Experienced social partners hinder learning performance in naïve clonal fish

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Abstract

Social learning can facilitate information spread within groups and is generally assumed to increase learning efficiency in animals. Here, we asked how individual learning is affected by skill level of a demonstrator present during learning. We predicted that both task-naïve and task-experienced individuals benefit from a task-experienced, conspecific demonstrator. We used the all-female clonal Amazon molly (Poecilia formosa) for our experiments. We further propose a model framework which lets us directly relate each research question to a single model parameter. First, following a classical conditioning paradigm over five days, half the fish were trained to find food inside a cylinder, while the others were provided with food randomly dispersed in their tank. As a result trained individuals visited the cylinder with a higher likelihood than randomly fed individuals and showed consistent individual differences in their learning performance. In a second step, we allowed these fish to observe a conspecific while we continued (for those trained) or started (for naives) individual training. We found that trained individuals did not benefit from a partner, regardless of the partners’ proficiency, but showed higher average performances compared to naives. Naïve individuals showed a decrease in learning performance when paired with experienced partners but not when paired with other naïve ones.
Our results suggest that Amazon mollies learn a foraging task individually and differ consistently in their individual learning ability. Further, social learning depends on own, as well as the demonstrator’s skill level in a way that observing informed conspecifics may hamper own learning.

**Keywords** Behaviour, Learning, Clonal, Individuality, Social Information

**Highlights**

- Naturally clonal fish learn operant conditioning tasks
- Individuals show consistent differences in learning abilities
- Experienced social partners are not helpful during learning

**Introduction**

In 1514 Machiavelli already stated that "Men nearly always follow the tracks made by others and proceed in their affairs by imitation". This is not unique to humans alone, as many gregarious animal species often acquire information about their environment from their social partners [Dall et al., 2005; Giraldeau and Caraco, 2018] and is commonly referred to as observational or social learning [Bikhchandani et al., 1998; Brown and Laland, 2003; Webster and Laland, 2008]. It contrasts private learning, where information is gained by exploring solutions alone and in absence of others [Laland et al., 2011; Kao et al., 2014]. In general, social learning involves the observation of others and the copying of the observed actions [Galef and Laland, 2005]. For example, task-naive Amazon Parrots (*Amazona amazonica*) have been shown to copy the behaviour of other, more experienced individuals in order to access an obstructed food source [Picard et al., 2017; Reader et al., 2003] demonstrated that wild guppies (*Poecilia reticulata*) could copy the food patch preference and predator avoidance behaviour from other conspecifics. However, how such social learning processes are affected by the initial skill levels of both, observer and demonstrator is only poorly understood.

For one, observed demonstrators may differ in performance skills and thus in the quality of the information they can provide. Variation in information quality can in turn lead to error propagation and accumulation, giving rise to a potential trade-off between individual and social information use [Giraldeau et al., 2002; Kendal et al., 2005]. Nevertheless, there is evidence that demonstrators’ skill levels *per se* do not determine the extent to which they are copied by less experienced observers. For example, in the guppy, familiarity
among observer and demonstrator seems to be much more important than demonstrator skills when it comes
to being copying [Kendal et al., 2005]. Similarly, Roy and Bhat [2017] found that utilizing social information
led to food income equality in zebrafish (Danio rerio), where observers relied on visual behavioural cues
of successful demonstrators to find food themselves. While these studies allowed for full contact among
individuals and targeted leader-follower interactions, it still remains unclear how an observer’s performance in
learning a complex task by pure visual interaction with a demonstrator is, in turn, affected by the performance
skills of this demonstrator. Nevertheless, some pioneering work has been done decades ago, on which the here
presented work heavily builds on, investigating the relationships and potential costs and mismatches between

Although numerous studies have highlighted the benefits of social learning to the observing or eavesdropping
individuals as it allows an individual to circumvent exploring all possible solutions on its own, and thus saves
time and energy, e.g., opportunity costs are reduced [Swaney et al., 2001, Pike et al., 2010, Webster and
Laland [2012], Brown and Laland [2001, 2003], Reader et al., 2003, Harpaz and Schneidman [2020], these
benefits might not be shared mutually with the observed and copied demonstrators [Toyokawa et al., 2019,
Zonca et al. [2021]. While the mere presence of more individuals is beneficial during predator encounters
[Krause and Ruxton [2002], experienced demonstrators may lose task solving performance when interacting
with inexperienced naive individuals, either due to distraction [Roy and Bhat, 2017] or changed time budgets
as more time is allocated to social interactions than to the task at hand [Gartland et al., 2021]. But also direct
negative effects of the copying behaviour are known. For example, in many fish species males copy the mate
choice decisions of other males by observing these copulating with females which may help the observer
determine high quality females. However, this behaviour will likely increase the risk for sperm competition
and thus is costly for the copied male that initially mated with the female [Plath and Bierbach [2011]. As a
counter strategy, males may change their mate choices to mislead others and conceal their real preferences,
which is referred to as audience effects [Plath et al., 2008, Zuberbühler [2008], a form of social deception
[Wiley [1994]. In the context of complex task learning by observation alone, the question remains of how a
demonstrator’s performance is affected by being copied and whether the observer’s skill levels play a role in
this.

In addition to situations where there is an information discrepancy among observers and demonstrators,
individuals may also face a social counterpart with the same prior experience as themselves. Here, one can
assume that individuals may spend more time exploring individually, thus leading to increased learning and
final performance. This assumption is based on the idea that no additional task-specific, social information can
be gained from observing such a partner, as the information would be highly correlated to the own experience
and therefore deemed redundant [Strandburg-Peshkin et al., 2013, Kao et al., 2014].

In the current study we aimed at testing how variation in skill levels between observers and demonstrators affected their learning performances (for naive observers), as well as overall task performances (for experienced demonstrators). We used the Amazon molly (Poecilia formosa), a naturally occurring clonal fish species that reproduces gynogenetically and gives birth to live offspring that are genetically identical to their sisters and mothers [Schartl et al., 1995, Lampert and Schartl, 2008, Stoeck et al., 2010]. Through its clonal genetic background as well as its gregarious life-style, this species has been proposed to represent a useful model organism for the study of individual behavioural differences and the influence of behavioural traits on the social functioning of groups [Doran et al., 2019, Laskowski et al., 2019, 2021, Makowicz et al., 2022]. However, to date no research has been conducted on the learning abilities of these fish. Due to this intricate natural history all individuals in this study were of same genetic composition and near identical rearing background. In a first step (private information acquisition), an operand conditioning procedure (5 days, 3 times training per day) was used to produce two differently experienced cohorts of otherwise genetically identical individuals: One cohort was trained to find food in a opaque cylinder (the task, see Figure 1) and therefore given the opportunity to learn to solve the task (task-experienced/trained individuals). The second cohort was trained to find food distributed randomly, with no ability to learn an association between food and cylinder location (task-inexperienced/naive individuals). In a second step (social information acquisition), we paired two individuals to have visual access to each other, enabling them to observe each other while we continued (for trained individuals) or started (for those naive) the conditional training (5 days, 3 times training per day). Our full factorial design allowed us to create pairs of fish with all possible experience combinations: naive-naive, naive-trained and trained-trained. With this design, we tested first whether Amazon mollies are able to learn the task and whether there were consistent individual differences in both the learning rate and overall task performance at the end of the private information acquisition phase. We then explored how the skill level of the partner affected learning and overall performance when social information becomes available. The prediction was that naive fish paired with a trained partner will have a higher probability to reach a novel food source compared to individuals that were paired with another task-naive partner. For experienced Amazon mollies, the prediction was that the task performance would be worse when paired with naive individuals, compared to those interacting with a similarly proficient individual. The reasoning behind this assumption being, that individuals paired with a similarly skilled partner which provides redundant information may allocate more time and efforts towards acquiring private information - this can outweigh the potential opportunity costs that arise through the social interactions and which should be apparent when paired with both naive and experienced partners.
Materials and Methods

Study organism and maintenance

For our experiments we used the Amazon molly (*P. formosa*), a naturally occurring clonal freshwater fish. This is an all-female species that originated from a rare hybridisation event between a male Sailfin molly (*P. latipinna*, ♂) and a female Atlantic molly (*P. mexicana*, ♀) about 100,000 years ago [Hubbs and Hubbs, 1932; Schartl et al., 1995; Schultz, 1973; Lampert and Schartl, 2008; Stoeck et al., 2010; Warren et al., 2018]. This species reproduces through gynogenesis which means that females require sperm from males of closely related Poeciliid species to induce embryogenesis [Evans et al., 2011]. However, no paternal genetic material is incorporated into the embryo, thus Amazon mollies produce broods of offspring that are genetically identical to each other and their mothers [Schartl, 1995]. The herein used clonal lineage has been reared for many generation in captivity and regular molecular checks confirm that individuals are clones. Fish were bred with Atlantic molly males as sperm donors at the animal care facilities of XXXXXX Fish were reared in 200-L tanks filled with aged tap water at a temperature of 26 °C and fed twice daily *ad libitum* with commercially available flake food as well as defrosted blood worms (*Chironomidae* sp.). All animal experiments were conducted under the animal experiment number #0089/21 of the XXXXXX.

Experimental design

For our experiment, we first generated two different treatment groups, one that was fed three times per day for one week only inside an opaque cylinder (‘trained cohort’, Figure 1), while the other one was fed with food dispersed randomly in the experimental tank (‘naive cohort’). In a second step, we visually paired fish with individuals from the same or a differing training regime and either continued (for those already trained) or started to feed only in the cylinder (for those habituated, but naive).

To start the experiment, we placed pairs of size-matched, unfamiliar fish (N=36, 23±2 mm) in each of six identical test aquariums (300 × 600 × 200 mm). Fish were taken from multiple husbandry tanks ensuring that familiarity was not given, and size-matched in order to reduce dominance effects and most importantly to account for any age differences. All individuals were randomly distributed across all experimental tanks.

An opaque divider separated each tank into two same-sized compartments, each containing one fish. This divider could be exchanged with a transparent one during experimentation to allow visual interactions (see Figure 2). Each two-compartment tank was externally filtered (EHEIM Professional 3 250) throughout the entire trial in order to maintain water quality and to provide olfactory cues to the fish. Water quality was...
checked weekly (SERA pH, NH$_3$/NH$_4$, NO$_2$, NO$_3$) and 50 percent of the water was exchanged at the same interval. The temperature was maintained within the range of 23-26 °C and adjusted through the ambient room temperature. Water levels were maintained at 70 mm, resulting in a total of 18.7 l per tank and 3.5 l per individual compartment. In order to enhance the learning outcome, the fish were kept on a continuous light cycle, which has been shown to have no effect on the stress level of a closely related species, while improving the learning abilities [Kurvers et al., 2018]. All fish were fed with frozen blood worms, which were thawed approximately 30 min. before each experiment.

**Figure 1.** A Schematic of the general recording setup. Each inlet and outlet was attached to an individual circulating filter system. B Concealed food source used in the conditioning trials. Food was presented within an opaque cylinder, that could only be accessed through a horizontal opening. Entry into the cylinder was monitored through the top opening, vertically facing the camera. The cylinders were glued to ceramic plates to ensure stability. This further ensured that food particles and olfactory cues were contained within the cylinder.

**Food conditioning experiments**

**Private Information Acquisition - Week 1**

For the individual conditioning, we randomly selected future demonstrators and observers within each of six simultaneously trained pairs. Demonstrators were then trained on six occasions per day, for at least five consecutive days without visual access to the conspecific partner. Each training instance, consisting of eight minutes, was recorded using consumer-grade webcams (c920 HD Pro Logitec, USB 3.0, 432 × 240 px, gray scale, 30 fps) mounted above each individual tank. It was ensured that the camera was centered precisely above the tank in order to keep occlusions and perspective distortion minimal and evenly distributed among both individuals being recorded. During a training instance the individual was either presented with an opaque, vertical PVC cylinder (height: 100 mm, Ø: 50 mm, see Figure[1]), containing food as stimulus or with a mock...
stimulus (50:50 - mock:real). This resulted in three mock treatments and 3 actual training instances per day, for every individual. To standardize the starting distance of the fish to the food source, individuals were limited to one side of the compartment at the beginning of each instance. This was done using a small separator (see Figure 2). For mock treatments the fish underwent all steps, as if it was an actual training instance, being constrained to one side of the compartment and having this separator subsequently removed, but without the following stimulus presentation. The choice for true conditional or mock stimulus was randomized over the course of the day, while ensuring that each accounted for 50% of the total daily tests (3 true, 3 mock). Mock treatments were introduced to reduce any association with other neutral stimuli of the procedure and to ensure that the focus was drawn to the actual task being learned [Courville et al., 2006, Robinson et al., 2014, Anselme and Güntürkün, 2019]. For the trained cohort of fish, the cylinder was stocked with blood worms (N ≈ 8) which were visually occluded from the fish and only accessible through a round opening in the side of the vertically oriented cylinder (see Figure 1). Fish of the naive cohort were treated with identical conditions as their trained counterparts, with the only difference being the location at which food was presented. Here, the same amount of food was distributed randomly within the tank and accessible for the duration of the test instance. At the end of each test instance the cylinder as well as any remaining food particles were removed from the tank using a pipette.

Social Information Acquisition - Week 2

In the second week of the experiment, individuals were regrouped with a new size-matched partner and randomly redistributed across the six experimental tanks. This was done to ensure that each individual was relocated to a new test tank. Regarding the individual’s own and the partner’s initial training, the following social treatments were created: trained paired with trained individuals (TT), naive paired with trained (NT) or trained with naive (TN) as well as naive paired with naive (NN). The previously opaque division, separating the two individuals was replaced by a clear one, enabling full visual access between both individuals (see Figure 2). This clear division was left in place for the entire duration of the social trial, which lasted for five consecutive days. During this period all individuals were being trained and tested according to the individual conditioning procedure previously described, receiving food only within the cylinder (see Figure 1).

Video Analysis

In order to quantify the learning outcome, fish were tracked using a custom developed tracking function (see Appendix - Python Code: track2h5) implemented in Python and using the computer vision library OpenCV.
The fish were detected by using frame-wise motion tracking, based on simple background averaging and subsequent background subtraction. Detected objects were further filtered based on size, speed and using an isolation forest algorithm to limit detections to actual fish and reduce noise due to reflections and moving particles to an absolute minimum. Individual positions were given as two-dimensional Cartesian coordinates, calculated as the center of mass of each filtered detection contour. Since background subtraction can result in missing observations due to little movement of the animal, all coordinates were interpolated linearly over time to account for this. The first 30 s of each test instance were considered the acclimation phase, in which the animals were allowed to settle after having the separator removed. This period was exempted from further analysis. To further standardize recordings, all recordings were restricted to a maximum duration of 433 s, leading to a total duration from start to end of 403 s. Given that each individual was restricted to its specific compartment, identities were maintained based on spatial discrimination. Presence and position of the stimulus cylinder were automatically determined by using an implementation of the Hough transformation, returning the coordinates of the center of mass and the radius of the detected cylinder. This enabled the exact measurement of the Euclidean distance of each individual to the cylinder center at each given time point. In addition to the automated process, all videos were manually checked for validity of cylinder detection and tracking results.
Statistical analysis

All statistical analysis was run in R (R version 3.6.3 ‘Holding the Windsock’) and statistical inference based on generalized mixed effects models (more specifically logit models) which were composed using the function `glmer` in library `lme4`. After tailoring models to the experiment and research questions, further model selection was done based on Akaike’s information criterion (AIC) or conditional AIC, where applicable, using the library `cAIC4`. Validation and estimation of accuracy was done using the `check_model` function in the `performance` library. Test statistics and calculations were done using `tab_model` in the library `sjPlot`. For testing variance components, we use the boundary correction described by Stram and Lee [1994] for linear mixed effect models. Significance is reported on a 95%-level and all confidence intervals (CIs) provided are given as 95% CIs.

Individuals $i = 1, \ldots, 36$, equipped with universal unique identifiers (UUIDs), are defined to have reached the region of interest (i.e. solved the task) in test instance $j = 1, \ldots, 15$ (response $y_{ij} = 1$) if their distance to the cylinder center was smaller than 2.5 cm over a duration of 1 s or more, and to fail otherwise ($y_{ij} = 0$). Predicting that fish should increase the likelihood to solve the task when being fed within the cylinder, we associate the learning performance of individual $i$ with its probability of reaching the region of interest and employ a statistical learning model based on logit regression reflecting each of our main hypotheses in a single model coefficient. Two slightly different model variants are used for experiments of Week 1 (Model 1) and Week 2 (Model 2). Model 1, addressing questions of private information acquisition, is given by

$$\log \text{ODDS}_{ij} = A_i + B_i t_{ij} = \alpha_0 + \alpha_1 x_{Ti} + a_i + (\beta_0 + (\beta_1 + b_i) x_{Ti}) t_{ij}$$

where probabilities $R_{ij}$ of success $y_{ij} = 1$ are modelled via odds $\text{ODDS}_{ij} = \frac{p_{ij}}{1 - p_{ij}}$ of ‘expected # solved : expected # failed’, allowing for interpretation via odds ratios (OR). The combined intercept $A_i$ determines the baseline odds of reaching the region of interest. This corresponds to the baseline likelihood of an individual reaching the region of interest, before having any prior experience on entering it (Test Instances 1-2, illustrated in Figure 3). The slope $B_i$ reflects the learning rate of individual $i$, with $\text{ODDS}_{ij}$ expected to increase with the number of visits $t_{ij}$ after initially solving the task (count variable, Time since solved $\leq 15$, illustrated in Figure 3). For the probability $p_{ij}$ of solving the task, this results in a sigmoidal learning curve in $t_{ij}$ (Figure 5). With $x_{Ti} = 1$ if individual $i$ is trained and 0 otherwise dummy-coding the training status, $B_i = \beta_0 + (\beta_1 + b_i) x_{Ti}$ is composed of a reference slope $\beta_0$ reflecting the learning behaviour of un-trained individuals and the gain in the learning rate $\beta_1$ for trained individuals as fixed effects, plus a random effect $b_i$ reflecting subject-specific deviations of trained individuals. This applies analogously for $A_i$ as well. The random effects $a_i$ and $b_i$ are
assumed normally distributed with standard deviations \( \tau_a \) and \( \tau_b \), respectively, and correlation \( \rho \). The random
slope \( b_i \) is restricted to trained individuals, which are of major interest. In this model, \( \beta_1 > 0 \) corresponds to
Hypothesis I that clonal fish are capable of learning to feed inside the provided cylinder, in that it reflects
deviation from zero in the learning rate, and \( \tau_b > 0 \) corresponds to Hypothesis II that learning behaviour is
subject specific, as it describes the variation among individual learning abilities. Including an indicator \( x_{solvedij} \)
as additional covariate into Model (1) which is 1 if the \( i \)th individual has reached the region of interest before
the \( j \)th training instance and 0 otherwise, has been considered to enable less gradual learning behaviour but
turned out unfavorable in AIC-based model selection.

Model 2, designed for comparing learning behaviour of individuals in pairs with different training history, is
given by

\[
\log\text{ODDS}_{ij} = A_i + B_i t_{ij} = \alpha_0 + \alpha_1 x_{NTi} + \alpha_2 1_{TN}(i) + \alpha_3 x_{TTi} + a_i + (\beta_0 + \beta_1 x_{NTi} + \beta_2 x_{TNi} + \beta_3 x_{TTi} + b_i) t_{ij}
\]  

where \( x_{NTi} = 1 \) if individual \( i \) is in group NT, i.e. was not trained in Week 1 but has an experienced partner,
and 0 otherwise. Analogously for TN and TT. Accordingly, \( \beta_0 \) describes the baseline learning rate in reference
group NN and \( \beta_1, \beta_2, \beta_3 \) reflect the deviation from that in the other treatment groups. In particular, \( \beta_1 \neq 0 \)
indicates differences in learning behaviour of naive fish with trained partners (Hypothesis III). Random effects
\( a_i \) and \( b_i \) are specified analogously to Model (1) to account for subject-specific variations.

### Results

**I. Amazon mollies are able to quickly learn foraging task**

Our first question was whether clonal fish were capable of learning to feed inside the provided cylinder. We
verify this based on Model (1) which captured the variance within the data well, while random effects accounted
for a large proportion of the variance (marginal \( R^2 \): 0.083, conditional \( R^2 \): 0.839, following Nakagawa et al. 2017). At baseline, we obtain odds of about 1 : 9 (probability \( P_j = 0.10 \)) for an untrained fish to reach the
region of interest within a test instance (given by intercept \( \alpha_0 = -2.18 \), CI = \([-2.80, -1.57]\), for \( b_1 = 0 \)). This

corresponds to the probability of an individual to enter the region of interest without having ever entered it
before (see Figure [3] Test Instance 0-2). For individuals being trained, and thus not being fed outside the
region of interest, we obtain a slightly higher baseline probability, with the odds increased by a factor of
Figure 3. Definition of the “time since solved” \( t_{ij} \) used as variable for individually describing the learning process. Until the food inside of the cylinder was first found by individual \( i \) at test instance \( J_i = \min \{ j : y_{ij} = 1 \} \), no training effect can occur and \( t_{ij} = 0 \) for \( j < J_i \). After that, individual training commences and training time monotonically increases as \( t_{ij} = j - J_i \).

Figure 4. Overview of space use across treatment groups in the second week of training (order from left to right: NN, NT, TN, TT). Only instances where the cylinder was present are shown. Darker coloration represents higher number of occurrences, lighter lower. Sample trajectories are shown for random individuals of each treatment group. All trajectories where centered on the cylinder, for better visualization.

\[ \exp(\alpha_1) = 1.55 \text{ (CI } [0.67, 3.56], p = 0.302), \text{ which is, however, not significantly different to those not being trained. While we even observe a slightly negative ‘learning effect’ of entering the cylinder (} \beta_0 = -0.14, \text{ CI } [-0.35, 0.065], \text{ not significant) for individuals not being trained, a significant positive learning effect is obtained for trained individuals (} \beta_1 = 1.37, \text{ CI } [0.60, 2.14], p < 0.001^{***}). \text{ The likelihood of trained individuals to reach the food source significantly increased, once they had solved the task for the first time (see}\]

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Figure 3: Test Instances > 3), with an odds ratio of \( \text{OR} = \exp(\beta_0 + \beta_1) = 3.42, \text{CI} = [1.60, 7.30] \) more than tripling the odds for the next visit (in a conditional ceteris paribus interpretation used also in the following).

Figure 5 depicts estimated mean learning curves with and without training, showing probabilities \( P_{ij} \) of solving the task in dependence on \( t_{ij} \), and illustrates how the time spent by fish in the region of interest increases with \( t_{ij} \).

**Figure 5.** Learning outcome of the two treatment groups (trained/naive) in the first week. Both graphs show results from 36 individuals: Naive: \( N = 18 \), Trained: \( N = 18 \).

**A:** Model output the first week of training in form of estimated marginal means (lines, thin: individual; bold: group mean) and raw data (points). Instances along the x-axis are in respect to the first time the goal was reached. Confidence intervals are based on the Upper Control Limit (UCL) and the Lower Control Limit (LCL) at a 95% confidence level. 

**B:** Visualization of time spend within goal area across both treatment groups (trained/naive) and over all test instances in the first week. A truncated linear fit is shown as trend line (between instance 5-11), estimated over all data points and for each treatment group. A slight jitter was applied along x in order to reduce overlap.
II. Clonal Amazon mollies individually differ in learning ability

We approached the question, whether individual variability was observable among the learning abilities, and more specifically the probability to reach the goal area, using the same model as in I (Equation (1)) by investigating the the random effect $b_i$ on the learning rate of trained individuals. A standard deviation of $\tau_0 = 0.74$ is estimated for $b_i$ which bespeaks considerable variation across individuals accounting for about $\tau_0/ (\beta_0 + \beta_1) = 60\%$ of their mean learning rate, and testing for $\tau_0 > 0$ confirms significant inter-individual differences in the learning behaviour ($p < 0.001^{***}$). Aside of differences in the learning rate, the standard deviation $\tau_a = 0.43$ of the random intercepts $a_i$ could be interpreted to reflect differences in the exploration behaviour of individual fish. It is, however, not significantly $> 0$ ($p = 0.386$). Inter-individual differences are also supported in terms of model selection, preferring Model 1 with random effects (marginal AIC = 314, conditional cAIC = 259) over an analogous model without random effects (AIC = 342).

III. Evident social effects of informed partner can hinder own learning

The pairwise interactions in the second week, allowed to assess whether task performance was worse in observers paired with naive demonstrators, compared to those interacting with task-proficient ones. For this purpose we refer to results of Model 2 which are also illustrated in Fig. 5A. Overall the model (see Model 2) to determine these effects captured the variance within the data well (marginal $R^2$: 0.716, conditional $R^2$: 0.903). In Week 2, naive individuals showed similar baseline probabilities for initially entering the region of interest when paired with naive partners as they did in Week 1 (reference group NN: odds $exp(\alpha_0) = 0.07$, CI = [0.02, 0.25]). The baseline probabilities are substantially increased for experienced individuals (TN vs. NN: OR = $exp(\alpha_1) = 24.74$, CI = [2.82, 216.76], $p = 0.004^{**}$) in accordance with the training effect affirmed above. However, there was no evidence for a positive effect of the partner’s experience on own probability of initially entering the cylinder. By contrast, our data indicates a negative effect of having an experienced partner on both naive and trained individuals (NT vs. NN: $OR = exp(\alpha_1) = 0.39$, CI = [0.04, 4.04], $p = 0.432$; TT vs. TN: $OR = exp(\alpha_3 - \alpha_2) = 0.92$, CI = [0.10, 7.79], $p = 0.938$) which is smaller for the trained: the odds to initially reach the goal area were decreased by $~61\%$ in naive individuals, when paired with an informed individual. For already trained individuals paired with another trained partner this effect was smaller, amounting for a $8\%$ decrease. Although these effects on the initial detection probability are subject to considerable estimation uncertainty and not significant, a significant negative effect of the partner’s experience on the learning rate (reference NN: $\beta_0 = 2.03$, CI = [1.14, 2.92]) is found for naive individuals ($\beta_1 = -1.77$, CI = $[-2.99, -0.56]$, $p = 0.004^{**}$). For NT vs. NN, the probability for reentering the region of
Figure 6. Learning outcomes of four treatment groups, depending on the focal individual and partner denoted in brackets: Naive (Naive): \( N = 12 \), Naive (Trained): \( N = 6 \), Trained (Naive): \( N = 6 \), Trained (Trained): \( N = 12 \). A: Model output the second week of training in a social context. Results are shown in form of estimated marginal means (lines, thin: individual; bold: group mean) and raw data (points). Instances along the x-axis are in respect to the first time the goal was reached. B: Visualization of time spent within goal area across all treatment groups and over all test instances in the second week. For better visibility, first solved instances are shown with large icons and higher contrast. All remaining data is shown with less contrast. A truncated linear fit is shown as trend line (between instance 5-11), estimated over all data points, for each treatment group independently. In order to reduce overlap in the plot a slight jitter was applied to the data.

interest after the first visit is, hence, significantly reduced with an odds ratio of \( OR = 0.17 \ (CI = [0.05, 0.57]) \), when paired with a experienced social partner. For experienced individuals, the negative effect of having an experienced partner is less distinctly expressed, yielding \( OR = 0.87 \ (CI = [0.20, 3.64]) \, p = 0.847 \, not
In our experimental setup, we thus consistently find performance decreased for individuals with experienced partners when comparing them to individuals with naive partners – an effect that is significant, however, only for the learning rate of naive individuals with experience partners, where it is also most pronounced.

**Discussion**

In the present study, we found that clonal Amazon mollies can be trained according to a classical operant conditioning task, that they exhibited among-inter-individual differences in their learning performance, and that the presence of a task-experienced social partner reduces own learning and task-solving performance, especially for task-naive individuals.

Clonal Amazon mollies can learn in an operant conditioning paradigm within a few days and a low number of repeated training sessions to associate food with a location in their laboratory environments. This is in line with current research on fish cognition, which shows that fish are avid learners and have sophisticated cognitive abilities [Brown et al., 2008, Kohda et al., 2019, Bshary and Triki, 2022, Bierbach et al., 2022]. Further, [Fuss and Witte, 2019] and [Fuss et al., 2021] found similar learning capabilities in both parental species of the Amazon molly, *P. latipinna* and *P. mexicana*, and also in the closely related guppy (*P. reticulata*).

It was shown that both mollies and guppies are capable of operant conditioning as well as reversal learning, thus it is not surprising that we found similar cognitive capabilities in the clonal Amazon molly. Our results suggest consistent individual variation in the learning curves during the solitary phase of the experiment. There is substantial knowledge about consistent individual differences in behavioural traits [Réale et al., 2007], including clonal animals like Amazon mollies [Schuett et al., 2011, Freund et al., 2013, Bierbach et al., 2017]. However, learning as an individual trait has only recently been shown in great detail in the fruit fly *D. melanogaster* [Smith et al., 2022]. Here, we show that this individuality in learning can also be found in a naturally-occurring clonal vertebrate. Eager learning can be seen as an adaptation, allowing individuals to respond to environmental changes and unforeseen circumstances. Why even genetically-identical individuals differ in their learning performance may have multiple reasons, including pre-birth processes like epigenetics differences, maternal effects [Kasper et al., 2017] and developmental stochasticity [Honegger and de Bivort, 2018], and may be due to post-birth processes like differences in previous experience [Kieffer and Colgan, 1992] and encountered, environment conditions [Freund et al., 2013, Akhund-Zade et al., 2019]. In the here presented study all individuals were genetically identical and reared under near identical conditions. However, we used individuals from different mothers and individual variability among our test subjects can thus be due
to a variety of these variance-inducing processes [Bierbach et al., 2017]. Further experimentation is needed in order to point out which factors are the most prominent drivers of among-individual variation in the learning performance of this clonal vertebrate species.

As shown here, the skill level and performance of a social partner indeed has a strong influence on own performance but in an unexpected way. We found that naive individuals paired with trained ones exhibited slowest learning, when compared to naive individuals paired with other naive ones. Trained individuals that were associated with naive partners did not significantly differ from trained individuals that were paired with other trained ones, although our results tend towards hindering, rather than supportive effects of observing trained partners. Therefore, it seems as though being accompanied by highly skilled conspecifics did not improve own learning performance, and that having a naive social partner was more beneficial during learning, when being naive to the task as well. So, how can such counter-intuitive effects be explained? First, the goal areas of both social partners were in mirrored locations (see Figure 2), such that the behaviour of the other would not necessarily lead to the same information, visual cues and ultimate learning outcome. Trained individuals have acquired experience and established a procedure of solving the task. This can manifest in behaviours such as accessing the goal from a certain direction, location or at a specific time, which in turn do not necessarily match those of the social partner leading to a dissonance between observed and performed behaviour. For two naive individuals performing the task together this could not have such an impact, since both individuals are acquiring the knowledge about the novel task at the same time, leading to more synchronous experience between both individuals. Following the logic that naive social partners simultaneously learning the task from initial non-proficiency show more undirected and variable behaviour, Kohn [1976] argued that a continued perception of change, as would be the case when watching another naive individual trying to perform a novel task, can maintain attention and act reinforcing to the observer. The experienced partner would merely repeat its already learnt behaviour and result in less variation and subsequent reinforcement for the naive observer. Second, our task was designed in a way that the observer did not see the demonstrator actually feed. In studies that found local or stimulus enhancement effects [Brown and Laland, 2003], observers could actually see demonstrators getting the benefit and we argue that a lack of seeing the direct benefit in our study hampered the social learning especially from experienced demonstrators that virtually disappeared when performing the task. This is underpinned by the fact that trained partners have little effect on initially reaching the cylinder and food source in their naive social partners, where we only observe a smaller, non-significant effect. However, the detrimental effect of having an experienced partner is clearly pronounced in the chance to subsequently re-visit the region of interest in the naive individuals, where we observe a strong and significant decrease in their learning rate. This indicates a more complex effect than pure spatial misguidance, due to mere
copying and also rules out positive effects such as stimulus enhancement [Spence, 1937] or local enhancement [Thorpe, 1956] due to the trained demonstrator.

**Conclusion**

In congruence with our results, weak or absent positive effects of highly skilled partners have been found in studies using full-contact designs during demonstrator-observer interaction and path learning tasks. In the guppy, naive individuals were following familiar, but less skilled partners more readily through unknown maze setups [Swaney et al., 2001]. Similarly, in zebrafish food income equality was enforced via social information, where observers relied on visual, behavioural cues of successful demonstrators to find food themselves [Roy and Bhat, 2017]. In pigeons, Biederman and Vanayan [1988] showed that naive individuals observing demonstrators performing a task at chance and gradually improving, outcompeted those observing well proficient demonstrators in speed of learning and overall task accuracy. Further, although near identical and clonal, our tested individuals show consistent differences in their learning behaviour which is in line with previous studies proposing consitent among-individual differences being common also in clonal animals [Schuett et al., 2011, Bierbach et al., 2017, Freund et al., 2013]. In sum, this study builds upon the well established field of operant learning and conditioning, utilizing a naturally clonal fish species as model organism, in which learning has not yet be studied. The here presented work adds a sleek and interpretable approach to analysing both the learning efficiency, as well as the inter-individual differences in the learning performance. This is done by carefully constructing a statistical model, along side the experimental design, in which all components represent key aspects of interest, and biologically relevant terms such as learning rate and overall exploration. The here highlighted insight, that prior knowledge, or information contained within one’s social partners has an effect on the own performance in certain contexts has broad implications for collective behaviour and group performance. It has already been shown that information differences can explain dynamics within animal collectives [Ioannou et al., 2011, MacGregor et al., 2020]. Information quality [Kao et al., 2014], such as uncertainty and redundancy, as well as the processes by which novel information is generated or affected by the social environment most likely play a key role in the learning behaviour of gregarious individuals [Hofmann et al., 2013, Rodriguez-Santiago et al., 2020]. In light of learning - a process of information uptake and integration over time - the here presented results give a concise approach to shed light on the timing of such events. The process of learning and timing of informational cues gives rise to a multitude of interesting questions, such as how information is being distributed in a multi agent system, or fish school, in order to...
achieve optimal exposure and learning for each of its individual members.

In more biological terms, what drives an individual to take on a certain role in the group, move to a specific location or perform a given behaviour is still very much an open question, which yearns to be answered. As shown here, the experience and prior knowledge of social partners has an effect on the learning performance of individuals. Therefore, the social environment during certain experiences likely effects the ability of individuals to learn and adapt to novel situations. These insights, as well as the unique modelling approach shown here to address such learning processes and their timing, should pave the way for more experiments in this exciting direction.

Supporting Material

All supplemental files, such as code for tracking and statistical analysis, as well as the data used in this study can be found here: https://github.com/XXXXX

1. [dataset] Author: F.Francisco, 2022, Learning Data, Data_AmazonMollyLearning.csv
2. Statistical Code, Rstats_AmazonMollyLearning.Rmd, 2022, Authors: F.Francisco, J. Lukas, A. Stöcker
3. Tracking Code, TrackingCode_AmazonMollyLearning.py, 2022, Author: F.Francisco

Author Contributions

FF, DB and PR derived the research question and experimental design. FF conducted the experiments. FF, JL and AS conducted the statistical analysis and designed the analytical structure. FF wrote the initial draft of the manuscript with input from all coauthors. All authors acknowledge no conflict of interests and have proofread the final version of the manuscript and agreed on submission.

Ethical Note

Animal experiments were conducted under the animal experiment number #0089/21 of the German State Office for Health and Social Affairs (LAGeSo). A total of N=36 fish were used over the course of this experiment. All animals used for this research were kept under best possible holding conditions, in order to assure healthy, natural behaviour. After the experiment, animals were released to designated holding tanks. All animal facilities and maintenance protocols were kept in accordance to the LAGeSo.
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