments, all fish were euthanized for brain dissections. All animal protocols were developed in accordance with guidelines established by the Canadian Council on Animal Care and were approved by the McMaster University Animal Research Ethics Board (AUP 17–45–12).

### 2.2. Aversive learning assay

To compare the learning performance of captive and wild fish, we modified a trawl assay designed by Lindeyer and Reader (2010) (Fig. 1 A). Each tank used in the aversive learning assay was 38 L (51 cm × 25.5 cm x 29 cm) and was divided in half by a transparent acrylic divider. On one side of the divider there was a black mesh trawl spanning the entire depth and width of the tank, and this trawl could be moved back and forth on gliders across the half-length of the tank. We called this half of the tank the “trawl zone”. The trawl net would move towards the divider and could come into physical contact with fish in the trawl zone if the fish did not escape first and move through the hole beyond the barrier; this experimental set up has previously been used as an aversive stimulus by Lindeyer and Reader (2010) and Bikker et al. (2024). Essentially the net would trap the fish against the barrier if they did not manage to escape. On the other side of the divider there was a sponge filter which maintained water quality but was also used as a refuge by the fish, and we called this half of the tank the “refuge zone”. Fish could escape from the aversive trawl zone into the refuge zone by swimming through one of two round exit holes in the divider (diameter = 3.2 cm; 1 cm from the bottom of the divider). We outlined these exit holes in black to make them more visibly obvious, and we added a plant illustration next to one exit hole and a rock illustration next to the other exit hole to make them even more visibly distinguishable (Bikker et al., 2024). The walls of the tank were lined with opaque paper to minimize visual contact with the experimenter and nearby tanks. Each aversive assay tank was maintained under the same environmental conditions (temperature and light) as the housing tanks.

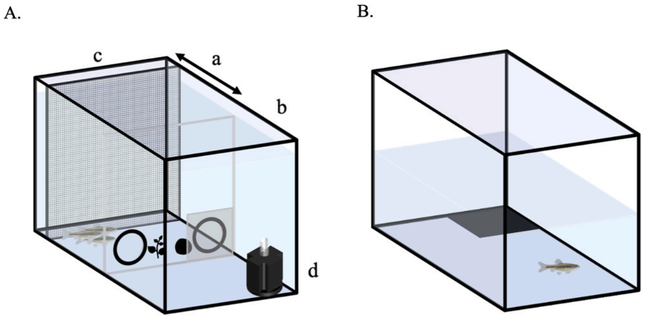

**Fig. 1.** (A) Illustration of the tank used in the aversive learning assay. The tank was split in half by a transparent barrier with two holes that the fish could use to escape from the trawl zone (a) to the refuge zone (b). One hole was covered with transparent plastic during associative learning, and the opposite hole was covered during reversal learning. The black mesh net (c) was moved back and forth in the trawl zone as an aversive stimulus. The sponge filter (d) was used as a shelter in the refuge zone. (B) Illustration of the tank used in the anxiety assay. The bottom of the tank was white except for a small black square in the corner. Figures are adapted from Bikker et al. (2024).

Based on pilot studies, we opted to perform the learning assay on groups of three fish rather than on individuals, as running fish in groups promoted movement and considerably shortened the duration for the fish to reach learning criteria. Individuals in each trio were deliberately chosen to have different body sizes, with the goal of allowing individual identification when scoring behaviour from video recordings. In total, we ran 18 trios of captive fish (n = 54) and 17 trios of wild fish (n = 51). After transporting a trio of fish into the assay tank we gave the fish 60 min to acclimate in the refuge zone before beginning the learning assays. During this 60-minute acclimation period we covered both exit holes with transparent plastic. The holding and assay testing tanks with captive fish and wild fish were spatially randomized to control for any possible position effects. We ran learning assays from 10 October—6 December 2021 and completed 100 % water change whenever a trio was finished the learning assay and before a new trio from the holding tanks was added to that learning assay tank.

#### 2.2.1. Associative learning

To begin an associative learning training session, we uncovered one of the two exit holes (either the rock or plant hole) and left the other exit hole covered with transparent plastic. Although fish could see through both exits, they needed to learn that only one was the "correct" exit to escape. We randomly assigned each trio to have either the rock or plant exit hole as the “correct” exit open for all associative learning trials, to control for possible side biases. At the start of each trial, we used a hand net to move the three fish from the refuge zone over the divider into the trawl zone, where we let them acclimate for 5 min. We then began a two-minute trawl trial where we moved the trawl back and forth in the trawl zone every 30 s for a total of four times within the two-minute period. At the end of the trawl trial period, we noted the number of fish that remained in the trawl zone and those that successfully escaped to the refuge zone. We used a hand net to guide any fish remaining in the trawl zone through the correct exit into the refuge zone, and this marked the end of the trial. We waited two minutes before repeating this procedure and did this for a total of four trials per training session. We completed two training sessions each day (8 trials per day), and each training session with four trials was always separated by a minimum of one hour. Between sessions we covered both exits, confining the fish to the refuge zone. The learning criterion we used to designate that a trio had completed the associative learning task was when all three fish escaped through the correct exit within the two-minute trawl period during four consecutive trials.

#### 2.2.2. Reversal learning

Once a trio had achieved the associative learning criterion (all three fish escaped through the correct exit in four consecutive trials), we switched from the associative learning phase to the reversal learning phase. In this reversal learning phase, we switched the correct exit, then we followed the same trawl procedure described in the associative learning phase. Our learning criterion for designating a trio as having completed the reversal learning task was when all three fish swam through the "newly correct" (and opposite) exit within the two-minute trawl period for four consecutive trials. The entire learning assay, including both associative and reversal phases, lasted a maximum of 14 days (108 trials, with four trials on the first day of testing and eight trials on each subsequent day). We discontinued trials after this period regardless of whether the trio had achieved the associative learning and reversal learning criteria or not (three trios never reached the associative learning criterion; one additional trio never reached the reversal learning criterion). We videotaped all the learning trials using GoPro or Sony HDR cameras mounted above the tanks.

### 2.3. Anxiety assay

After the aversive learning assay, we tested the anxiety level of a subset of captive fish (n = 17) and wild fish (n = 18) using a scototaxis assay modified from Polverino et al. (2021), Figure 1B). The anxiety test was conducted by placing individual fish in 20 L tanks (41 cm × 21 cm x 25 cm) filled with 10 L of dechlorinated, aerated municipal tap water. The bottom and sides of each tank were lined with white adhesive, except for one small black square (17 cm × 17 cm) in the bottom corner. Since fish are often more conspicuous to predators on lighter backgrounds and thus commonly prefer darker, "safer" backgrounds (Maximino et al., 2010), we measured anxiety by recording the number of visits to the black square and the total time spent in the black square. We acclimated fish to the anxiety test tanks by placing them in a black PVC tube positioned vertically in the middle of the tank for two minutes. We began a trial by removing the PVC tube, and then the fish's activity was video recorded for 15 min using either a GoPro or Sony HDR camera.

### 2.4. Video scoring

We analyzed our video recordings of the aversive learning assay and extracted the data on two endpoints. First, we counted the number of trials each fish trio required to reach the associative learning criterion and the reversal learning criterion. Second, we measured the latency for each fish in the trios to escape the trawl zone through the correct exit in each trial. If a fish did not escape through the correct exit during a trial (i.e. it needed to be guided through the correct exit using a net), that fish was given the maximum latency of 120 s. All video footage was analyzed by a single scorer (AY) who was blind to whether the trio was from the wild or captivity. After reviewing the videos, for a number of reasons we needed to exclude some trials from our analysis: including procedural errors during a trial (e.g., leaving an exit open between sessions), video corruption errors, and trial timing errors. See Appendix A and B for a detailed description of exclusion criteria in the aversive learning assay and a breakdown of how many trials and groups were excluded overall and the reasons for these exclusions. In total, we analyzed 1279 trials (71 % of all total trials) from 16 captive trios and 13 wild trios during the two phases of the aversive learning assay. From the video recordings of the anxiety assay, a different observer (HS) blind to each fish's captive or wild status extracted (1) the amount of time each fish spent on the black square and (2) the number of times the fish left the black square.

### 2.5. Dissections and brain mass measurement

After completing all assays, we euthanized the fish using an overdose of benzocaine and recorded their body mass and sex. We severed each fish's head at the back of the opercula and placed their heads in a 4 % formalin solution and then four days later removed their brains, placing the brains in a 4 % formalin solution for storage. The same dissector was used for each fish, and we took great care to remove the entire brain from the telencephalon to hind brain as well as the olfactory bulbs before storing the brain. The brains were then transferred into Eppendorf tubes with 70 % ethanol for 9—10 days before taking the mass. To weigh the brains, we removed the ethanol from the Eppendorf tubes and left the brains to dry out overnight in the open tubes. We then weighed each brain on a microbalance (to the nearest microgram, 0.001 mg), weighing each brain three times. We took an average of these three brain masses and used this average as the brain mass for that fish. In total, we dissected and collected the brains from 48 captive fish and 39 wild fish. Two captive fish had to be excluded from our analysis after accidentally storing their brains in the same container, leaving us with 46 brains for captive fish and 39 for wild fish.

### 2.6. Statistical analysis

We analyzed our data using R version 4.2.2 (R Core Team, 2021), with $\alpha = 0.05$ determining statistical significance. We first inspected the data for normality and homogeneity of variance using Shapiro-Wilk tests and Bartlett tests. If data did not meet assumptions of normality and homogeneity of variance, it was transformed using either a square root, log10, or cube root transformation, and if the transformed data still did not meet the assumptions, non-parametric tests were used. To assess the probability of captive trios versus wild trios achieving the learning criteria over time, we conducted a survival analysis using a proportional hazards regression model in the R *survival* package (Therneau, 2024). This is a semiparametric model that is increasingly being used for modeling probabilities of learning and innovation (Sol et al., 2012; Prasher et al., 2019; Bikker et al., 2024; Latchem et al., 2025). While other statistical models might exclude observations from individuals that never achieved the learning criteria or assign them an artificial ceiling value (e.g., requiring 108 trials to learn), survival analysis has the advantage that they are able to account for these "censored" observations and still include them in analysis without modification or exclusion to make valid inferences (Huang et al., 2023). We built separate models for associative and reversal learning. In our survival analysis of reversal learning, we included the number of trials required to complete the associative learning task as a covariate because trios that required more trials to complete the associative learning task had fewer opportunities to complete the reversal learning task before we stopped experiments after 108 trials. For example, if Trio_A required 100 trials to complete the associative learning task, then this trio would only have 8 possible trials to complete the reversal learning task before trials were cut off, whereas if Trio_B required 10 trials to complete the associative learning task, then this trio would have 98 possible trials to complete the reversal learning task before trials were cut off. Therefore, some trios could be more likely to complete the reversal learning task simply because they were given more opportunities to complete the reversal learning task. The addition of this covariate (number of trials required to complete the associative learning task) to the model did not significantly improve the model's fit (Likelihood ratio test, p = 0.068), but its inclusion was necessary to account for the number of possible opportunities to complete the reversal learning task being dependent on the number of trials required to complete the associative learning task, as described above.

For each trio, we also calculated the mean latency for the last fish to escape through the correct exit during associative learning and reversal learning. We used a square root transformation to meet assumptions of normality, then built linear models with the mean latency to escape through the correct exit as a response variable, and origin (captive or wild) as a fixed effect. As a measure of group cohesion, we calculated each trio's average following duration as the difference between the first fish and last fish's latency to escape through the correct exit each trial.

We used a logarithmic transformation on this data to meet assumptions of normality, then built linear models with average following duration as a response variable and trio origin (wild or captive) as a fixed effect. We included only trios which successfully reached the associative or reversal learning criteria and had to remove three trios (1 captive and 2 wild trios) from our associative learning analysis and one captive trio from our reversal learning analysis given that these trios had not successfully learned. Due to a video corruption error, we also had to remove one wild trio from our analyses of following duration and latency during associative learning, but this trio could still be included in our analysis of latency during reversal learning.

To compare the anxiety of captive and wild fish, we analyzed the amount of time each fish spent in the black square as well as the number of times each fish entered the black square. We used a cube root transformation to meet assumptions of normality, then built a linear model with the response variable as the time each fish spent in the black square, and the fish's origin (wild or captive) as a fixed effect. We could not transform the data to meet assumptions of normality for the number of times each fish entered the black square, so we used a non-parametric Mann-Whitney *U* test to compare the number of times captive and wild fish entered the black square. To compare the brain mass of captive and wild fish, we created a linear model of brain mass relative to body mass, with positive residuals representing individuals with relatively larger brains (controlling for body size) and negative residuals indicating individuals with relatively smaller brains. These residuals were not normally distributed and we could not transform them to reach normality, therefore, we used a non-parametric Mann-Whitney test to compare the median residual brain mass of captive versus wild fish.

## 3. Results

### 3.1. *Aversive learning assay*

We did not detect a difference in the likelihood that wild (n = 13) versus captive trios (n = 16) achieved the associative learning criterion over time (Cox PH model, HR=0.94, p = 0.88, Fig. 2A). Captive fish trios required an average of 19 trials to reach this learning criterion while wild trios required an average of 15 trials. However, in the more challenging reversal learning assay, wild fish trios (n = 13) were 3.4 times more likely to achieve the learning criterion compared to captive trios (n = 13; Cox PH model, HR=3.4, p = 0.025, Fig. 2B). Captive fish trios required an average of 26 trials to complete this task. Wild fish trios only required an average of 15 trials to complete this harder task, and interestingly the same number of trials that was required for them to learn the first and supposedly easier associative task.

### 3.1.1. *Escape latency and following duration*

Overall, we did not detect a difference in the average latency taken for captive and wild fish trios to escape through the correct exit. During associative learning, the third and last fish to escape through the correct exit had an average latency of 72.2 s in wild trios (n = 10) while this latency was similar at 75.2 s in captive trios (n = 15; LM, $F_{(1,23)}$= 0.102, p = 0.75). During the more challenging reversal learning task, the third fish to escape had an average latency of 72.2 s in wild trios (n = 11; a similar latency observed during associative learning), while in captive trios (n = 11) the third fish took longer to escape with an average latency of 81.3 s. But again the difference in latency was not statistically significant (LM, $F_{(1,20)}$= 0.105, p = 0.75). Similar results were found when we analyzed the first fish's escape latency. As a measure of group cohesion, we analyzed the difference between the latency of the first fish to escape and the third fish to escape. Captive trios (n = 11) had an average following duration of 128.8 s and wild trios (n = 10) had an average following duration of 137.5 s, but this difference was not statistically significant (LM, $F_{(1,19)}$= 0.2, p = 0.66).

### 3.1.2. *Trade-offs between associative learning and reversal learning*

We found a negative correlation between the number of trials that trios required to complete the associative learning task and reversal learning task (Spearman correlation, $r_s$ (20)= -0.56, p = 0.007, Fig. 3). This correlation was driven primarily by wild trios (Spearman correlation, $r_s$(9)= -0.71, p = 0.014) since we did not detect a significant correlation between the number of trials required to complete the associative learning task and reversal learning task when considering the captive trios alone (Spearman correlation, $r_s$(9)= -0.44, p = 0.17).

### 3.2. *Brain mass differences between wild and captive fish*

We did not detect a difference between the relative brain mass of captive fish (n = 46) and wild fish (n = 39; Mann-Whitney *U* test, Z = 1.16, p = 0.25, Fig. 4).

### 3.3. *Anxiety differences between wild and captive fish*

Overall, we did not detect a difference between captive fish and wild fish in anxiety. Captive fish (n = 17) spent a mean of 225 s on the black square while wild fish (n = 18) spent a mean of 187 s on the black square, but this difference was not significant (LM: $F_{(1,33)}$= 0.020, p = 0.89). Captive fish entered the black square a median of 7 times and wild fish entered a median of 16 times, but this difference did not reach significance (Mann-Whitney *U* test, Z = -1.8, p = 0.08).

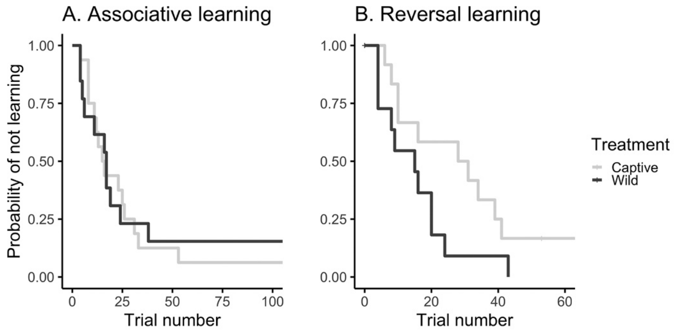

**Fig. 2.** The number of trials until trios achieved the associative learning criterion (A) and reversal learning criterion (B), plotted as Kaplan-Meier survival probabilities. Grey lines indicate captive trios and black lines represent wild trios; see Appendix A and B for exclusion criteria.

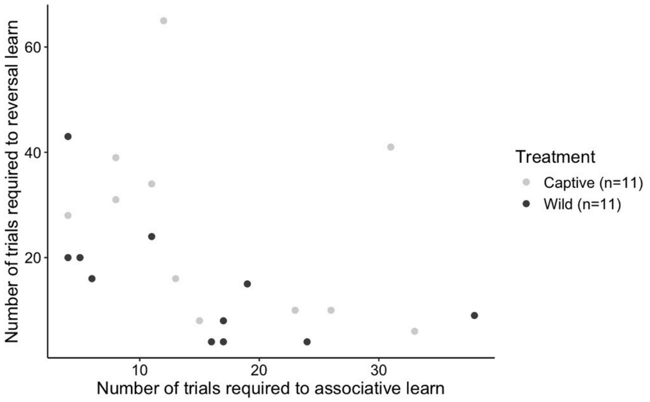

**Fig. 3.** The number of trials required for each fish trio to complete the associative learning task is plotted against the number of trials required for that trio to complete the reversal learning task. Each point represents one trio. Captive fish trios (n = 11) are represented by grey points, and wild fish trios (n = 11) are represented by black points.

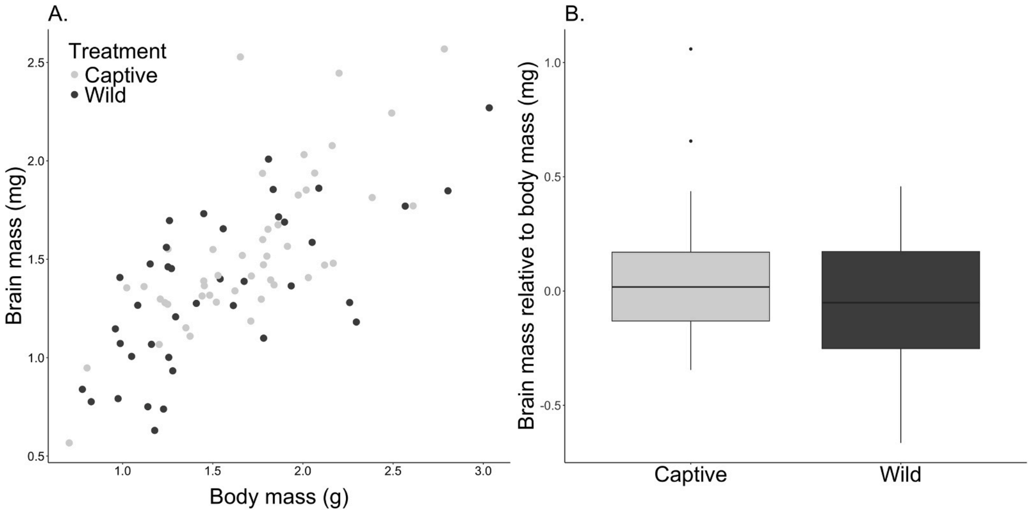

**Fig. 4.** The brain-body mass relationship in captive and wild fish. (A) Each point represents an individual fish's brain mass plotted against that individual's body mass. Light grey points represent captive individuals, while dark grey points represent wild individuals. (B) Median brain mass relative to body mass for captive and wild fish. Positive values indicate brains larger than expected given body mass, and negative values indicate brains smaller than expected given body mass.

## 4. Discussion

In this study, we compared the cognitive abilities of captive and wild fathead minnow by measuring their aversive learning performance, and we evaluated if anxiety and brain size could contribute to these abilities. We found no evidence that captive fish and wild fish differed in associative learning, anxiety, or brain mass. However, wild fish were more than three times more likely to solve the reversal learning task compared to the captive fish, indicating that captive fish may have some cognitive deficits.

Why would wild fish outperform captive fish in reversal learning but not associative learning? Reversal learning, "forgetting" one solution and learning a new one, is considered a more complex and challenging task than associative learning, requiring more cognitive functions such as inhibition and cognitive flexibility to allow individuals to adjust behaviour to shifting conditions (Buechel et al., 2018). The cognitive flexibility involved in reversal learning is likely important to wild fathead minnows, as they are a generalist species capable of adapting to various environmental conditions and exist across a wide geographic range spanning North America (Ankley and Villeneuve, 2006). Environmental conditions often vary in the wild (e.g., across a species' range and between seasons), and if cognitive flexibility increases an individual's ability to adapt to these varied conditions, then reversal learning would be expected to be selected for in wild populations. In contrast, captive environments tend to have low environmental variability (Bhat et al., 2015; Hirakawa and Salinas, 2020), so cognitive flexibility may not be needed or confer fewer fitness benefits in these homogeneous and relatively benign captive environments. Our captive population was an active breeding commercial population for 30 years (Lisa Taylor, personal communication, 2023). Therefore, we suggest that the reversal learning deficit observed in captive fish may be related to their life in captivity over many generations, as wild populations experience much higher levels of environmental variability, and so the cognitive flexibility involved in reversal learning likely is adaptive. Alternatively, the benign and unenriched conditions typical of captive environments could affect cognition via plasticity without selection, but we were not able to distinguish between these two mechanisms of phenotypic change in our study. Initially, we predicted that both associative and reversal learning would be impacted negatively by captivity. However, the associative task may not have been difficult enough to reveal any deficits in associative learning. Increasing the complexity of the associative task could help clarify this point.

We found a significant negative relationship between the number of trials required to complete the associative learning task and the number of trials required to complete the reversal learning task, suggesting that there could be a trade-off between these two types of learning. Similar results have been observed in Florida scrubjays (*Aphelocoma coerulescens:* Bebus et al., 2016), common Myna (*Sturnus tristis*; Griffin et al., 2013), zebrafish (*Danio rerio:* De Russi et al., 2024), and guppies (*Poecilia reticulata*: De Russi et al., 2025). In contrast, a positive correlation between associative learning and reversal learning has been reported in the buff-tailed bee (*Bombus terrestris,* Raine and Chittka, 2012). In fathead minnow, our observation that captive and wild fish differed in reversal learning performance but not associative learning performance further supports the idea that these two types of learning represent distinct cognitive mechanisms that may be traded off for each other. Alternatively, variable side preferences among the fish trios could potentially explain the negative correlation between performance in the associative learning task and performance in the reversal learning task, although we had no evidence of a side bias and counter balanced which sides were used as the "correct" exit in the learning assays. Future studies could further investigate which type of learning is supported by different rearing or natural environments, with particular attention to why reversal learning may be favoured in wild populations compared to captive populations.

We expected that poorer reversal learning performance in captive fish would be associated with a reduced relative brain mass, since cognitive ability is often positively correlated with brain size (Buechel et al., 2018; Kotrschal et al., 2013; Kotrschal and Kotrschal, 2020), and previous studies have found reduced brain size in captive animals (Burns et al., 2009; Guay and Iwaniuk, 2008; Marchetti and Nevitt, 2003; Kihslinger et al., 2006). However, we did not detect any differences in the relative brain mass of captive and wild fish. The contrasting results between our study and previous studies might be explained by differences in methodology (e.g., we measured the mass of the entire brain while other researchers focused on specific brain regions). Furthermore, some studies have observed a reduction in the brain mass of wild-caught adult fish within a few weeks (Park et al., 2012; Turschwell and White, 2016), however, to allow our wild fish to calm down and participate in the learning trials we needed to acclimate them for seven weeks. It is therefore possible that any original differences in brain mass between our wild and captive fish were extinguished by the laboratory acclimation period. Alternatively, our observations might indicate that relative brain mass is conserved between wild and captive populations, and even after many generations in captivity there appear to be strong constraints on changes in brain size. Selection pressures in captivity may not always be strong enough to decrease brain size, or it may be that our particular captive environments exerted enough selection pressure to favor the maintenance of brain size. Our finding that captivity appears to affect cognition without affecting brain size is in line with previous work showing that cognition can be plastic and respond to the environment in which individuals are raised without necessarily involving effects at the level of brain size (Lucon-Xiccato et al., 2023; De Russi et al., 2024; Brunet et al., 2023; Strand et al., 2010; Salvanes et al., 2013; Gatto et al., 2024).

In addition to brain size, we compared the anxiety levels of captive and wild fish since previous studies have shown that anxiety can negatively impact learning performance (Mandler and Sarason, 1952; Darcet et al., 2014) but we did not detect a difference. This suggests that differences in anxiety were not responsible for observed differences in learning performance. We were unable to analyze correlations between anxiety and learning performance since we measured anxiety at the individual level but learning performance at the group level. Future work that directly links the two (anxiety and learning) would be worthwhile. Future studies could also attempt to distinguish between social learning and individual learning, as we were only able to assess learning at the group level in our learning assays.

Although we found differences in aversive learning performance between captive and wild fathead minnow, we only compared fish from two populations (one captive and one from the wild). In learning studies, training time is usually very long and testing multiple populations is rarely logistically practical. As we had only 12 aversive learning tanks that needed to be re-used and our learning assay was extremely time consuming, we did not prioritize the comparison of multiple wild populations with multiple captive populations. However, future studies could expand on the generalizability of our findings by incorporating comparisons between multiple captive and wild populations, and better ensure that our results represent a general difference between wild and captive animals. Future studies could also attempt to determine whether phenotypic differences between wild and captive animals are primarily driven by genetic changes or by plasticity, as we were unable to distinguish between these two mechanisms of phenotypic change in our study.

The slower reversal learning we observed in captive fish could have several implications for animal welfare and the validity of animal-based research. Fish with poor learning abilities (e.g., those raised in captivity) may be less able to escape predators, forage, or choose quality mates, all of which are important to fitness in the wild (Dukas, 2004). Thus, our finding that captive fish had dampened or slower learning may partly explain why many captive animals show reduced survival and reproduction once released to the wild for conservation reasons or even fish stocking programs (Griffith et al., 1989; Jule et al., 2008; Beck et al., 1994). Although fathead minnow are not imperiled or currently reintroduced, they could be used as a surrogate species to test whether the result we observed is generalizable and could apply to other captive breeding programs used for fisheries stocking or conservation reintroduction programs.

Second, the slower reversal learning we observed in captive fish demonstrates that there may be differences between captive and wild populations and therefore research conducted on captive populations may not be applicable to wild populations. Fathead minnow are an ecotoxicological model species and are often used to guide legislation about acceptable levels of pollutants (Ankley and Villeneuve, 2006). While the use of sublethal endpoints (e.g., altered behaviour or cognition) is slowly becoming more accepted in ecotoxicology (Leonard et al., 2014, Saaristo et al., 2018, Ågerstrand et al., 2020), the common use of captive, lab-bred animals for these exposure experiments could fail to accurately predict effects of pollutants on wild populations since we demonstrated that this model organism differs in their cognitive abilities depending on whether or not they come from captivity. Clearly determining if similar concentrations of pollutants cause mortality in both captive and non-captive populations is essential, but our study shows that baseline functions like cognition can differ between captive and non-captive populations. Without considering captive-wild differences, scientists and regulators could erroneously develop environmental protection policies that are less meaningful to wild populations.

Finally, fishes are increasingly being used as model organisms in the studies of development and senescence, processes which in all organisms involve many cognitive changes (Burns et al., 2009; Adams and Kafaligonul, 2018). Many of these studies make use of captively bred fish, such as zebrafish (*Danio rerio)*. Researchers should be aware of any existing cognitive deficits in captive fish before using such animals as models, as captive fish may not be representative of normal cognitive function. When unaccounted for in research studies, cognitive deficits of captive fish may limit our ability to understand basic mechanisms of cognition (Burns et al., 2009), how these mechanisms impact survival, and how they are likely to be impacted by anthropogenic factors such as chemical pollutants.

To our knowledge, this was the first study to compare aversive learning in wild versus captive fathead minnow, a widespread North American freshwater fish species and a common model in ecotoxicology. Captive fish appeared to require more trials to achieve a reversal learning task, suggesting that captivity may lack certain selection regimes or appropriate developmental inputs essential for normal cognitive function (e.g., cognitive flexibility). We believe that our findings will strengthen our understanding of how captivity impacts learning, a sublethal endpoint with high relevance to fitness in the wild. Our findings also contribute to the growing realization that captive conditions can change individuals in important ways; if we are wanting to extrapolate from lab studies on captive organisms to the real world, future studies will need to take better account of the different possible responses resulting from captivity.

## CRediT authorship contribution statement

**Megan Dorothy Cyr:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Jacqueline Bikker:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Adrienne Yau:** Writing – review & editing, Methodology, Investigation. **Sigal Balshine:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2025.105322.

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