Behavioural Processes 189 (2021) 104421

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Tone deaf: Association of an auditory stimulus with predation risk by zebrafish *Danio rerio* does not generalize to another auditory stimulus

Alex R. Seigel, Isabelle G. DeVriendt, Savanna J. Hohenstein, Mark B. Lueders, Ananda Shastri, Brian D. Wisenden *

Biosciences Department, Minnesota State University Moorhead, United States

ARTICLE INFO

Keywords:
Learned predator recognition
Alarm cues
Learning generalization
Auditory cue
Zebrafish

ABSTRACT

Predator recognition by small fishes can be acquired when chemical alarm cues released from damaged skin (by a predator attack) are paired with a novel stimulus, such as the appearance or odor of a predator. Once learned, fish can extend recognition of risk by generalizing to associate risk with additional stimuli that are similar to the conditioned novel stimulus. Here, we trained zebrafish to associate a novel auditory stimulus with predation risk, and then tested to see if they generalize risk to all sound stimuli or whether the conditioned response is limited to the sound frequency of the conditioning stimulus. We found that zebrafish *Danio rerio* readily associated risk of predation with Tone 1 (285 Hz), as evidenced by reduction in activity, increased time spent near the substratum and increased shelter use, but fish conditioned to fear Tone 1 completely ignored presentation of a second tone of 762 Hz. These data suggest that generalization does not occur as easily for auditory cues as they do for olfactory and visual cues, perhaps due to differences in the properties of sensory biology or the cognitive mechanisms that process information in different sensory modalities.

1. Introduction

Predation is a ubiquitous component of all communities and has sweeping effects on the distribution, morphology, life history and behavior of prey (e.g., Kerfoot and Sih, 1987; Lima and Dill, 1990; Reznick et al., 1996; Tollrian and Harvell, 1999). There is steep selection promoting the fitness of prey that effectively detect and evade encounters with predators. In aquatic ecosystems, semiochemicals, such as predator odor or chemical alarm cues released from conspecifics injured by a predator, are used by a wide range of taxa to detect risk of predation (Dodson et al., 1994; Ferrari et al., 2010a). Behavioral responses to these cues reduce the probability of predation (Berejikian et al., 1999; Mirza and Chivers, 2000; Polo-Cavia and Gomez-Mestre, 2014; Wisenden et al., 1999).

Although fishes innately evade rapidly approaching objects (Domenici and Hale, 2019), they generally do not have innate recognition of predator odor or appearance. Predator recognition is often acquired through associative learning through social learning (e.g. Brown and Laland, 2001; Manassa and McCormick, 2012; Manassa et al., 2013) or when novel stimuli are presented simultaneously with chemical alarm cues released during a predation event (Brown, 2003; Ferrari et al., 2010a). Associative learning facilitated by chemical alarm cues is known as releaser-induced recognition learning (Suboski, 1990) whereby alarm cues serve as a releaser of rapid and near-permanent association of the unconditioned stimulus (US, i.e., alarm cue) with the conditioned stimulus (CS, novel stimulus). After a single pairing event of the US and CS, prey respond to the novel stimulus with a full suite of antipredator behaviors (conditioned response, CR). Releaser-induced recognition learning provides prey with a flexible and adaptable mechanism for tracking predator identity when predator identity varies temporally over ontogeny, and ecological and evolutionary time scales and spatially across a species' geographic distribution. Laboratory experiments have demonstrated releaser-induced recognition learning of novel stimuli in olfactory, visual and auditory sensory modalities (Ferrari et al., 2010a; Wisenden, 2015).

Generalization of acquired predator recognition from one conditioned stimulus (signature odor, appearance) to a range of species that match some, but not all, aspects of the conditioned stimulus allows prey to recognize a broader range of potential predators. This phenomenon has been documented in mammals (Griffin et al., 2001; Stankowich and Coss, 2007), amphibians (Ferrari et al., 2009) and fish (Chivers et al., 2013; Ferrari et al., 2007, 2010b). Generalization increases with

---

* Corresponding author at: Biosciences Department, Minnesota State University Moorhead, 1104 7th Ave S, Moorhead, MN, 56563, United States.
E-mail address: wisenden@mnstate.edu (B.D. Wisenden).

https://doi.org/10.1016/j.beproc.2021.104421
Received 16 November 2020; Accepted 11 May 2021
Available online 13 May 2021

decreasing phylogenetic distance presumably because closely related species share similar chemical profiles and/or visual outlines and behavior (Brown et al., 2011; Chivers and Ferrari, 2013; Chivers et al., 2013; Ferrari and Chivers, 2009; Ferrari et al., 2016, 2010b; Ferrari et al., 2008, 2009; Griffin et al., 2001; Griffin, 2004; Mitchell et al., 2013). For example, tammar wallabies (*Macropus eugenii*) trained to fear a model red fox (*Vulpes vulpes*) later responded to both a red fox model and a model of a feral cat (*Felix catus*), but not to a model of a goat (*Capra hircus*) (Griffin et al., 2001). In aquatic ecosystems, juvenile rainbow trout (*Oncorhynchus mykiss*) conditioned to fear the odor of pumpkinseed sunfish (*Lepomis gibbosus*), responded strongly to the odor of the congener longear sunfish (*Lepomis megalotis*), weakly to the odor of a more distantly related species in the same family (rockbass *Ambloplites rupestris*) but not to the odor of a fish outside of the Centrarchidae (yellow perch *Perca flavescens*) (Brown et al., 2011). Lemon damselfish (*Pomacentrus moluccensis*) conditioned to recognize moon wrasse (*Thalassoma lunare*) odor as an indicator of predation risk, responded to the odor of congeneric wrasses, but not heterogeneric wrasses, or fish species outside the wrasse family (Mitchell et al., 2013). Fathead minnows (*Pimephales promelas*) conditioned to fear the visual appearance of rainbow trout or brook trout (*Salmo trutta*) respond with antipredator behavior to either trout species but not to the appearance of yellow perch (Chivers et al., 2013). To date, generalization of auditory indicators of predation risk has not been tested in fishes.

Many fishes have a well-developed sense of hearing (Amorim, 2006; Fine and Parmentier, 2015; Kasumyan, 2008; Ladich, 2019; Popper and Fay, 1993). The role of the auditory sense in fish ecology is relatively understudied compared to olfactory and visual modalities. Many species produce and use sound during courtship and territorial displays, and in times of distress (Popper and Fay, 1993; Kasumyan, 2008). The otophysi are freshwater fishes that include speciose families of small schooling species such as minnows and characins (family Cyprinidae has 3160 species, Characidae has 1135 species [www.fishbase.in accessed 16 July 2020]). These fish have specialized vertebrae, called Weberian ossicles, that connect the gas bladder to the inner ear. The gas bladder serves as a resonating chamber that greatly extends the ability of these fishes to hear auditory stimuli of low amplitude and across a broad range of frequencies (Yan et al., 2000). It is not known if these small fishes can detect the sound of approaching predators however fathead minnows (Cyprinidae) and glow light tetras (Characidae) can associate a sound stimulus with predation risk through releaser induced recognition learning (Wisenden et al., 2008).

The zebrafish (*Danio rerio*) is a model organism in molecular genetics that has clear behavioral responses to conspecific alarm cues (Barkhymer et al., 2018; Korpi and Wisenden, 2001; Mathuru et al., 2012; Speedie and Gerlai, 2008). Zebrafish (Cyprinidae, *Danio rerio*) are in the otophysi and hear sounds from 100 to 8000 Hz with peak sensitivity between 600 and 800 Hz (Higgs et al., 2011).

In this study, we conditioned zebrafish to associate predation risk with one tone and then re-tested them with either the same tone or a tone of a different frequency to see if tone recognition was generalized to other tones, or if the conditioned response was specific to the frequency of the conditioning stimulus.

2. Methods

Adult zebrafish were purchased from a commercial supplier of wild-type research-grade animals (EkkWill Farm, Florida, USA) and held in our aquatic research facility for six months before being used in this study. The fish were held in 190-L glass aquaria filled with dechlorinated tap water maintained at 23 °C on a 12:12 L:D (900–2100) cycle and fed TetraMin® Tropical flake food supplemented with occasional feedings of brine shrimp nauplii.

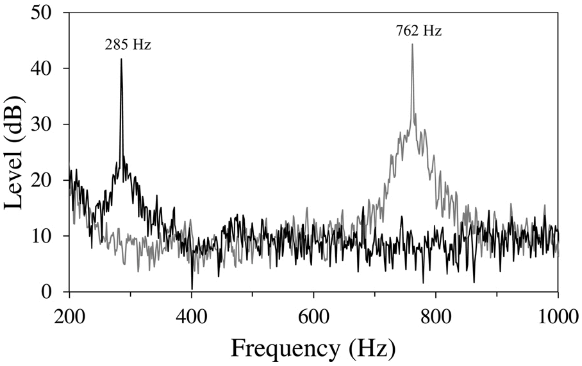

Fig. 1. Spectrogram of the stimulus tones used to condition and test zebrafish. Tone 1 was 285 Hz and Tone 2 was 762 Hz.

2.1. *Experimental set-up*

We used fifteen, 37.9-L glass aquaria (50.5 × 25.7 × 30.8 cm) with a 5 × 5.25 cm grid drawn on the short panel to aid in recording zebrafish activity and vertical distribution. Tanks were filled with dechlorinated water to a depth of 26 cm. Each tank was equipped with an air-powered sponge filter with two standard airline tubes, one tube for compressed air and one tube to serve as an injection tube for surreptitious delivery of chemical stimuli (alarm cue or water). Each tank contained a shelter made from a ceramic tile (10.8 × 10.8 cm) supported by cylindrical legs 4.8 cm in length. The stimulus injection tube was secured to the front of the shelf to prevent movement during cue injection that might affect fish behavior. Rigid black plastic panels were placed on either side of every tank to visually isolate each tank. Care was taken to acoustically isolate test subjects by ensuring that adjacent tanks did not touch one another and tests were conducted on every second tank to further reduce transmission of sound stimuli to non-focal tanks.

2.2. *Auditory stimuli*

A pair of waterproof earbud earphones (PyleHome marine grade IP-7 model PWPE10B) were placed inside the tank on the bottom in the center of the short, gridded panel facing the observers. Tones were generated using the Tuner Lite application loaded onto an iPhone 8, played with the phone’s volume set to maximum. Two tones were used in this experiment. Tone 1 was 285 Hz. This tone was used for all of the conditioning trials and half of the test trials. The second tone was 762 Hz, that was used as the test for generalization of auditory stimuli for half of the test trials (Fig. 1).

It was verified that the tone amplitudes were not significantly different from one another. To do this, test stimuli were recorded using a kithub hydrophone (kithub.cc) in a 37-L aquarium arranged in a similar manner as conditioning and test trials. The hydrophone output was fed into an amplifier with gain of 1000 and band pass filtering from 1 Hz to 5 kHz (Land et al., 2001). Audacity software (version 2.4.1) was used to produce a power spectrum for each tone in dB, relative to the ambient acoustic background noise level (Fig. 1).

2.3. *Alarm cue preparation*

Conspecific alarm cue was made from 33 adult zebrafish (mean ± SE total length = 36.93 ± 0.51 mm). Zebrafish were killed with an overdose of tricaine mesylate (MS-222) in accordance with Minnesota State University Moorhead Institutional Animal Care and Use Committee protocol 19-R/T-BIO-018-N-Y-C. Whole zebrafish were then placed into a 500-mL beaker containing 100 mL of dechlorinated tap water resting on a bed of ice. We used a handheld blender (Cuisinart® Smart Stick 2 Speed Hand Blender) to homogenize the fish for 30 s. The mixture was filtered through a wad of polyester wool, diluted to a final volume of

Table 1
Timeline and experimental design of treatment groups.

	Treatment group			
Time line	A:1−1	A:1−2	W:1−1	W:1−2
Day 1: Acclimation				
Day 2: Conditioning	Alarm cues + Tone 1 ($n = 30$)		Water + Tone 1 (n = 30)	
Day 3: Acclimation				
Day 4: Test	Tone 1 ($n = 15$)	Tone 2 ($n = 15$)	Tone 1 ($n = 15$)	Tone 2 ($n = 15$)
Hypothesis tested	Recognition learning	Generalization	Negative control	Negative control

330 mL, aliquoted into 33 10-mL doses and frozen at −20 °C until needed. Thirty-three 10-mL doses of blank dechlorinated water controls were prepared and frozen at −20 °C until needed.

2.4. Experimental protocol

Each fish was tested twice; once in a conditioning trial and again in a test trial (Table 1). Individual adult zebrafish were placed in each test aquarium and allowed 24 h to acclimate. Conditioning trials were conducted on the second day, then the water was changed and 24 h allowed to elapse to acclimate to the disturbance of the water change and the effects of conditioning trials. Test trials were conducted on the fourth day (Table 1).

Behavioral trials began by withdrawing 60 mL of tank water through the injection tube to rinse it of residues, discarding the water, then drawing another 60 mL of tank water, which was retained. Conditioning trials consisted of 5 min of prestimulus behavioral observation, followed by 30 s of Tone 1 (285.31 Hz) while simultaneously injecting either 10 mL of alarm cue or 10 mL of water followed by 60 mL of previously-retained tank water to flush the test cue completely out of the injection tube. This was followed immediately by 5 min of poststimulus behavioral observation. We ran 60 conditioning trials, 30 in which conspecific alarm cue was injected and 30 for which water was injected. Injection tubes were replaced after every trial. Behaviors observed were *Activity*, scored as the total number of grid lines crossed in 5 min, *Vertical Distribution*, scored as the grid row occupied by the fish at 10-s intervals, with “1” assigned to the row near the surface and “5” for the row nearest the substratum, and *Shelter Use* scored as time spent under the shelter or behind the filter.

Test trials were conducted in the same manner except that only sound stimuli were presented (Table 1). For half of the trials, Tone 1 was replayed ($n = 15$ for alarm cue-conditioned fish and $n = 15$ for water-conditioned fish), while the other half of the trials were presented with Tone 2 (761.74 Hz) ($N = 15$ for alarm cue-conditioned fish and $n = 15$ for water-conditioned fish).

2.5. Data analysis

Response variables failed to meet the assumptions of the normal distribution (Kilmogorov-Smirnoff tests for conditioning trials Change in Vertical distribution $D = 0.172$, $P < 0.001$; Change in Activity $D = 0.242$, $P < 0.001$; Change in Shelter Use $D = 0.381$, $P < 0.001$; and for test trials Change in Vertical Distribution $D = 0.234$, $P < 0.001$; Change in Activity $D = 0.213$, $P < 0.001$; Change in Shelter Use $D = 0.338$, $P < 0.001$). Therefore, treatment groups were compared using non-parametric Kruskal-Wallis one-way ANOVA. The effect of chemical cue (water versus alarm cue) in conditioning trials, and tone type (test of recognition learning and generalization for fish pre-conditioned on Tone 1) in test trials, were compared using Mann-Whitney *U* tests.

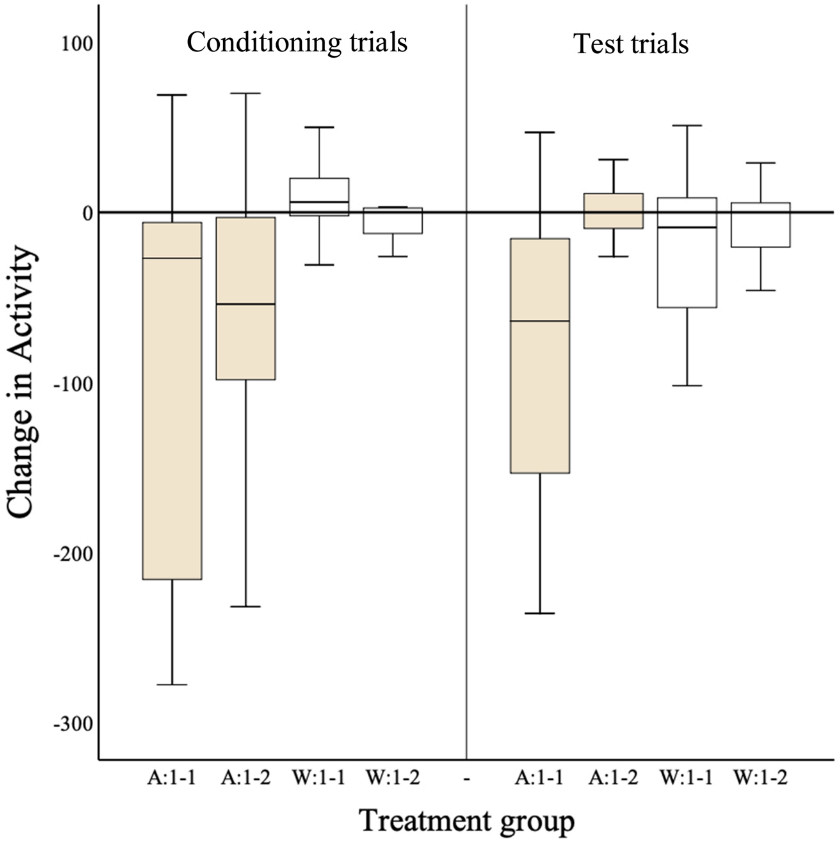

Fig. 2. Median ± quartiles and range for change (poststimulus - prestimulus) in Activity for conditioning trials (left panel) and test trials (right panel) for each of the four treatment groups. The four treatment groups were conditioned with either conspecific alarm cues (A, shaded fill) or water (W, open fill) and Tone 1. In test trials, fish were presented with either Tone 1 or Tone 2. Labels on the x-axis indicate conditioning chemical cue: conditioning tone – test tone, hence A:1-2 indicates fish that were conditioned with alarm cue + Tone 1, and tested with Tone 2.

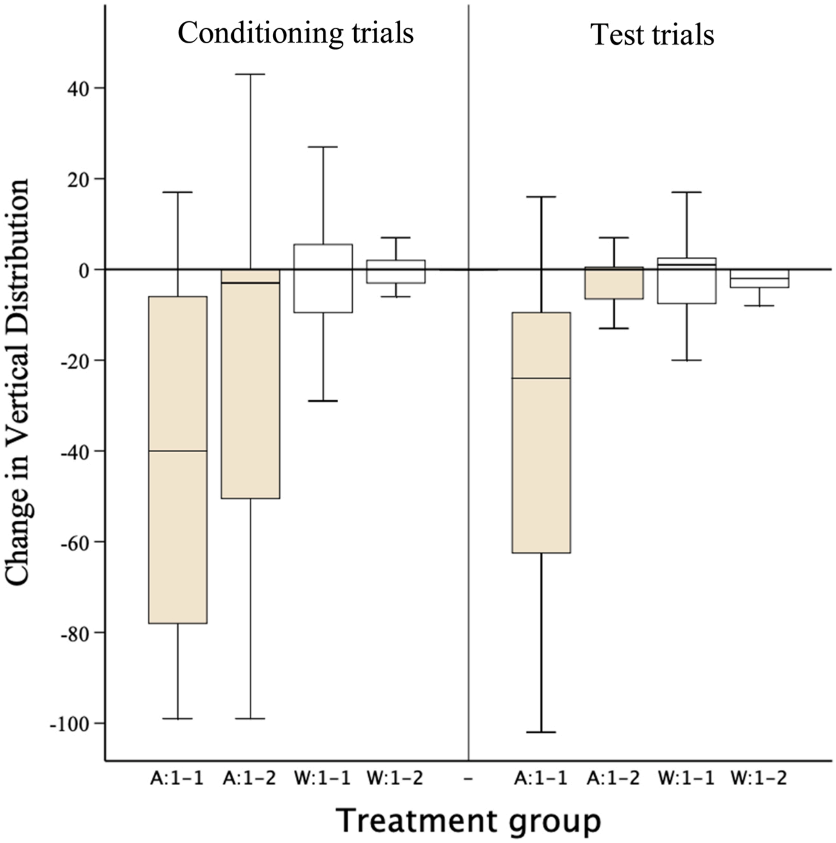

Fig. 3. Median ± quartiles and range for change in (poststimulus - prestimulus) Vertical Distribution for conditioning trials (left panel) and test trials (right panel) for each of the four treatment groups. The four treatment groups were conditioned with either conspecific alarm cues (A, shaded fill) or water (W, open fill) and Tone 1. X-axis labels are as described for Fig. 2.

3. Results

In conditioning trials, the four treatment groups (Conditioning cue * Test tone combinations) differed significantly in poststimulus-prestimulus change in Activity ($KW_3 = 14.42$, $P = 0.002$; Fig. 2), Vertical Distribution ($KW_3 = 9.53$, $P = 0.023$; Fig. 3) and Shelter Use ($KW_3 = 7.91$, $P = 0.048$; Fig. 4). Zebrafish responded to conspecific alarm cue, relative to water controls, with antipredator behaviors in terms of reduction in activity (Mann-Whitney U test $U = 199$, $N = 60$, $P < 0.001$), increased time spent near the substratum ($U = 262$, $N = 60$, $P = 0.005$), and increased time in shelter ($U = 287$, $N = 60$, $P = 0.006$). There was no -pre-existing difference between fishes that would later receive Tone 1 or Tone 2 in test trials (Table 2).

In test trials, conditioning experience had a significant effect on change in Activity ($KW_3 = 13.84$, $P = 0.003$; Fig. 2), Vertical Distribution ($KW_3 = 14.45$, $P = 0.002$; Fig. 3) and Shelter Use ($KW_3 = 19.43$, $P < 0.001$; Fig. 4). Fish conditioned with alarm cues + Tone 1 showed significant reduction in activity, more time at the substratum and more time in shelter when presented with Tone 1 than when presented with Tone 2 (Table 2). There was no evidence that fish conditioned to fear Tone 1 generalized fear to Tone 2. Fish conditioned with Water + Tone 1 did not show any behavioral changes when retested with either tone (Table 2, Figs. 2–4).

4. Discussion

We showed that zebrafish learned to associate a novel auditory stimulus with predation risk by pairing the sound of a tone with conspecific alarm cues. While releaser-induced recognition learning has been demonstrated many times for many species (Ferrari et al., 2010a), the present study is only the second time this phenomenon has been demonstrated for auditory stimuli (Wisenden et al., 2008) and the first demonstration of releaser-induced auditory learning in zebrafish. Zebrafish can be trained to respond to sounds through positive rewards (e.g. Cervi et al., 2012) but unlike releaser-induced recognition learning, positive reinforcement requires multiple reinforcement events for an association to form.

Zebrafish conditioned to fear Tone 1 did not generalize their fear response to another sounds, as evidenced by the complete lack of recognition of Tone 2, even though Tone 2 was similar in structure in being a steady tone, and was delivered at the same volume by the same speaker device in the same apparatus where conditioning took place. Both tones used in this experiment are well within the range of frequencies detectable by adult zebrafish (Higgs et al., 2011).

Generalization is likely facilitated by complexity of the conditioned stimulus. Predator odor, a common conditioning stimulus in these studies, is a mixture of compounds (Wisenden, 2015), ensuring that some components will be shared among closely related species. Strength of generalized responses to predator odor decreases with phylogenetic distance in fathead minnows (Ferrari et al., 2007, 2008; Chivers et al., 2013), wood frog tadpoles, *Rana sylvatica* (Ferrari et al., 2009) and their embryos (Ferrari and Chivers, 2009), lemon damselfish (Mitchell et al., 2013) and juvenile rainbow trout (Brown et al., 2011). Visual appearance of a predator is also a mixture of different angles of view, lighting and turbidity (Chivers et al., 2013; Ferrari et al., 2010b). We predict that conditioning fish with a mixture of sounds, or sounds that vary in frequency and amplitude may increase the probability of generalization to an auditory model. Similarly, conditioning fish with pure chemical compounds (olfactory) or a static cutout model predator (visual) may shrink the generalization “window” (Chivers et al., 2016) to include only those stimuli that hew closely to the conditioning stimulus.

One outcome of this study is demonstration of a convenient

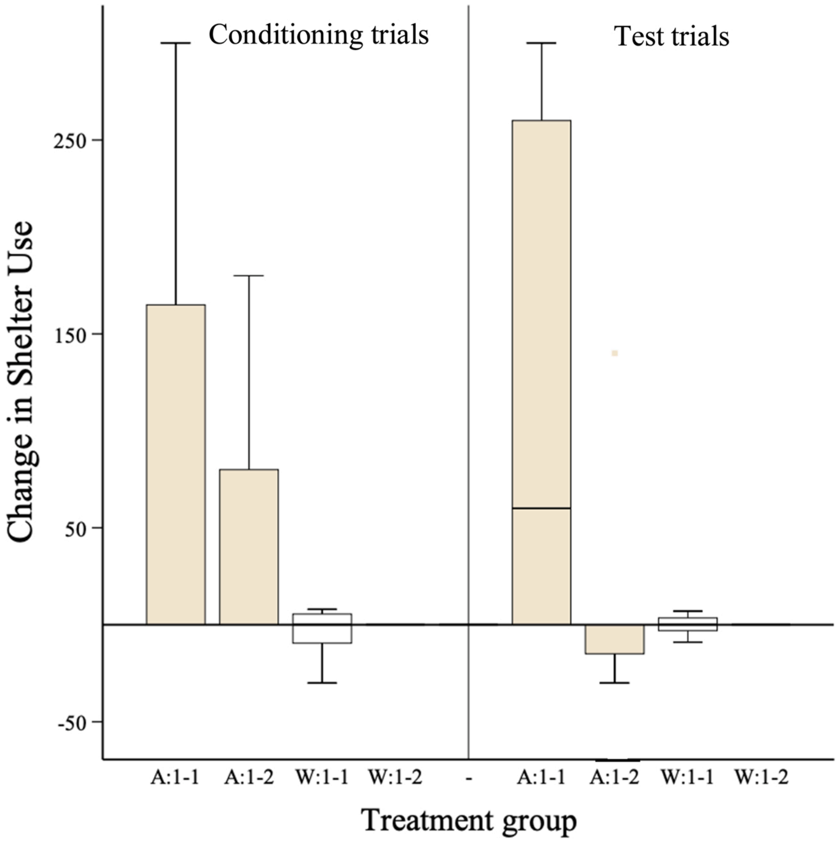

Fig. 4. Median ± quartiles and range for change (poststimulus - prestimulus) in Shelter Use for conditioning trials (left panel) and test trials (right panel) for each of the four treatment groups. The four treatment groups were conditioned with either conspecific alarm cues (A, shaded fill) or water (W, open fill) and Tone 1. X-axis labels are as described for Fig. 2.

Table 2
Outcome of Mann-Whitney *U* tests comparing responses to different tones. Behaviors are change (poststimulus – prestimulus) in Activity, Vertical Distribution and Shelter Use. In conditioning trials all fish received Tone 1 (285 Hz) and either conspecific alarm cues (A) or blank control water (W). In test trials, fish received either Tone 1 to test for a conditioned response or Tone 2 (762 Hz) to test for a generalized response. Significant effects ($P < 0.05$) are bolded.

Trial series	Behavior	Statistic	W:1−1 v W:1−2	A:1−1 v A:1−2
Conditioning trials	Activity	*U*	87.5	111
		n	15	15
		P	0.298	0.950
	Vertical Distribution	*U*	112.5	81.5
		n	15	15
		P	~1	0.197
	Shelter Use	*U*	112.5	97.5
		n	15	15
		P	~1	0.491
Test trials	Activity	*U*	95	29
		n	15	15
		P	0.467	**0.001**
	Vertical Distribution	*U*	96.5	42.5
		n	15	15
		P	0.504	**0.003**
	Shelter Use	*U*	112	33
		n	15	15
		P	0.98	**< 0.001**

experimental tool with which to explore the mechanisms of generalization. Auditory stimuli offer greater experimental control of conditioning stimuli than do chemical or visual stimuli as models. Future work could test tones closer to each other in frequency than the two tones used in this study to explore the threshold for generalization to auditory cues. It remains untested if there are aspects of sensory detection and processing of auditory stimuli that differ from olfactory or visual modalities that affect the cognitive processes involved in generalization.

The ability to associate fear with sounds raises questions about auditory signatures of predators and/or predation events in aquatic soundscapes (Rafael and Vasconcelos, 2019). Little is known about the role of sound stimuli in mediating predator-prey interactions in aquatic habitats (Ladich, 2019). Many fish species are known to produce distress sounds when grasped by a predator (Permentier et al., 2017), but zebrafish are not among them. Sounds produced during agonistic and reproductive interactions by predatory black drum (*Pogonias cromis*), hardhead catfish (*Ariopis felis*) and oyster toadfish (*Opsanus taur*) cause reduction in foraging behavior in their prey, mud crabs (*Panopeus* spp.) (Hughes et al., 2014). Humpback whales (*Megapterus novaeangliae*) flee from the distant sounds of approaching killer whales (Orcinus orca) (Curé et al., 2015). Sonar clicks used by hunting cetaceans are detected and evaded by American shad *Alosa sapidissima* fish (Kraus et al., 1997; Mann et al., 1998).

5. Conclusions

In this study we demonstrated releaser-induced recognition learning to an auditory stimulus by zebrafish, but found no evidence that zebrafish generalize antipredator responses to sound stimuli other than the frequency of the tone used for conditioning. Many questions are raised by this finding about sensory and cognitive processing of auditory cues, and the role of auditory cues in mediating predator-prey interactions in fishes.

Funding

Funding to support this research was provided by faculty research grants to BDW from the MSUM College of Science, Health and the Environment, and from the Judith Strong Fund for undergraduate research to ARS, IGD, SJH, and MBL.

CRediT authorship contribution statement

Alex R. Seigel: Conceptualization, Investigation. **Isabelle G. DeVriendt**: Conceptualization, Investigation. **Savanna J. Hohenstein:** Conceptualization, Investigation. **Mark B. Leuders:** Conceptualization, Investigation. **Ananda Shastri:** Auditory recording, Supervision. **Brian D. Wisenden**: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

References

Amorim, M.C.P., 2006. Diversity of sound production in fish. In: Ladich, F., Collin, S.P., Moller, P., Kapoor, B.G. (Eds.), Communication in Fishes. Science Publishers Inc., Enfield, NH US, pp. 71–105.

Barkhymer, A.J., Garrett, S.G., Wisenden, B.D., 2018. Olfactorily-mediated cortisol response to chemical alarm cues in zebrafish *Danio rerio*. J. Fish Biol. 95, 287–292. https://doi.org/10.1111/jfb.13860.

Berejikian, B.A., Smith, R.J.F., Tezak, E.P., Schroder, S.L., Knudson, C.M., 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Can. J. Fish. Aquat. Sci. 56, 830–838. https://doi.org/10.1139/f99-010.

Brown, G.E., 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. Fish Fish. 4, 227–234.

Brown, C., Laland, K., 2001. Social learning and life skills training for hatchery reared fish. J. Fish Biol. 59, 471–493.

Brown, G.E., Ferrari, M.C.O., Malka, P.H., Russo, S., Tressider, M., Chivers, D.P., 2011. Generalization of predators and nonpredators by juvenile rainbow trout: learning what is and is not a threat. Anim. Behav. 81, 1249–1256.

Cervi, A.L., Poling, K.R., Higgs, D.M., 2012. Behavioral measure of frequency detection and discrimination in the zebrafish, *Danio rerio*. Zebrafish 9. https://doi.org/10.1089/zeb.2011.0720.

Chivers, D.P., Ferrari, M.C.O., 2013. Tadpole antipredator responses change over time: what is the role of learning and generalization? Behav. Ecol. 20, 1114–1121. https://doi.org/10.1093/beheco/art038.

Chivers, D.P., Al-Batati, F., Brown, G.E., Ferrari, M.C.O., 2013. The effect of turbidity on recognition and generalization of predators and non-predators in aquatic ecosystems. Ecol. Evol. 3, 268–277.

Chivers, D.P., Mitchell, M.D., Lucon-Xiccato, T., Brown, G.E., 2016. Background risk influences learning but not generalization of predators. Anim. Behav. 121, 185–189.

Curé, C., Sivle, L.D., Visser, F., Wensveen, P.J., Isojunno, S., Harris, C.M., Kvadsheim, P. H., Lam, P.H.A., Miller, P.J.O., 2015. Predator sound playbacks reveal strong avoidance responses in a fight strategist baleen whale. Mar. Ecol. Prog. Ser. 526, 267–282. https://doi.org/10.3354/meps11231 https://.

Dodson, S.I., Crowl, T.A., Peckarsky, B.L., Kays, L.B., Covich, A.P., Culp, J.M., 1994. Non-visual communication in freshwater benthos: an overview. JNABS 13, 268–282.

Domenici, P., Hale, M.E., 2019. Escape responses of fish: a review of the diversity in motor control, kinematics and behaviour. J. Expt. Biol. 222 https://doi.org/10.1242/jeb.166009.

Ferrari, M.C.O., Chivers, D.P., 2009. Sophisticated early life lessons: generalization of predator recognition by frog embryos. Behav. Ecol. 20, 1295–1298.

Ferrari, M.C.O., Gonzalo, A., Messier, F., Chivers, D.P., 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. Proc. Roy. Soc. Lond. Ser. B. 274, 1853–1859.

Ferrari, M.C.O., Messier, F., Chivers, D.P., 2008. Can prey exhibit threat-sensitive generalization of predator recognition? Extending the predator recognition continuum hypothesis. Proc. Roy. Soc. Lond. Ser. B. 275, 1811–1816.

Ferrari, M.C.O., Brown, G.E., Messier, F., Chivers, D.P., 2009. Threat-sensitive generalization of predator recognition by larval amphibians. Behav. Ecol. Sociobiol. 63, 1369–1375.

Ferrari, M.C.O., Wisenden, B.D., Chivers, D.P., 2010a. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Can. J. Zool. 88, 698–724.

Ferrari, M.C.O., Lysack, K.R., Chivers, D.P., 2010b. Turbidity as an ecological constraint on learned predator recognition and generalization in a prey fish. Anim. Behav. 79, 515–519.

Ferrari, M.C.O., Crane, A.L., Chivers, D.P., 2016. Certainty and the cognitive ecology of generalization of predator recognition. Anim. Behav. 111, 207–211.

Fine, M.L., Parmentier, E., 2015. Mechanisms of fish sound production. In: Ladich, F. (Ed.), Sound Communication in Fishes. Springer-Verlag, Wien, pp. 77–126.

Griffin, A.S., 2004. Social learning about predators: a review and prospectus. Learn. Behav. 32, 131–140.

Griffin, A.S., Evans, C.S., Blumstein, D.T., 2001. Learning specificity in acquired predator recognition. Anim. Behav. 62, 577–589.

Higgs, D.M., Souza, M.J., Wilkins, H.R., Presson, J.C., Popper, A.N., 2011. Age- and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). J. Assoc. Res. Otolaryngol. 3, 174–184. https://doi.org/10.1007/s101620020035.

Hughes, A.R., Mann, D.A., Kimbro, D.L., 2014. Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proc. Roy. Soc. Ser. B. 281, 20140715 https://doi.org/10.1098/rspb.2014.0715.

Kasumyan, A.O., 2008. Sounds and sound production in fishes. J. Ichthyol. 48, 981–1030.

Kerfoot, W.C., Sih, A., 1987. Predation: Direct and Indirect Impacts on Aquatic Communities. University of New England Press, Hanover, p. 386.

Korpi, N.L., Wisenden, B.D., 2001. Learned recognition of novel predator odour by zebra danios, *Danio rerio*, following time-shifted presentation of alarm cue and predator odour. Environ. Biol. Fish. 61, 205–211.

Kraus, S.D., Read, A.J., Solow, A., Baldwin, K., Spradlin, T., Anderson, E., Williamson, J., 1997. Acoustic alarms reduce porpoise mortality. Nature 388, 525.

Ladich, F., 2019. Ecology of sound communication in fishes. Fish Fish. 20, 552–563. https://doi.org/10.1111/faf.12368.

Land, B.R., Wyttenbach, R.A., Johnson, B.R., 2001. Tools for physiology labs: an inexpensive high-performance amplifier and electrode for extracellular recording. J. Neurosc. Meth. 106, 47–55.

Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640.

Manassa, R.P., McCormick, M.I., 2012. Social learning improves survivorship at a life history transition. Oecologia. 171, 845–852. https://doi.org/10.1007/s00442-012-2458-x.

Manassa, R.P., McCormick, M.I., Chivers, D.P., 2013. Socially acquired predator recognition in complex ecosystems. Behav. Ecol. Sociobiol. 67, 1033–1040. https://doi.org/10.1007/s00265-013-1528-3.

Mann, D.A., Lu, Z., Hastings, M.C., Popper, A.N., 1998. Detection of ultrasonic tones and simulated dolphin echolocation clicks by a teleost fish, the American shad (*Alosa sapidissima*). J. Acoust. Soc. Am. 104, 562–568.

Mathuru, A.S., Kibat, C., Cheong, W.F., Shui, G., Wenk, M.R., Friedrich, R.W., Jesuthasan, S., 2012. Chondroitin fragments are odorants that trigger fear behavior in fish. Curr. Biol. 22, 1–7. https://doi.org/10.1016/j.cub.2012.01.061.

Mirza, R.S., Chivers, D.P., 2000. Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. Can. J. Zool. 78, 2198–2208.

Mitchell, M.D., McCormick, M.I., Chivers, D.P., Ferrari, M.C.O., 2013. Generalization of learned predator recognition in coral reef ecosystems: how cautious are damselfish? Funct. Ecol. 27, 299–304. https://doi.org/10.1111/1365-2435.12043.

Permentier, E., Diogo, R., Fine, M.L., 2017. Multiple exaptation leading to fish sound production. Fish Fish. 18, 958–966. https://doi.org/10.1111/faf.12217.

Polo-Cavia, N., Gomez-Mestre, I., 2014. Learned recognition of introduced predators determines survival of tadpole prey. Funct. Ecol. 28, 432–439.

Popper, A.N., Fay, R.R., 1993. Sound detection and processing by fish: critical review and major research questions. Brain Behav. Evol. 41, 14–38.

Rafael, A.L., Vasconcelos, R.O., 2019. Characterization of the natural soundscape of zebrafish and comparison with the captive noise conditions. Zebrafish 16, 152–164. https://doi.org/10.1089/zeb.2018.1654.

Reznick, D.N., Butler, M.J.4th, Rodd, F.H., Ross, P., 1996. Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism of natural selection. Evolution 50, 1651–1660. https://doi.org/10.1111/j.1558-5646.1996.tb03937.x.

Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav. Brain Res. 188, 168–177.

Stankowich, T., Coss, R.G., 2007. The re-emergence of felid camouflage with the decay of predator recognition in deer under relaxed selection. Proc. Roy. Soc. Ser. B. 274, 175–182.

Suboski, M.D., 1990. Releaser-induced recognition learning. Psychol. Rev. 97, 271–284.

Tollrian, R., Harvell, C.D., 1999. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, New Jersey, USA.

Wisenden, B.D., 2015. Chemical cues that indicate risk of predation. In: Sorensen, P.W., Wisenden, B.D. (Eds.), Fish Pheromones and Related Cues. Wiley-Blackwell Press, NY, US, pp. 131–148.

Wisenden, B.D., Cline, A., Sparkes, T.C., 1999. Survival benefit to antipredator behavior in the amphipod *Gammarus minus* in response to injury-released chemical cues from conspecifics and heterospecifics. Ethology 105, 407–414.

Wisenden, B.D., Pogatshnik, J., Gibson, D., Bonacci, L., Schumacher, A., Willett, A., 2008. Sound the alarm: learned association of predation risk with novel auditory stimuli by fathead minnows (*Pimephales promelas*) and glowlight tetras (*Hemigrammus erythrozonus*) after single simultaneous pairings with conspecific chemical alarm cues. Environ. Biol. Fish. 81, 141–147.

Yan, H.Y., Fine, M.L., Horn, N.S., Colon, W.E., 2000. Variability in the role of the gasbladder in fish audition. J. Comp. Physiol. A 186, 435–445.