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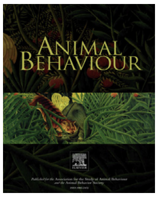

Mosquitofish use the past experiences of others with risk to make shoaling decisions

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In social species, individuals can use a variety of cues to inform their association patterns. For example, individuals might use information about the rank, recent diet and disease state of potential partners to make association decisions. However, whether individuals use the past experiences of others with predation risk to inform their association decisions remains unknown. Associating with experienced individuals might enable social transmission of predator information and prepare individuals for dangers they have not yet encountered personally. Alternatively, individuals might avoid such predator-experienced individuals and the potential high predation risk nearby. Here, I manipulated the exposure to predation risk of laboratory-born mosquitofish, *Gambusia affinis*, creating high-risk individuals (exposed to alarm cues 9 × over 18 days) and low-risk individuals (exposed to water only). I tested preference of naïve laboratory-born focal fish to associate with these high-risk and low-risk individuals, allowing both visual and chemical cues, in a choice test. Under safe conditions, focal fish associated equally with the high-risk and low-risk individuals. I then added alarm cue to the testing tank and again measured association preferences. Under these risky conditions, the focal fish increased their time spent associating with the low-risk partner. Importantly, high-risk and low-risk partners did not differ in swimming activity. These results suggest that during interactions, individuals can not only detect the past experiences of others with predation risk, but that they use this information to alter their shoaling decisions under certain conditions, specifically when they themselves encounter cues of predation risk.

Making the appropriate trade-offs between antipredator behaviour and other necessary activities (e.g. foraging, mating) requires prey to assess predation risk, but due to spatial and temporal variability in predator densities, prey vary in their personal experience with predators (Lima & Dill, 1990). Learning about the likelihood of predator encounters within an area from others without having to encounter a predator personally would be particularly beneficial and occurs in a variety of taxa (Crane & Ferrari, 2013; Hoppitt & Laland, 2008; Kendal et al., 2018). For example, Indian mynahs, *Sturnus tristis*, become more cautious in locations where they have witnessed conspecifics being chased and captured by humans (Griffin & Boyce, 2009; Griffin & Haythorpe, 2011). Social transmission of information about predation risk occurs in many aquatic species and can be surprisingly complex (reviewed in Crane & Ferrari, 2013). For example, naïve individuals can learn that a predator cue is dangerous by interacting with experienced individuals encountering that cue (Ferrari, Trowell, Brown, & Chivers, 2005; Kelley, Evans, Ramnarine, & Magurran, 2003; Mathis, Chivers, & Smith, 1996) and can socially acquire fear-induced neophobia similar to that displayed by experienced partners (Crane, Bairos-Novak, Sacco, & Ferrari, 2018). Thus, choosing to associate with experienced individuals might enable social transmission of predator information and prepare individuals for dangers they have not yet encountered personally.

However, it is also possible that given a choice, individuals might avoid such predator-experienced individuals whose presence might be indicative of a high predation risk environment or of vulnerability to predator encounters. Instead, individuals might prefer to associate with unstressed individuals indicative of a low predation environment, similar to the common preference to associate with unparasitized individuals to reduce one's risk of parasite exposure (Behringer, Karvonen, & Bojko, 2018; Hoare & Krause, 2003). Additionally if one has not yet encountered a predator, associating with similarly naïve individuals might increase the phenotypic homogeneity of the group, thereby decreasing individual vulnerability to predation risk (Landeau & Terborgh, 1986; Penry-Williams, Ioannou, & Taylor, 2018). There

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is substantial evidence that fishes prefer to shoal with individuals that are similar to them in a variety of traits, such as pattern, size and sex (Cattelan & Griggio, 2018; Hoare & Krause, 2003; Hoare, Krause, Peuhkuri, & Godin, 2000; Pitcher & Parrish, 1993).

Decisions to associate with particular individuals can also be affected by the social and environmental experiences of partners. For example, individuals in many social taxa often choose to associate with more experienced and dominant individuals (Jaatinen & Öst, 2011; Jordan, Wong, & Balshine, 2010; Smith, Memenis, & Holekamp, 2007). In fishes, shoaling decisions can be based on a variety of partner attributes that are tied to their previous experiences, such as their previous diet (Morrell, Hunt, Croft, & Krause, 2007; Ward, Hart, & Krause, 2004) and recent habitat (Ward et al., 2004). Shoaling preferences can also be affected by witnessing the performance of potential partners in competitive foraging tasks (Metcalfe & Thomson, 1995) and in their inspection of a nearby predator (Dugatkin & Alfieri, 1991). However, it remains unknown whether shoaling decisions based on partner experiences extend to the past experiences of potential shoaling partners with predation risk.

In this study, I examined whether western mosquitofish, *Gambusia affinis*, a social shoaling fish, use information on the past experiences of others with risk to inform their own shoaling decisions. I manipulated the background risk of individuals who then acted as potential shoaling partners. With both visual and olfactory cues available, naïve individuals could choose between associating with a partner who had experienced cues of predation risk in the past (high-risk partner) or a partner that had not (low-risk partner). I tested this association pattern under both safe and potentially dangerous conditions.

METHODS

Fish Collection and Housing

In summer 2016, I collected pregnant female western mosquitofish from Lake Cheston, Sewanee, Tennessee, U.S.A., which has many piscivorous fishes including largemouth bass, *Micropterus salmoides*. The fish used in this study were laboratory-born (F1) sexually immature juveniles from these field-impregnated females (>24 families). After birth, full/half siblings were spread over >80 1.9-litre tanks in small groups of two to four siblings. A month before starting treatments, all families were combined and >250 juveniles were housed across at least eight 38-litre tanks with plastic plants and sponge filters. Before starting treatments (see below), a subset of similarly sized juveniles was randomly pulled from these multiple tanks and redistributed in new groups of 10 and put into 10 new 19-litre treatment tanks (filled with approximately 16 litres of water) covered on three sides with black opaque plastic with a sponge filter and plastic plants. I used the fish in these 10 treatment tanks as partners for focal fish and they received water or alarm cues based on their treatment (see Past Experiences with Risk Section). I housed focal fish separately from the treatment fish and they were left in their original 38-litre housing tanks (in large groups with plastic plants and sponge filters). Focal fish received no treatment cues and were left undisturbed throughout the treatment time period (see Past Experiences with Risk Section). I randomly distributed tanks of treatment fish and focal fish across several shelves and put opaque plastic between tanks to prevent interactions.

I fed fish daily ad libitum (frozen *Daphnia*, tetramin flakes and live *Artemia*) and the room was kept at 22 °C on a 14:10 h light:dark cycle. Since juveniles were not sexually mature, males and females could not be distinguished. I tested fish when they were approximately 10–15 mm standard length, but fish were not measured to minimize handling stress for subsequent studies. Within trials, I attempted to size-match focal and partner fish within 1–2 mm.

Alarm Cue

Most aquatic animals, including fishes, can detect chemical cues of damaged conspecific tissue (i.e. alarm cues) and respond with a variety of antipredator behaviours, such as increased shoaling and use of refuges, and decreased swimming activity (Ferrari, Wisenden, & Chivers, 2010). To make alarm cue, I euthanized six field-collected adult female mosquitofish by decapitation with sharp scissors, removed all gut and reproductive organs, and sonicated the entire body (excluding organs and head) with deionized water (following standardized protocols, Brown & Godin, 1999). I filtered the ground tissue through polyester floss, adding deionized water until approximately 0.04 cm^2 of fish tissue per 1 ml was achieved. Whether mosquitofish react differently to adult versus juvenile cues is unknown. While some fishes respond similarly to alarm cues from differently aged conspecifics (Brown, Foam, Cowell, Guevara-Fiore, & Chivers, 2004; Carreau-Green, Mirza, Martínez, & Pyle, 2008), others do not (Lönnstedt & McCormick, 2011). I made alarm cue from adult females to maximize the amount of tissue while minimizing the number of animals euthanized. I stored alarm cue and deionized water (for controls) in 10 ml tubes in a −20 °C freezer. Freezing alarm cues minimizes the total number of animals euthanized and is common practice across studies (e.g. Brown & Godin, 1999; Ferrari et al., 2005; Joyce, Demers, Chivers, Ferrari, & Brown, 2016).

Past Experience with Risk

I exposed the 10 treatment tanks (five of each treatment) containing 10 juveniles each to either alarm cue (resulting in a 'high-risk treatment') or deionized water (resulting in a 'low-risk treatment') by adding 2.5 ml of either alarm cue or water to their tank every other day for 18 days. Thus, over the course of 18 days, individuals in the high-risk (or low-risk) treatment had nine additions of alarm cue (or water). Studies often use repeated exposure to alarm cues to alter the background risk level and induce 'high-risk' phenotypes, with exposures occurring more than once a day (2–3×) and over multiple sequential days (4–7 days) (e.g. Brown, Ferrari, Elvidge, Ramnarine, & Chivers, 2013; Chivers, McCormick, Mitchell, Ramasamy, & Ferrari, 2014; Crane et al., 2018). The experienced treatment used here had a similar total number of alarm cue exposures, but exposures were spread out over a longer period and interspersed with periods of no risk. Accumulation of cues in treatment tanks was unlikely as all tanks had bacterial sponge filters and alarm cue degrades within hours (Chivers, Dixson, White, Mccormick, & Ferrari, 2013; Wisenden, Rugg, Korpi, & Fuselier, 2009). I did not add any cues for 2 days prior to testing. During this time period, focal fish tanks did not receive any cues/treatment.

Preference Assay

I conducted preference assays in multiple 38-litre tanks (50 × 25 cm) filled to 5 cm depth (with a total of 7.5 litres of water) and covered on three sides with opaque black plastic. Tanks had two 20 cm choice zones at either end and a middle 10 cm 'no-choice' zone. At either end, within each 20 cm choice zone, I placed a treatment fish in a fine-mesh breeding box (Marina©: 16 × 12.5 × 13 cm, ~1 mm mesh size made up of transparent nylon threads of <0.1 mm thickness) that allowed the exchange of visual and chemical cues. Both visual and chemical cues have been shown to be important in shoaling decisions based on habitat and diet

(Ward et al., 2004). I first determined the side of the tank to place each treatment partner (high- versus low-risk) fish by coin toss and then alternated treatment partners between trials. I rotated through each treatment tank as the source for high-risk and low-risk partners, and I used only a subset of potential partners from each tank (six of the 10 fish available). I transferred fish between tanks in water-filled cups. Trials were videorecorded from several feet away to minimize disturbance.

I placed one focal fish in an opaque cylinder in the centre of the preference tank for 1 min to acclimate. Upon release, I measured the shoaling behaviour of the focal fish in terms of its willingness to approach and spend time near each partner. Specifically, I measured the number of times the focal fish entered the different choice zones and the total time the focal fish spent associating with the high-risk and low-risk partner over 3 min. I defined an entrance into a choice zone as occurring when the focal fish's head crossed the choice-zone line, and I defined the association time as the total time spent in either choice zone over the 3 min. I then added 1 ml of alarm cue (0.04 cm^2 tissue per ml) to the centre of each tank (regardless of the focal fish position). Note that this is roughly half of the daily alarm cue that had been released into the treatment tanks (2.5 ml), but the volume of water in the preference assay tank (7.5 litres) was approximately half that of the treatment tanks (16 litres). After adding the alarm cue, I again measured the time the focal fish spent associating with each partner for 3 min. I separately measured the time each partner spent actively swimming (i.e. not ‘frozen’) during these 3 min trials, both before and after the alarm cue was added.

After each trial, I replaced the focal fish and both treatment partners, and I did not reuse any fish. I rinsed choice tanks and completely replaced the water between trials. I conducted all trials between 0930 and 1700 hours on a single day in January 2017, prior to feeding fish. Videos were scored using JWatcher (Blumstein, Daniel, & Evans, 2010). A student blind to the experimental design scored the focal behaviour and I scored the partner behaviour purposely blind to focal fish behaviour and in a haphazard order with respect to partner treatment and alarm cue presence. I tested 30 focal fish but excluded one trial because the focal fish moved under a partner box, resulting in a final sample size of 29 focal fish (with 58 different partners).

Data Analysis

Since the time spent with each partner within a trial are not independent, either within or between time periods, I compared the time focal individuals spent with the low-risk versus high-risk partner, as well as the number of times the focal individual entered the choice areas of the low-risk versus high-risk partner, within a single 3 min assay (before or after the alarm cue) with paired nonparametric Wilcoxon signed-rank tests. I opted for nonparametric tests based on the distribution of the data. The amount of time each partner spent actively swimming over the 3 min assays was examined in a similar way. I also examined whether the addition of alarm cue affected the total time individuals spent shoaling with a partner, regardless of their identity, with paired nonparametric Wilcoxon signed-rank tests. I calculated Cohen's *d* estimates to provide insight into the biological significance of the patterns (Cohen, 1988). I show means ± SE throughout. All analyses were conducted in R (v.1.1.453, R Core Team, 2018) and figures in ggplot2 (Wickham, 2016).

Ethical Note

All animal collections were approved by the Tennessee Wildlife Resources Agency (TWRA permit number 3896) and all experiments were approved by the ACUC at the University of the South (protocol number McGhee 1–2016). Field collections and housing conditions in the laboratory adhered carefully to Guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2018). Adult fish (the parents of the F1 offspring used here) were collected with dip-nets and transferred immediately to coolers of lake water with plant cover. Coolers were transferred by car to the laboratory within approximately 1 h. In the laboratory, field-collected fish remained in the coolers for ~24 h and were gradually acclimated to the temperature and water conditions in the laboratory before being transferred to new tanks. In addition to daily care, weekly water testing and ad libitum feeding with diverse food types, all tanks had enrichment (e.g. plastic plants) and constant water movement with bacterial filtration (e.g. sponge filters and airstones), animals were housed in groups unless being tested in an assay, and transferring to and from test tanks was via water-filled cups. Tennessee scientific collection permits prohibit release of any wild-caught aquatic animal after 12 h. Thus, the animals that were euthanized to make alarm cue in this experiment could not have been released and had been living in ‘retirement’ tanks from earlier field collections. To minimize pain and stress during euthanasia, females were housed in water-filled containers with plastic plants that were slowly cooled by surrounding the holding container with ice in a darkened box prior to decapitation. The laboratory-born fish used in this experiment were transferred to multiple large stock tanks for future experiments.

RESULTS

Focal individuals often interacted with their partners through the net boxes, following each other up and down during the trials. Partners spent >75% of trials actively swimming throughout their container regardless of their past experiences and presence of alarm cue (proportion of time moving before alarm cue: low-risk partner = 0.79 ± 0.05; high-risk partner = 0.76 ± 0.03; Wilcoxon signed-rank test: $V = 159$, $N = 58$ partners, $P = 0.213$, Cohen's $d = 0.14$; after alarm cue added: low-risk partner = 0.79 ± 0.05; high-risk partner = 0.78 ± 0.05, $V = 185$, $N = 58$ partners, $P = 0.495$, Cohen's $d = 0.04$). To examine how the added alarm cue might have dispersed during the trial and whether it might have affected partner behaviour directly, I conducted a follow-up test using food colouring as a proxy for the alarm cue. I found that 1 ml of food colouring dispersed into the choice areas in 40 s and reached the breeding boxes in 45 s. Although the food colouring dispersed throughout 75% of each choice area within 90 s, it remained concentrated along the outer edges of the breeding box with very limited movement into the box, suggesting that partners might not have encountered the added alarm cue during the testing assay.

Before the alarm cue was added, focal individuals spent similar amounts of time associating with both partners (Wilcoxon signed-rank test: $V = 218$, $N = 29$, $P = 1$, Cohen's $d = 0.24$; Fig. 1). After the alarm cue was added, focal individuals significantly increased their total shoaling time (i.e. less time in no-choice zone) (before: 116.9 ± 8.4 s; after: 143.5 ± 4.9 s; Wilcoxon signed-rank test: $V = 93$, $N = 29$, $P = 0.006$, Cohen's $d = 0.72$). Interestingly, this increased time spent shoaling was not divided equally between low-risk and high-risk partners. Instead, after the alarm cue was added, focal individuals spent significantly more time with the low-risk partner compared to the high-risk partner (Wilcoxon signed-rank test: $V = 116$, $N = 29$, $P = 0.027$, Cohen's $d = 0.82$; Fig. 1). Although the time spent in the choice areas differed based on partner treatment in the presence of alarm cue, focal fish entered both choice areas equally (number of enters before alarm cue: low-risk partner = 2.2 ± 0.2; high-risk partner = 1.9 ± 0.3; Wilcoxon

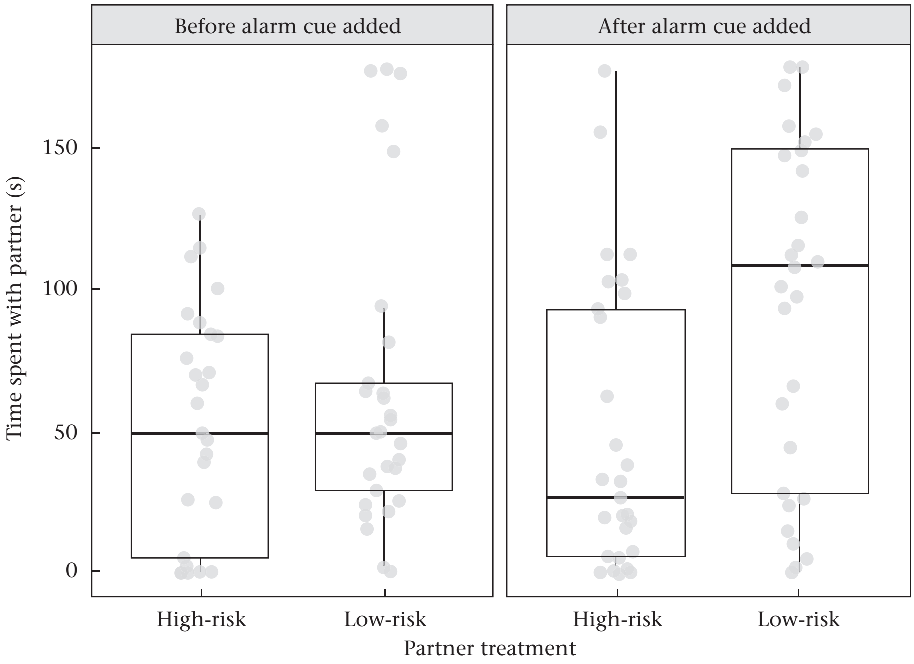

Figure 1. Association time of naïve focal individuals with an alarm cue-exposed, high-risk partner or a water-exposed, low-risk partner before and after alarm cue was added ($N = 29$ focal fish, grey symbols). Boxes enclose interquartile range with median indicated as a thick line and whiskers extending to 1.5 × the interquartile range.

signed-rank test: $V = 65$, $N = 29$, $P = 0.111$, Cohen's $d = 0.19$; after alarm cue added: low-risk partner = 2.7 ± 0.3; high-risk partner = 2.8 ± 0.5, $V = 162.5$, $N = 29$, $P = 0.748$, Cohen's $d = 0.05$). All focal fish entered both choice areas over the course of the entire 6 min assay.

All data have been deposited in the Mendeley Data Repository (https://data.mendeley.com/datasets/64bj3t26fx/draft?a=37944553-1975-4f99-8a54-6d76686ebd4e).

DISCUSSION

In a novel environment without risk cues, individuals spent similar amounts of time associating with both low-risk and high-risk partners. However, under conditions of risk (i.e. alarm cue present), focal individuals increased the time they spent associating with their water-exposed, low-risk partner. Increased shoaling behaviour in response to predator cues is consistent with previous studies (Ferrari et al., 2010; Lima & Dill, 1990), and being in a group rather than alone offers prey protection and decreases an individual's own vulnerability to predators (Hoare & Krause, 2003; Landeau & Terborgh, 1986; Pitcher & Parrish, 1993). That individuals preferred to associate with low-risk partners instead of partners experienced with high-risk when alarm cues were present suggests that individuals can not only detect the past risk experiences of others based on their interactions with them, but that they use this information to alter their shoaling decisions under potentially risky conditions.

How individuals assess the past risk exposure of others remains unclear. Both visual and chemical cues could be involved (Dellinger, Zhang, Bell, & Hellmann, 2018; Ward et al., 2004), and this experiment allowed for both modes of communication. It is possible that high-risk partners reacted differently to the alarm cue than low-risk partners and this affected the shoaling decisions of focal individuals. For example, high background levels of risk due to repeated exposure to alarm cues can cause individuals to react to subsequent alarm cue with a reduced antipredator response, consistent with the risk allocation hypothesis (Ferrari et al., 2010), or alternatively, high-risk individuals might react more strongly to alarm cue exposure (Chivers et al., 2014). Interestingly, in my study, low-risk and high-risk partners showed similarly high levels of swimming activity during the trials, both before and after the alarm cue was added (actively swimming 76–79% of time). Based on the limited dispersal of food colouring into the net breeding boxes, it seems likely that little alarm cue moved into the net boxes. Thus, the lack of response to the alarm cue by the partners might be due to only weak cues (or subthreshold levels) being detected, which can result in no overt behavioural response (Brown, Poirier, & Adrian, 2004), or detection of no alarm cue at all. Partner fish might also have behaved similarly due to potentially seeing one another and influencing one another's behaviour, although they were separated by two layers of fine mesh and at least 20 cm. Regardless of the underlying cause of the partner behaviour, the differential association patterns of focal individuals cannot be explained by focal individuals choosing to associate with the most active or visible partner. Shoaling decisions can also be affected by relatedness and familiarity (Jordan et al., 2010; Ward & Hart, 2003), with preference for familiar individuals persisting for >2 weeks to >2 months (Ward & Hart, 2003). Although partners and focal individuals had been mixed from >24 families, redistributed multiple times since birth across multiple tanks, and were all housed separately for >2 weeks, some of the fish within trials might have been full/half siblings and could have interacted in the past. Thus, it is possible that relatedness and/or familiarity might also have played a role in shoaling decisions.

It seems likely that subtle behavioural or chemical differences might allow focal individuals to distinguish the partners of each treatment from one another. The stress hormone cortisol can passively diffuse across the gills in fishes (Sadoul & Geffroy, 2019) and thus could be detectable in the water, potentially during the close interactions between partners. Chronic exposure to predation cues tends to elevate cortisol levels (Dellinger et al., 2018), but whether any diffusion would be detectable within the short trials used here is unknown, as is the type of information that would be gained by its detection. Interestingly, male threespine stickleback, *Gasterosteus* *aculeatus*, can detect the previous predator-exposure status of potential mates and show less courtship to predator-exposed females (Dellinger et al., 2018; McGhee, Feng, Leasure, & Bell, 2015). This response is primarily due to visual behavioural cues from females, but when predator-exposed females release more cortisol into the water than control females, olfactory cues seem to play an additional role (Dellinger et al., 2018). The exact behavioural and/or chemical mechanisms that enable individuals to distinguish between low-risk and high-risk partners remain unclear but could be particularly important for issues of animal welfare and housing, and the transmission of stress (Crane et al., 2018).

Why choose to associate with low-risk partners over high-risk partners under conditions of risk? Presumably if danger is detected, shoaling decisions should minimize an individual's vulnerability to predation (Hoare & Krause, 2003; Lima & Dill, 1990; Pitcher & Parrish, 1993). Focal individuals might perceive a partner's reaction to alarm cue as informative about the location or intensity of the danger (although see above about the weak movement of alarm cue into the net boxes). For example, focal individuals might infer that danger is closer to high-risk partners and further from low-risk partners, and avoid the high-risk partner (and the potential nearby danger) in response. Other studies have indeed found that prey can extrapolate information from chemical cues to different habitats (Mitchell, Crane, Bairos-Novak, Ferrari, & Chivers, 2018) as well as to future time points (Crane & Ferrari, 2017). Individuals also prefer to shoal with morphologically similar individuals (Cattelan & Griggio, 2018; Hoare et al., 2000), with dissimilar, odd individuals in a group being more likely targeted by predators (Landeau & Terborgh, 1986; Penry-Williams et al., 2018). It is possible that assessment of ‘similarity’ extends beyond morphological traits to past experiences and behaviour. For example, focal individuals, naïve to alarm cues, might prefer to shoal with similarly naïve low-risk partners and avoid dissimilar high-risk partners. Consistent with this are studies in which individuals prefer to associate with others that have experienced similar diets and habitats (Morrell et al., 2007; Ward et al., 2004). Future studies extending this work to larger groups under conditions of variable predation threat would help us understand how these shoaling decisions might affect vulnerability to predation.

Predation risk affects the formation of social networks (Hasenjager & Dugatkin, 2017; Kelley, Morrell, Inskip, Krause, & Croft, 2011), interaction rules (Herbert-Read et al., 2017) and the stability of social relationships (Heathcote, Darden, Franks, Ramnarine, & Croft, 2017) in groups where all members have had similar experiences. This study suggests that shoaling patterns could also be shaped by the past experiences of individual group members with predation risk, potentially leading to the separation of individuals based on their previous encounters with predators. Avoidance of previously stressed individuals could also further increase the vulnerability of those individuals to predation if they remain solitary and cannot rely on the safety of a group. Furthermore, cultural transmission is an important aspect of learned predator recognition (Ferrari et al., 2005; Kelley et al., 2003; Mathis et al., 1996), and association patterns among individuals will affect the flow of this information within and among groups. Thus, an individual's encounters with predators in the past can extend further than simply affecting their own behaviour (Clinchy, Sheriff, & Zanette, 2013). These experiences can affect the shoaling decisions of others, with the potential to indirectly shape association patterns and change how individuals interact with the larger ecological community.

Declaration of Interest

None.

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References

ASAB/ABS. (2018). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour, 147*. i–x.

Behringer, D. C., Karvonen, A., & Bojko, J. (2018). Parasite avoidance behaviours in aquatic environments. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*(1751), 20170202. http://doi.org/10.1098/rstb.2017.0202.

Blumstein, D. T., Daniel, J. C., & Evans, C. S. (2010). *JWatcher (Version 1.0)*. Retrieved from http://www.jwatcher.ucla.edu/.

Brown, G. E., Ferrari, M. C. O., Elvidge, C. K., Ramnarine, I., & Chivers, D. P. (2013). Phenotypically plastic neophoia: A response to variable predation risk. *Proceedings of the Royal Society B: Biological Sciences, 280*, 20122712. http://doi.org/10.1098/rspb.2012.2712.

Brown, G. E., Foam, P. E., Cowell, H. E., Guevara-Fiore, P., & Chivers, D. P. (2004a). Production of chemical alarm cues in convict cichlids: The effects of diet, body condition and ontogeny. *Annales Zoologici Fennici, 41*, 487–499.

Brown, G. E., & Godin, J.-G. (1999). Chemical alarm signals in wild Trinidadian guppies (*Poecilia reticulata*). *Canadian Journal of Zoology, 77*(4), 562–570. http://doi.org/10.1139/cjz-77-4-562.

Brown, G. E., Poirier, J. F., & Adrian, J. C. (2004b). Assessment of local predation risk: The role of subthreshold concentrations of chemical alarm cues. *Behavioral Ecology, 15*(5), 810–815. http://doi.org/10.1093/beheco/arh084.

Carreau-Green, N. D., Mirza, R. S., Martínez, M. L., & Pyle, G. G. (2008). The ontogeny of chemically mediated antipredator responses of fathead minnows *Pimephales promelas*. *Journal of Fish Biology, 73*(10), 2390–2401. http://doi.org/10.1111/j.1095-8649.2008.02092.x.

Cattelan, S., & Griggio, M. (2018). Within-shoal phenotypic homogeneity affects shoaling preference in a killifish. *Biology Letters, 14*(8). http://doi.org/10.1098/rsbl.2018.0293.

Chivers, D. P., Dixson, D. L., White, J. R., Mccormick, M. I., & Ferrari, M. C. O. (2013). Degradation of chemical alarm cues and assessment of risk throughout the day. *Ecology and Evolution, 3*(11), 3925–3934. http://doi.org/10.1002/ece3.760.

Chivers, D. P., McCormick, M. I., Mitchell, M. D., Ramasamy, R. A., & Ferrari, M. C. O. (2014). Background level of risk determines how prey categorize predators and non-predators. *Proceedings of the Royal Society B: Biological Sciences, 281*(1787), 20140355 http://doi.org/10.1098/rspb.2014.0355.

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology, 27*(1), 56–65. http://doi.org/10.1111/1365-2435.12007.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum.

Crane, A. L., Bairos-Novak, K. R., Sacco, L. H., & Ferrari, M. C. O. (2018). The socially mediated recovery of a fearful fish paired with periodically replaced calm models. *Proceedings of the Royal Society B: Biological Sciences, 285*, 20180739. http://doi.org/10.1098/rspb.2018.0739.

Crane, A. L., & Ferrari, M. C. O. (2013). Social learning of predation risk: A review and prospectus. In K. B. Clark (Ed.), *Social learning theory* (pp. 53–82). Hauppauge, NY: Nova Science.

Crane, A. L., & Ferrari, M. C. O. (2017). Evidence for risk extrapolation in decision making by tadpoles. *Scientific Reports, 7*, 1–7. http://doi.org/10.1038/srep43255.

Dellinger, M., Zhang, W., Bell, A. M., & Hellmann, J. K. (2018). Do male sticklebacks use visual and/or olfactory cues to assess a potential mate's history with predation risk? *Animal Behaviour, 145*, 151–159. http://doi.org/S000334721830294X.

Dugatkin, L. A., & Alfieri, M. (1991). Guppies and the tit for tat strategy: Preference based on past interaction. *Behavioral Ecology and Sociobiology, 28*(4), 243–246. http://doi.org/10.1007/BF00175096.

Ferrari, M. C. O., Trowell, J. J., Brown, G. E., & Chivers, D. P. (2005). The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Animal Behaviour, 70*(4), 777–784. http://doi.org/10.1016/j.anbehav.2005.01.009.

Ferrari, M. C. O., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: A review and prospectus. *Canadian Journal of Zoology, 88*(7), 698–724. http://doi.org/10.1139/Z10-029.

Griffin, A. S., & Boyce, H. M. (2009). Indian mynahs, *Acridotheres tristis*, learn about dangerous places by observing the fate of others. *Animal Behaviour, 78*, 79–84.

Griffin, A. S., & Haythorpe, K. (2011). Learning from watching alarmed demonstrators: Does the cause of alarm matter? *Animal Behaviour, 81*(6), 1163–1169. http://doi.org/10.1016/j.anbehav.2011.02.022.

Hasenjager, M. J., & Dugatkin, L. A. (2017). Fear of predation shapes social network structure and the acquisition of foraging information in guppy shoals. *Proceedings of the Royal Society B: Biological Sciences, 284*(1867), 20172020. http://doi.org/10.1098/rspb.2017.2020.

Heathcote, R. J. P., Darden, S. K., Franks, D. W., Ramnarine, I. W., & Croft, D. P. (2017). Fear of predation drives stable and differentiated social relationships in guppies. *Scientific Reports, 7*(1), 1–10. http://doi.org/10.1038/srep41679.

Herbert-Read, J. E., Rosén, E., Szorkovszky, A., Ioannou, C. C., Rogell, B., Perna, A., et al. (2017). How predation shapes the social interaction rules of shoaling fish. *Proceedings of the Royal Society B: Biological Sciences, 284*(1861), 20171126. http://doi.org/10.1098/rspb.2017.1126.

Hoare, D. J., & Krause, J. (2003). Social organisation, shoal structure and information transfer. *Fish and Fisheries, 4*(3), 269–279. http://doi.org/10.1046/j.1467-2979.2003.00130.x.

Hoare, D. J., Krause, J., Peuhkuri, N., & Godin, J.-G. (2000). Body size and shoaling in fish. *Journal of Fish Biology, 57*(6), 1351–1366. http://doi.org/10.1006/jfbi.2000.1446.

Hoppitt, W., & Laland, K. N. (2008). Social processes influencing learning in animals: A review of the evidence. *Advances in the Study of Behavior, 38*(08), 105–165. http://doi.org/10.1016/S0065-3454(08)00003-X.

Jaatinen, K., & Öst, M. (2011). Experience attracts: The role of age in the formation of cooperative brood-rearing coalitions in eiders. *Animal Behaviour, 81*(6), 1289–1294. http://doi.org/10.1016/j.anbehav.2011.03.020.

Jordan, L. A., Wong, M. Y. L., & Balshine, S. (2010). The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biology Letters, 6*, 301–303. http://doi.org/10.1098/rsbl.2009.0732.

Joyce, B. J., Demers, E. E. M., Chivers, D. P., Ferrari, M. C. O., & Brown, G. E. (2016). Risk-induced neophobia is constrained by ontogeny in juvenile convict cichlids. *Animal Behaviour, 114*, 37–43. http://doi.org/10.1016/j.anbehav.2016.01.007.

Kelley, J. L., Evans, J. P., Ramnarine, I. W., & Magurran, A. E. (2003). Back to school: Can antipredator behaviour in guppies be enhanced through social learning? *Animal Behaviour, 65*(4), 655–662. http://doi.org/10.1006/anbe.2003.2076.

Kelley, J. L., Morrell, L. J., Inskip, C., Krause, J., & Croft, D. P. (2011). Predation risk shapes social networks in fission–fusion populations. *PLoS One, 6*(8). http://doi.org/10.1371/journal.pone.0024280.

Kendal, R. L., Boogert, N. J., Rendell, L., Laland, K. N., Webster, M., & Jones, P. L. (2018). Social learning strategies: Bridge-building between fields. *Trends in Cognitive Sciences, 22*(7), 651–665. http://doi.org/10.1016/j.tics.2018.04.003.

Landeau, L., & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour, 34*, 1372–1380.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology, 68*, 619–640.

Lönnstedt, O. M., & McCormick, M. I. (2011). Chemical alarm cues inform prey of predation threat: The importance of ontogeny and concentration in a coral reef fish. *Animal Behaviour, 82*(2), 213–218. http://doi.org/10.1016/j.anbehav.2011.04.015.

Mathis, A., Chivers, D. P., & Smith, R. J. F. (1996). Cultural transmission of predator recognition in fishes: Intraspecific and interspecific learning. *Animal Behaviour, 51*, 185–201.

McGhee, K. E., Feng, S., Leasure, S., & Bell, A. M. (2015). A female's past experience with predators affects male courtship and the care her offspring will receive from their father. *Proceedings of the Royal Society B: Biological Sciences, 282*(1819), 20151840. http://doi.org/10.1098/rspb.2015.1840.

Metcalfe, N. B., & Thomson, B. C. (1995). Fish recognize and prefer to shoal with poor competitors. *Proceedings of the Royal Society B: Biological Sciences, 259*(1355), 207–210. http://doi.org/10.1098/rspb.1995.0030.

Mitchell, M. D., Crane, A. L., Bairos-Novak, K. R., Ferrari, M. C. O., & Chivers, D. P. (2018). Olfactory cues of habitats facilitate learning about landscapes of fear. *Behavioral Ecology, 29*(3), 693–700. http://doi.org/10.1093/beheco/ary024.

Morrell, L. J., Hunt, K. L., Croft, D. P., & Krause, J. (2007). Diet, familiarity and shoaling decisions in guppies. *Animal Behaviour, 74*(2), 311–319. http://doi.org/10.1016/j.anbehav.2006.10.021.

Penry-Williams, I. L., Ioannou, C. C., & Taylor, M. I. (2018). The oddity effect drives prey choice but not necessarily attack time. *Ethology, 124*(7), 496–503. http://doi.org/10.1111/eth.12754.

Pitcher, T. J., & Parrish, J. K. (1993). Functions of shoaling behaviour in teleosts. In T. J. Pitcher (Ed.), *Behaviour of teleost fishes* (2nd ed., pp. 363–439). London, U.K.: Chapman & Hall.

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Sadoul, B., & Geffroy, B. (2019). Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology, 94*(4), 540–555. http://doi.org/10.1111/jfb.13904.

Smith, J. E., Memenis, S. K., & Holekamp, K. E. (2007). Rank-related partner choice in the fission–fusion society of the spotted hyena (*Crocuta crocuta*). *Behavioral Ecology and Sociobiology, 61*(5), 753–765. http://doi.org/10.1007/s00265-006-0305-y.

Ward, A. J. W., & Hart, P. J. B. (2003). The effects of kin and familiarity on interactions between fish. *Fish and Fisheries, 4*(4), 348–358. http://doi.org/10.1046/j.1467-2979.2003.00135.x.

Ward, A. J. W., Hart, P. J. B., & Krause, J. (2004). The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. *Behavioral Ecology, 15*(6), 925–929. http://doi.org/10.1093/beheco/arh097.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag. Retrieved from http://ggplot2.org.

Wisenden, B. D., Rugg, M. L., Korpi, N. L., & Fuselier, L. C. (2009). Lab and field estimates of active time of chemical alarm cues of a cyprinid fish and an amphipod crustacean. *Behaviour, 146*(10), 1423–1442.