Minimizing predation risk, especially for young or naïve individuals, can be achieved by learning to recognize predators. Embryonic learning may optimize survival by allowing for the earliest possible response to predation threats posthatch. However, predatory threats often change over an individual’s lifetime, and using old information can be detrimental if it becomes outdated. Adaptive forgetting allows an individual to discount obsolete information in decision-making and instead emphasize newer, more relevant information when responding to predation threats. Little is known about the extent to which young individuals can learn and forget information about predation threats. Here we demonstrate that rainbow trout 1) are capable of learning from both conspecific and heterospecific alarm cues as embryos, newly hatched larvae, and free-swimming larvae, 2) exhibit adaptive forgetting of predator information at all stages, and 3) display dynamic adaptive forgetting based on the ontogeny of learning. Specifically, fish that learned information as embryos retained the information for longer periods than those that learned the same information as newly hatched alevins.

**Key words:** embryonic learning, ontogeny, predator recognition, memory, sensitive periods.

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**INTRODUCTION**

Animals must constantly weigh the risk of predation against the rewards of behaviors such as foraging or mating (Lima and Dill 1990). Although increased foraging maximizes growth and future reproductive success, death negates any such benefits; therefore, living with a degree of caution is paramount (Bonskila and Blumstein 1992). Animals may mitigate predation risk through a variety of antipredator behaviors (Lima and Dill 1990), including avoiding high-risk areas, seeking refuge, decreasing movement and reducing foraging behaviors, all of which are most effective when predation risk is detected early (Pfeiffer 1977). For aquatic organisms, for instance, chemicals present in the environment may provide information regarding imminent predation risk. It is, therefore, not surprising that risk-mediated alarm cues elicit dramatic and consistent antipredator responses in many aquatic species, ranging from coral to vertebrates (Ferrari, Wisenden, and Chivers 2010).

Damage-released chemical cues, commonly referred to as alarm cues, are an effective warning system for aquatic organisms (Chivers and Smith 1998). These cues can only be released when epidermal cells are damaged (Pfeiffer 1977), making them a highly reliable indicator of risk. Unsurprisingly, these alarm cues elicit an innate antipredator response in nearby conspecifics. Although each species possesses its own alarm cues, individuals can sometimes respond to alarm cues from another species via one of two mechanisms: phylogenetic relatedness or sympathy. First, if the responders are phylogenetically closely related to the cue donor, then the alarm cue homology between the species will allow the responders to innately recognize the cues as risky; such heterospecific cues will typically elicit a weaker antipredator response than would their own alarm cues (Mirza and Chivers 2001; Mitchell et al. 2012). Second, two unrelated species may respond to one another’s alarm cues if they are sympatric and share predator threats. Such responses to heterospecific alarm cues increase the likelihood of early predator detection and may be the result of learning (Chivers et al. 2002; Pollock et al. 2003; Pollock and Chivers 2004).

Although some prey species innately recognize cues from certain predators (Goth 2001), most prey fish do not (Brown 2003). Instead, they must learn to recognize the sight or smell of such potential predators. Alarm cue learning is a widespread and efficient learning mechanism in aquatic species, known to occur in a variety of species, from flatworms to aquatic vertebrates (reviewed in Ferrari, Brown, et al. 2010). After simultaneous exposure to a novel predator cue (sight, smell, or sound) and alarm cues, fish learn to recognize novel threats (Suboski 1990), thus increasing their chances of survival (Mirza and Chivers 2000; Lönnstedt et al. 2012). This type of learning, called releaser-induced recognition learning, is a variant of classical Pavlovian conditioning (Suboski...
90). Several studies have demonstrated successful learned predator recognition after a single conditioning event (Magurran 1989; Mathis and Smith 1993; Chivers and Smith 1994).

Alarm cue learning has been shown to occur as early as the embryonic stage. For instance, wood frog (Lithobates sylvaticus, LeConte 1823) embryos exposed to a novel predator odor in combination with alarm cues responded to predator odor when subsequently tested as tadpoles, demonstrating learning in the embryonic stage (Mathis et al. 2008). Embryonic learning has also been demonstrated in convict cichlids (Amphilophus nigrofasciata, Gunther 1867; Nelson et al. 2013). Atherton and McCormick (2015) also showed that alarm cue-mediated embryonic learning in cinnamon clownfish (Amphiprion melanopus, Bleaker 1852) was accompanied by an increase in embryonic heart rates at the time of conditioning. The capacity to learn to recognize predators while in the embryonic stage may provide an advantage for new hatchlings living in high-risk environments.

In spite of abundant research focusing on the acquisition of novel information via learning, few studies have investigated the retention of such information. After a certain period of time, fish stop responding to their conditioned stimuli. This phenomenon, often referred to as adaptive forgetting (Bouton 1994; Kraemer and Golding 1997; Ferrari, Brown, et al. 2010), is essential for an animal to maintain accurate responses in an environment where cues only predict environmental conditions for short periods of time. Such “forgetting” has been used to describe situations where an individual does not demonstrate the response it has been conditioned to display. However, a lack of response does not necessarily indicate that the learned information is no longer available to, or retrievable by, the individual. Woodfrogs, for instance, can adjust their behavioral response based on information to which they have ceased to respond (Chivers and Ferrari 2013), demonstrating that even if the information is not necessarily acted on, it still exists in their memories. In this paper, we use the term “forgetting” to refer to the phenomenon whereby previously learned information is not actively used for subsequent decision-making, without making inferences as to whether or not the information could be retrieved by the individual.

Little is known about the period for which individuals retain a response to their conditioned behaviors, in part because it varies so greatly depending on ecological context. Rainbow trout (Oncorhynchus mykiss, Walbaum 1792) may cease to respond to conditioned predator odors in as little as 8 days (Brown et al. 2011) but retain homing cues for more than a year and a half (Cooper et al. 1976). Additionally, within ecological contexts, a number of factors may affect the longevity of such a memory, including the duration or intensity of learning. Ferrari, Brown, et al. (2010) propose a number of different factors, both environmental and intrinsic, which may affect how long an individual retains the information about a predator. Environmental factors, such as the presence and appetite of the predator, may reinforce learned predator information. Each encounter, dangerous or safe, adds to the available information about potential predators and increases its reliability for use in future threat assessment. For instance, with increased risk of predation, tadpoles and fish retained their learned predator recognition longer, whereas in cases where the tadpoles were less certain of the predator risk, they stopped responding to the predator cues after a shorter period of time (Ferrari, Brown, et al. 2010; Ferrari, Brown, Jackson, et al. 2010). Intrinsic factors include a shift in the risk posed by a predator due to either changes in body size (growth) or habitat shifts.

Rainbow trout maintained on a higher growth rate trajectory while they underwent conditioning retained the information for a shorter time than those growing more slowly (Brown et al. 2011). Physiological and behavioral changes that occur as the individual grows may also increase the likelihood of successful escape from predators. As a result of a decreased growth rate, adult prey may maintain a relatively constant community of predators and may, consequently, benefit from maintaining their responses to predation threats for longer than embryos or juveniles whose predators risk is changing more rapidly.

Another hypothesis is that the longevity of the memory is affected by the ontogeny of the learner. Sensitive periods in development have been observed in many species (West-Eberhard 1989; Travis 1994; Panchanathan and Frankenhuysen 2016; Arnett and Kinnison 2017). Sensitive periods refer to periods of development which are particularly susceptible, or plastic, to environmental or experiential effects. These periods of plasticity allow organisms to change or adapt more rapidly than they might at other times. Such adaptations may be physical, such as the alteration of morphological traits to reduce predator damage (Januszakiewicz and Robinson 2007), or behavioral, such as adaptive antipredator behaviors (Dill 1983; Robinson et al. 2008). The extent to which an individual can learn and retain information may, thus, be impacted by these sensitive periods. A prime example of a sensitive period of learning is observed in salmonids. During a sensitive period early in their lives, these fish imprint on their home streams so that they may travel great distances and ultimately return to their natal sites to spawn (Cooper et al. 1976; Dittman et al. 1996). Streams and lakes visited later in life do not make this same indelible mark as those visited during the sensitive period.

The goal of this study was two-fold, with each goal tested in a separate experiment. First, we investigated whether rainbow trout are capable of embryonic learning from either conspecific or heterospecific alarm cues and tested the duration of information retention. Second, we investigated whether learning at different life stages, specifically as an embryo, newly hatched larvae, or free-swimming juveniles, would lead to different retention of learned information.

**METHODS**

**Holding conditions**

Fertilized rainbow trout (Oncorhynchus mykiss) eggs were purchased from Troutlodge Inc., Sumner, WA. Embryos take approximately a month to hatch at a temperature of 10 °C and were shipped to us around 3 weeks postfertilization. Embryos were held in groups of approximately 60 in 140 hatching tanks equipped with an airstone and filled by flow-through dechlorinated water (0.5 L/min, 10 °C). After hatching, the trout alevins remain as larvae for a few weeks, during which time they continue to rely on an external yolk sac for nutrition. At this time, they were transferred into mosquito netting baskets in four large flow-through tanks (500 L, flow rate 4 L/min). After hatching, the trout alevins were fed floating commercial trout food six times daily.
Collection of cues

Ten 6-month-old juvenile rainbow trout (fork length: mean ± standard error [SE]: 19.3 cm ± 0.5) and four 8-month-old brook trout (Salvelinus fontinalis, Mitchell 1814; 23.6 cm ± 0.6) were used to collect conspecific (CAC) and heterospecific (HAC) alarm cues, respectively. The fish used to procure cues were approximately four times the length of the fish used in behavioral testing in the first part of Experiment 1 and in Experiment 2 and about 30% longer than the 5–6-month-old trout tested in the second half of Experiment 1, but they still had visual the Markus characteristic of juveniles (oval spots along their sides). Trout were euthanized with a single, lethal blow to the head following approved institutional ethics protocols (2007083, as per guidelines from the Canadian Council on Animal Care) and a thin layer of skin was removed from each side of their body and measured. Skin fillets were placed in chilled water and homogenized using a Polytron homogenizer (Brinkmann Instruments, Mississauga, ON, Canada). The solution was filtered through cotton batting to remove the remaining tissue. A concentrated solution of 0.1 cm² of skin/mL water was stored frozen at −20 °C in 30-mL aliquots. Prior to use, the alarm cue aliquots were thawed overnight in the dark at temperature (approximately 14 °C).

Predator odor (PO) was collected from three northern pike (Esox lucius, Linnaeus 1758; 19.1 cm ± 0.2). The pike were held in 60-L glass tanks (Hagen, Montreal, QC, Canada) apart from other fish for a full week prior to odor collection and were not fed during this time to avoid any chemical cues that might result from the consumption of related prey species (Mirza and Chivers 2001). Each pike was then placed individually in a tank containing 60 L of clean, dechlorinated water, with an air stone but no filtration system. They remained in these tanks for 24 h with no food, after which they were returned to their original holding tanks. The water in which they were kept for 24 h was filtered through cotton batting and then frozen at −20 °C in 125-mL aliquots until needed, at which point it was allowed to thaw in the dark overnight at ambient temperature (approximately 14 °C).

Experiment 1: embryonic learning and memory of predator information

Experimental outline

Trout embryos were taught (or not) to recognize the odor of a novel predator, a northern pike, as a threat, using conspecific or heterospecific alarm cues, and their antipredator response to pike odor was recorded after 2–3 months. To further investigate the potential memory window of the fish, only trout taught with conspecific alarm cues were tested for their response to pike odor after 5–6 months.

Conditioning phase

Trout embryos were each conditioned once a day on 3, 4, 5, and 6 May (four conditioning events each) and began to hatch on 7 May 2013 (approximately 31 days postfertilization at 10 °C). Prior to conditioning, the trout embryos were placed in groups of approximately 60 into 450-mL plastic cups filled with 300 mL of dechlorinated water. After an hour of acclimation, one of five different conditioning treatments was applied to each cup: 1) a water control (W + W) consisting of 1 mL distilled water to control for disturbance associated with cue injection; 2) a predator odor control (W + PO) consisting of 1 mL of distilled water and 10 mL predator odor to control for potential sensitization or habituation arising from multiple exposures to predator odor; 3) an alarm cue control (CAC + W) consisting of 1 mL conspecific alarm cue control and 10 mL of distilled water to control for behavioral biases arising from embryos being exposed to risk stimuli during early ontogeny; 4) the conspecific alarm cue conditioning (CAC + PO) consisting of 1 mL conspecific alarm cue and 10 mL predator odor; and finally, 5) the heterospecific alarm cue conditioning (HAC + PO) consisting of 1 mL heterospecific alarm cue and 10 mL predator odor. There were 36 replicates (cups) of the W + W control and 27 replicates of each of the other treatments. The cues were injected gently into each cup to minimize disturbance. The final concentration of skin was 1 cm²/3 L, a concentration known to elicit an overt antipredator response in this species, even early life stages (Mirza and Chivers 2003; Horn and Chivers 2017). After 1 h, each egg cup underwent two complete water changes, whereby the conditioning treatment and water were drained and the cup was refilled with fresh water (twice), and then the eggs were returned to their respective hatching tanks.

Testing phase

Fish from all five treatments were tested for their responses to water or predator odor alone 33–67 days (2–3 months) after receiving their last conditioning treatment (fork length: mean ± SE: 45 mm ± 0.7). Based on the results we obtained, we decided to further investigate the duration of the memory window by testing fish from the CAC + PO group between 5 and 6 months after their last conditioning treatment (14.1 cm ± 0.17).

Behavioral assays followed established methodology (Chivers and Smith 1998), consisting of observing the behavior of the fish for 5 min prior to and 5 min after the injection of stimuli into the test arena. The smaller juveniles were tested in round plastic containers (745 mL, No Name brand, Loblaw, Brampton, ON, Canada) filled with 600 mL of dechlorinated water and visually divided into quarters by external perpendicular lines. Larger juveniles were tested in 37-L glass tanks (Hagen) partly filled with 25 L of dechlorinated water and divided by a three-by-four grid (8.9 × 12.4 cm quadrats) on the front of the tank. Each large tank was also equipped with a semicircular shelter cut from PVC piping and an airstone to provide aeration. Unfortunately, airstones created too much vibration for the small test bowls and were, thus, not used; time in the test bowls was, therefore, limited to a maximum of 2 h. The outer sides of each arena were darkened by heavy black plastic to minimize visual disturbance. All test arenas were equipped with airline tubing attached to the side of the arena, which facilitated the injection of stimuli into the tanks while minimizing disturbance of the fish. Observations were recorded from above the small arenas and from the side of the larger ones.

Typical antipredator behaviors in fish include decreases in movement and foraging behavior and increases in shelter use. We recorded movement as number of lines crossed using the grids in the testing arenas. We considered a line to be crossed when three quarters of a fish’s body crossed a dividing line. Foraging was quantified by counting the number of feeding bites or strikes an individual attempted during the test period. Foraging behavior and time spent using shelter (in seconds) were only evaluated with the larger individuals due to differences in behavior between the two ontogenetic groups.

Trout were placed in their individual testing tanks and left undisturbed for at least an hour prior to testing. Before each trial, either 30 or 60 mL of water (depending on arena size, see below) was drawn from each tank and set aside to be used at a later time.
to flush the stimuli into the testing arenas. Thirty seconds prior to the prestimulus observations, a small quantity of food (1 mL of live Artemia for the small and 15 pellets of food—1.5 mm FinFish slow sinking pellets, Zeigler, Gardners, PA—for the large) was added. Behaviors were recorded for 5 min. A second allotment of the same quantity of food was added immediately prior to the injection of the test stimulus. The test stimulus (distilled water or predator odor) was injected slowly into the tanks via blue airline tubing, which was then completely flushed into the arena with the previously reserved tank water. For small arenas, we injected 20 mL of stimulus followed by a 30-mL flush, whereas 30 mL of stimulus with a 60-mL flush was used for the larger tanks. Given the time elapsed between conditioning and testing of the older juveniles, we needed a positive control to ensure that the fish were capable of showing significant antipredator response, in case they failed to respond to the other cues. As a positive control, we exposed some of them to a third test stimulus: a CAC solution (7.5 mL of CAC mixed with 22.5 mL of distilled water). Behaviors were recorded for another 5 min poststimulus injection.

**Experiment 2: the role of ontogeny in the learning and retention of predator-related information**

**Experimental outline**

Trout were conditioned to recognize a novel predatory threat at one of three ontogenetic stages: as embryos, newly hatched alevins, or free-swimming larvae and were tested at a later date for their response to predator odor.

**Conditioning phase**

Trout embryos were each conditioned once a day on 11, 12, 13, and 14 January, approximately 3 weeks postfertilization, and began to hatch on 24 January 2014. The newly hatched alevins were conditioned approximately 1 week after hatching on 1, 2, 3, and 4 February, while still in the low-mobility stage. Free-swimming larvae were treated on 22, 23, 24, and 25 February after their yolk sacs were completely resorbed and they were fully mobile (approximately 3 weeks posthatch). Conditioning protocol followed that used in Experiment 1 with the difference that the fish received only three types of conditioning treatments: W + W, CAC + PO, and HAC + PO.

**Testing phase**

Trout were tested 28–155 days (1–5 months) after their last conditioning treatment (fork length: mean ± SE: 46 mm ± 1.1). Fish were tested in the small arenas using the same protocol outlined above for Experiment 1, but testing was limited to PO to decrease the complexity of the analysis as the capacity for learning by this protocol was established in Experiment 1.

**Statistical analyses**

Prestimulus and poststimulus behavioral data (numbers of line crosses, feeding strikes, and time spent unsheltered, as applicable) were computed into proportional change in behavior from the prestimulus baseline ([poststimulus − prestimulus]/prestimulus). Proportional change in behavior was used as a response variable in subsequent analyses. Prestimulus activity, foraging, and shelter, along with fish length (fork length in mm), were also analyzed to ensure no baseline biases between experimental groups. Any fish that crossed fewer than 30 or more than 200 lines remained completely stationary for longer than 2 min, or failed to forage at all during the prestimulus test, and any fish that increased their movement by more than 100% poststimulus was eliminated from analysis to avoid the use of fish with particularly aberrant behavior that might signify an external disturbance. Removing particularly inactive or active fish may bias the results by excluding the extremes inherent to any population of individuals; however, including fish who are already frightened would create a greater bias by obfuscating any potential reactions with an altered baseline. The remaining fish provided groups of 14–90 for each sample subset, depending on the experiment.

**Experiment 1**

For the small juveniles, a two-way general linear model (GLM) was used to test the effect of conditioning treatments (W + W, W + PO, CAC + W, CAC + PO, and HAC + PO) and testing cues (W vs PO) on the line crosses of the fish, both for prestimulus data and proportional change data (n = 14–15 per subset). For the large juveniles, one-way multivariate GLM was performed to evaluate the effect of testing cues (W, PO, or CAC) on prestimulus and proportional change data for line crosses, foraging, and, time spent unsheltered (n = 16). To use the most conservative measure, we reported P-values for Pillai’s Trace (Olson 1976). Shapiro–Wilks’s test was used to assess normality and Levene’s test was performed to assess for heteroscedasticity. Where significant interactions were observed, data was split to further investigate the nature of the interaction. Where necessary, nonnormal data were rank transformed before analysis was performed. Although rank transformation removes the magnitude information from the data, it has the benefit of still allowing for a complex analysis.

**Experiment 2**

Length, prestimulus, and proportional change in activity levels were each assessed using a univariate GLM testing the effects of ontogenetic stage at conditioning (embryos, newly hatched, and free-swimming larvae), conditioning (W + W, CAC + PO, and HAC + PO) and testing latency (number of days since conditioning, included as a continuous variable) tested with PO. Outliers were assessed using a Cooks Distance (4/n). From an initial 671 individuals, 27 outliers were identified, leaving a total of 644 individuals for assessment (n = 30–90 per subset). Normality and homoscedasticity were visually assessed using Q-plots and residual plots. Where significant interactions were assessed by the GLM, subsequent analyses were performed by splitting the data to further investigate the nature of the interaction. Only P-values of interest have been reported in the text of the results section; the remainder can be found in Supplementary Table 1. All analyses were performed using SPSS Statistical Software [Version 17.0] IBM.

**RESULTS**

**Experiment 1**

Fish tested at 1–2 months of age did not differ in length or baseline activity among conditioning treatments or testing cues (length: treatment: $F_{4,148} = 0.507$, $P = 0.672$; cue: $F_{4,148} = 0.927$, $P = 0.365$; treatment × cue: $F_{4,148} = 0.035$, $P > 0.99$; prestimulus: treatment: $F_{4,148} = 1.48$, $P = 0.211$; cue: $F_{4,148} = 0.129$, $P = 0.720$; treatment × cue: $F_{4,148} = 1.22$, $P = 0.307$). When exposed to the testing cues, their change in activity depended on both the conditioning treatment they received and the testing cue to which they were exposed (treatment × cue: $F_{4,148} = 0.157$, $P > 0.99$). Fish in the three control
groups did not respond differently to testing with water versus testing with predator odor (cue: W + W; F_{1,29} = 1.38, P = 0.25; W + PO: F_{1,29} = 0.29, P = 0.60; CAC + W: F_{1,29} = 0.17, P = 0.68), indicating that they failed to learn the pike as a threat. However, both alarm cue learning groups showed a significant antipredator response to the predator odor as compared with water (cue: CAC + PO: F_{1,29} = 39.81, P < 0.001; HAC + PO: F_{1,29} = 25.55, P < 0.001, Figure 1).

Embryos conditioned with alarm cue and predator odor (CAC + PO) and tested after 5–6 months did not differ in length (F_{2,25} = 0.707, P = 0.503) or baseline activity level (MANOVA: Pillai’s Trace: F_{6,68} = 0.823, P = 0.555), indicating no pre-existing bias among the groups. Fish altered their behavior depending on the testing cue (W, PO, or AC) they received (MANOVA: Pillai’s Trace: F_{6,68} = 5.943, P < 0.001). All types of activity were affected by testing cue (line crosses: F_{2,47} = 13.72, P < 0.001; bites: F_{2,47} = 10.07, P < 0.001; time unsheltered: F_{2,47} = 3.337, P = 0.045; Figure 2). Juveniles displayed an antipredator response to alarm cues compared with water control and predator odor based on their movement and foraging activity (line crosses, bites: all P ≤ 0.001), with no difference between predator odor and water (line crosses: P = 0.465; bites: P = 0.669, time unsheltered: P = 0.167), indicating that the fish failed to respond to pike odor as a threat. In terms of time unsheltered, the fish responded more to alarm cue than predator odor (P = 0.013) but not to water (P = 0.246).

**Experiment 2**

Neither length nor prestimulus activity was affected by interactions between testing latency (day), ontogenetic stage, and conditioning treatment (length: F_{3,537} = 2.21, P = 0.067; prestimulus activity: F_{1,643} = 0.72, P = 0.50), but both were affected by two-way interactions involving day (length: stage × day: F_{2,537} = 3.42, P = 0.034, conditioning × day: F_{2,537} = 2.21, P = 0.067, stage × conditioning: F_{2,537} = 2.21, P = 0.067; prestimulus activity: stage × day: F_{2,643} = 4.48, P = 0.012, conditioning × day: F_{2,643} = 3.74, P = 0.024, stage × conditioning: F_{2,643} = 1.00, P = 0.406). The effects of day on length and prestimulus activity are expected; as the fish grow over time, their size and baseline activity levels increase.

**Figure 1**

Mean (±SE) proportional change in movement (line crosses) for 1–2-month-old trout exposed to water (white bars) or predator odor (gray bars). The fish received one of five conditioning treatments as embryos: water only (W + W), predator odor only (W + PO), conspecific alarm cues only (CAC + W), conspecific alarm cues paired with predator odor (CAC + PO), or heterospecific alarm cues paired with predator odor (HAC + PO).

Our use of proportional change in behavior for analysis should minimize bias from this unavoidable consequence of our experimental design.

The proportional change in activity of the fish was affected by an interaction between conditioning treatment and ontogenetic stage at conditioning (F_{1,643} = 2.47, P = 0.044) and conditioning treatment and testing latency (F_{2,643} = 9.48, P < 0.001) and a near significant three-way interaction among the three factors (F_{2,643} = 2.36, P = 0.052). Individuals conditioned with W + W were not affected by ontogeny or testing latency (F_{2,224} = 2.38, P = 0.093). Conditioning with CAC + PO resulted in an interaction between ontogeny and day (F_{2,233} = 3.98, P < 0.001; Figure 3), indicating that individuals conditioned at different stages retained the information for different amounts of time. Specifically, embryos and free swimmers did not differ in their rate of forgetting (F_{1,156} = 0.063, P = 0.80). However, newly hatched individuals displayed a steeper decline of response over time as compared with embryos (F_{1,156} = 9.71, P = 0.002) but not significantly when compared with free swimmers (F_{1,153} = 2.81, P = 0.096).

Individuals conditioned with heterospecific cues were not affected by the interaction between ontogeny and testing latency (F_{2,181} = 0.17, P = 0.84) but were affected by testing latency (HAC + PO: F_{1,181} = 37.86, P < 0.001), indicating that all individuals, regardless of ontogeny, show a similar rate of forgetting (Figure 3).

**DISCUSSION**

Embryonic rainbow trout exposed to a combination of either conspecific or heterospecific alarm cues and predator odor showed a significant posthatch antipredator response to pike odor, indicating that they successfully learned to recognize a novel predator odor as a threat while in the egg. To the best of our knowledge, this study is the first to demonstrate embryonic learning of predator recognition in salmonids and to show that such learning can occur via heterospecific alarm cues. This result concurs with previous studies in amphibians demonstrating highly sophisticated learning abilities in embryos, including the ability to learn the time of day at which a predator poses the greatest danger or the ability to match intensity of response to the risk level posed by the predator (Ferrari and Chivers 2010).

Unlike the only two previous experiments looking at embryonic learning of predator recognition in fish (Nelson et al. 2013; Atherton and McCormick 2015), we used alarm cues from significantly older juveniles (6–8-month-old) rather than larval cues. This is of interest because learned responses may be stronger when conditioned with cues from conspecifics that are from a more similar ontogenetic group (Mirza and Chivers 2002; Lönnstedt and McCormick 2011; Mitchell and McCormick 2013). The capacity of embryonic fish to learn predators using alarm cues from fish from significantly different ontogenetic groups (and thus representing a very different prey guild) is also novel. The adaptive value of this depends on the closeness of the fish to which it responds. In this case, the rainbow trout responded to fish that were larger but in the same life stage and, thus, probably from a similar prey guild. They also responded to brook trout, a visually similar fish of comparable size, with similar habitats, so the response is adaptive. However, if this response also occurs with less similar species, it may prove detrimental by causing a fish to miss out on important foraging opportunities when a different prey is at risk.

In terms of information retention, our study indicates that embryos that learned to recognize pike using conspecific alarm cues...
displayed an overt antipredator response to pike odor 2–3 months after training, but the antipredator response is absent after 5 months. The significant response to alarm cues by this age class confirmed the ability of fish to display an antipredator response in a perceived risky situation. Adaptive forgetting is essential to allow prey to stop responding to former predators that are no longer a threat, as individuals grow and move through different prey guilds. Prey that maintain responses to predators that they have outgrown may miss out on important foraging and mating opportunities.

Our second experiment provided valuable insights into the effects of ontogeny on the retention of learned predator recognition. Regardless of their ontogenetic stage at conditioning, all fish taught to recognize the predator with either conspecific or heterospecific alarm cues displayed strong antipredator responses to pike odor when tested 5 weeks postconditioning. The intensity of this response decreased over time, a trajectory leading to extinction of the learned response after 4–6 months. For conspecific alarm cue learning, the rate of forgetting was slower when the information was learned as embryos as compared with the newly hatched alevins, with free swimmers displaying an intermediate rate, leading to a fluctuation in the predicted retention times depending on age at learning. In contrast, the antipredator response intensity decreased evenly in all age groups that learned from heterospecific alarm cues.

Fish may have a period of cognitive sensitivity early in their development, as they are taking in vast quantities of crucial information about their habitat, their kin, their food, and their predators—a sort of predetermined period of sensitivity built into their development. Ferrari et al. (2019) found that tadpoles who learned predator recognition as embryos retained it longer than conspecifics that learned as larvae. The article further proposes that, although young organisms generally have greater cognitive pliability than older ones, embryonic learners may suffer from “cognitive resonance,” a phenomenon by which information learned during certain stages of development have a relatively augmented impact on later life as compared with similar information learned later on. Specifically, information learned during these periods of cognitive resonance is better conserved, for better or for worse, than similar information gathered at different ontogenetic stages. Similarly, in Experiment 2, we also observed a longer retention in embryonic learners as compared with the newly hatched. However, by adding a third early ontogenetic stage, we observed a complexity than might not be entirely explained by a single period of sensitivity. Intrinsic factors such as growth rate, vulnerability to predation, hormone levels, and rate of developmental change are all greatly affected by ontogeny (Sibly et al. 2015) and may all have an effect on learning and memory. Although multiple periods of sensitivity are possible, we instead propose that relevant extrinsic and intrinsic factors modulate periods of sensitivity creating a complex landscape of learning and retention.

Embryos and newly hatched alevins are both limited in their mobility. Embryos cannot move at all and, although physically capable of some movement, alevins have tiny fragile bodies, encumbered by large egg sacs and scanty muscled. Neither stage has much hope of dashing into a hiding spot or out of the reach of their predators.
of a predator, whereas free swimmers, slender and muscular, do. Low-mobility, high-vulnerability prey may put a higher premium on predator information and, thus, retain it for a longer time, as they are at greater overall risk for predation. A parallel trend is represented by growth rate. Brown et al. (2011) found that faster-growing trout extinguished their learned predator recognition more rapidly, which is logical as these fish outgrow their predators more rapidly. If growth rate alone were the predictor, we would expect that embryos would retain the information longest, followed by alevis, and then free swimmers, as alevis grow faster than their egg-bound embryonic counterparts but more slowly than the free-swimming conspecifics who are able to feed rather than relying entirely on their yolk sacs for nutrition (Alami-Durante et al. 2014). Because the free swimmers retained the information longer than the alevis, growth rate alone cannot explain the trend we observed. Although this trend may justify why embryos might retain predator information better than newly hatched counterparts, it does not explain the lack of significant difference between free swimmers and embryonic learners.

Other factors might bias free swimmers to retain information better than embryos. Specifically, the rate of growth of the nervous system in the early days of a larval fish is inverse to that of their general mass, such that their brains grow fastest immediately posthatch and the development slows over time (Alami-Durante 1990). This high rate of neural development can most likely be projected back into the embryonic stage as well. During periods of rapid brain growth and development, there is often a high level of plasticity, such that new information may more easily replace older information (Hattori and Wasterlain 1990). Additionally, a more fully developed brain may have a greater physical capacity to retain information than a less developed brain, as the structures involved are more complete and less neural restructuring is occurring (Akers et al. 2014).

Perhaps the pattern we observed is a consequence of a nexus of these opposing trends. Although the embryos have a lower growth rate and higher vulnerability, which induce greater retention of information, and the free swimmers have a slower neural development to reduce the extinction of memory, alevis are less influenced by these factors. The net result may be that, by not retaining the information as well as the embryos and possibly by extinguishing it more rapidly than the free swimmers, the intensity of newly hatched learners’ reactions decrease more quickly.

Another factor which may have played a less predictable role in the retention is the semipermeable membrane surrounding the trout eggs (Gray 1932; Groot and Alderdice 1985). Although our first experiment demonstrates that alarm cues and predator odor can permeate the membrane, we cannot predict from this experiment the degree to which this membrane impacted the complete profile or the relative quantities of the chemicals that diffused through. It is possible that the concentration of alarm cues permeating the embryo was significantly lower than that surrounding the alevis or free swimmers, though that would have the opposite effect of what we observed, decreasing retention rather than increasing it. Alternatively, the cues might have gotten trapped in the membrane of the embryo, causing a much longer exposure period, potentially increasing the duration of their learned response. Unfortunately, without a greater understanding of the extremely complex chemistry of alarm cues and without related testing of membranes, it is impossible to predict what role embryonic membranes might play in the relative retention of cue-learned information.

Interestingly, the differentiated retention discussed above occurred only in individuals that learned from conspecific cues. Perhaps, heterospecific alarm cues carried some uncertainty as to their relevance to the juveniles, thus giving a lower value to the learned information at the time of conditioning. Mitchell et al. (2012) showed fish respond most strongly to cues from the most closely phylogenetically related donors. Information from a closely related yet distinct species may be considered less relevant and may, therefore, elicit a weaker antipredator response. The lower relevance of heterospecific alarm cues may have prevented the increased retention observed in the embryonic trout that learned from conspecific cues.

**CONCLUSION**

For any potential prey species, the ability to recognize predators is essential to survival. If an organism is able to learn to recognize its predators while it is still an embryo, it will have an immediate advantage over others who do not have that ability. However, to retain that information indefinitely would be detrimental as it would remove opportunities for growth and reproduction. Here, we have demonstrated that embryonic trout can not only learn from conspecifics, but also heterospecifics, and that they cease to respond as the information loses value. The information retention varies depending on ontogeny at the time of learning when fish learn from conspecific alarm cues, most likely as a result of a variety of growth and vulnerability factors that impact a period of sensitivity, or cognitive resonance, in the immature trout.

**SUPPLEMENTARY MATERIAL**

Supplementary data are available at *Behavioral Ecology* online.

**Supplementary Table 1** Statistical table of the GLM performed on the proportional change in activity over time, by ontogenetic stage and conditioning cue (Experiment 2)

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Marianna Horn (2019).

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