The Dose Makes the Poison: The Anti-predator Responses of the Southern Redbelly Dace

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Erin Schwarzbach 12/03/2020

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ABSTRACT

Social organisms use chemical alarm cues, which warn conspecifics of danger. A well-known case of this is seen in Ostariophysan fishes in the form of *Schreckstoff*. Historically, an extract made from skin was used to test fish for their fright response, however it is difficult to determine *Schreckstoff* concentrations and ensure consistency among trials and across species. The objectives of this study were to: (1) to determine if chondroitin, an easily measurable hypothesized replacement for skin extract, is an effective alarm substance for our focal species *Chrosomus erythrogaster*; and (2) assess anti-predator behaviors in the wild. We hypothesized that chondroitin would elicit the same fright response as conspecific skin extract. We also hypothesized that wild *C. erythrogaster* would respond to conspecific skin extract with anti-predator behaviors that are detected during aquarium-held experiments. We exposed fish to conspecific skin extract and two concentrations of chondroitin, and compared the responses; neither concentration of chondroitin elicited similar responses to conspecific skin extract. Fish exposed to conspecific skin extract spent significantly more time *Darting* ($F_{2, 32} = 5.01$, $p = 0.0128$) and *Burrowing* ($F_{2, 32} = 3.31$, $p = 0.049$) than fish exposed to either chondroitin concentration. There were no instances of any other typical anti-predator behaviors in the chondroitin trials. These results do not support the hypothesis that chondroitin is an effective alarm substance for *C. erythrogaster*. In the field, fish were exposed to both a water control and conspecific skin extract. We did not find any *Burrowing* or *Freezing* behaviors in response to skin extract, nor was there a significant difference in number of fish pre and post exposure to conspecific skin extract ($p = 0.718$). In addition, we observed a *Scatter* behavior in response to conspecific skin extract in the natural environment, similar to that of *Darting* in laboratory experiments. Behavioral responses to chondroitin may be species-specific as a function of the

concentration of chondroitin in the skin, or interplay between chemicals released from tissue damage. Further, we encourage future studies to focus on the *Scatter* behavior performed by this fish to elucidate the anti-predator responses in natural environments.

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Introduction

Communication, the transmission of information from one animal to another (Green and Marler 1979), is well documented (Wilson 1972, Endler 1993, Seyfarth and Cheney 2003). It can lead to either fitness benefits or losses (Gardner and West 2004), and is used to convey messages involving sociality, mating, defense or predator avoidance (Hay 2009). Generally, animals use visual, auditory, tactile, or chemical signals for communication. Signals are a specific mode of communication which contain information that will trigger the receiver to modify their behavior, often categorized into four parts: a reason for signaling, a voluntary reaction, a receiver reaction, and a fitness benefit to either the sender, receiver, or both (Barios-Novak et al. 2019). Signals can be honest, in which both sender and receiver benefit; deceitful, in which the sender benefits and the receiver loses; or eavesdropping, where the sender loses and the receiver benefits. If neither sender nor receiver benefits the signal is considered spiteful (Gardner and West 2004).

Alarm signals are anti-predator adaptations, emitted by animals to alert others of the presence of predators (Chivers and Smith 1998, Evans et al. 1993, Koenig et al. 1991, Müeller-Schwarze et al. 1984). It is a form of honest signaling, which takes cooperation from both sender and receiver. Unlike signals, cues are released involuntarily (Barios-Novak et al. 2019), although they still influence the future action of the receiver (Maynard Smith and Harper 1995). Chemical alarm cues are released during predator-induced damage, leading to anti-predator behaviors performed by the receivers. When the skin of the Western Toad larvae (*Anaxyrus boreas*) is damaged in an attempted predation event, they release chemical alarm cues. These cues lead to conspecifics increasing activity and avoiding the area (Hews 1988). Additionally, when sea urchins, *Diadema antillarium*, encounter crushed conspecifics, they move away from the stimulus (Müller-Schwarze 1980).

Schreckstoff

Schreckstoff is the chemosensory alarm cue substance found in fishes of the superorder Ostariophysi and order Gonorhynchiformes (Pfeiffer 1967). The superorder Ostariophysi contains approximately 64% of all freshwater fish species and includes catfish, loaches, suckers, and minnows (Nelson 1994). Receivers of the alarm cue display numerous anti-predator behaviors, which are often species-specific and vary within their habitat (Smith 1992). These responses also depend on concentration of the alarm cue; Roach (*Rutilus* rutilus) can be seen swimming at low concentrations but hide at high concentrations (Jachner and Rydz 2002). In the case of Zebrafish, the intensity of the anti predator response is positively related to the dosage (Speedie and Gerlai 2008).

The *Schreckstoff* substance of Ostariophysan fishes is located in the epidermal club cells, a diagnostic characteristic of the superorder. These club cells can be identified via staining methods as unstained oval or round cells with the nucleus in the center (Chivers et al. 2007). They are deeply embedded in the epidermal matrix and lack ducts to the external environment (Chivers et al. 2007, Chivers et al. 2012). Therefore, for the contents of club cells to be released, the skin must be physically abraded, such as in a predation event (Smith 1992).

Identification of which chemicals are released from epidermal clubs cells has been challenging. Specifically, identifying which components may be responsible for eliciting the fright reaction is crucial to understanding the proximate pathways of the Ostariophysan alarm system. While numerous chemicals are released upon tissue damage, the Ostariophysan anti-predator response is restricted to certain components of the epidermal club cells (Smith 1986). This may be an advantage by limiting confusion caused by multiple chemicals released at a single time (Smith 1986). Further, it was proposed that Ostariophysan alarm cues were

composed of species-specific combinations of purine molecules (Kelly et al. 2006), indicating the importance of determining the active components in the Schreckstoff substance. Hypoxanthine-3(N)-oxide was proposed as the alarm chemical (Argentini 1976), and tested on multiple species (Pfeiffer et al. 1985, Mathis et al. 1995, Brown et al. 2000). The chemical was often tested at high concentrations in order to produce a response, however when high performance liquid chromatography was used, it did not match the peaks of the skin extract of Fathead Minnows (Smith 1999). This suggested another chemical component might elicit the response. Biochemical fractionation revealed chondroitin-4-sulfate and chondroitin-6-sulfate as major components of Zebrafish (*Danio rerio*) club cells. Both these chemicals elicited typical fright response behaviors in the species as well as activated the olfactory bulb the same way conspecific skin extract does (Mathuru et al. 2012). Chondroitin is a glycosaminoglycan, which plays a significant role in immune homeostasis and in the inflammatory response (Ronca et al. 1998; Vailliéres and Souich 2010). In addition to eliciting anti-predator behaviors in Zebrafish, chondroitin also elicited anti-predator behaviors in the Northern Studfish (*Fundulus catenatus*) (Farnsley et al. 2016), suggesting that chondroitin may lead to anti-predator behaviors in other species, and may be the primary alarm component of Ostariophysan club cells.

Wild vs. Aquarium Held Behavioral Responses

While fright responses have been well documented from fish in laboratory settings (von Frisch 1938, Pfieffer 1967, Pfieffer 1977, Smith 1992, Dupuch 2004, Bairos-Novak et al 2019), there are few studies that explicitly examine responses of individuals in native habitats to the putative alarm cue, and the few studies which have done so have mixed results. Wild European Minnows (*Phoxinus phoxinus*) did not modify their behavior when skin extract was introduced into a small pond, nor did they attempt to escape the exposed area (Magurran et al 1996).

However, wild Northern Redbelly Dace (*Chrosomus eos*) did avoid areas exposed to conspecific skin extract in a native lake (Wisenden and Barbour 2005). Finescale Dace and Fathead Minnows also avoid areas in which conspecific skin extract is released (Friesen and Chivers 2006). While these studies focused on number of fish in an exposed area and if they attempted to escape the area, a lack of direct comparisons among behaviors seen in laboratory experiments (dashing, freezing, increased shoal cohesion) to those in native environments reduces our ability to determine the relevance of aquarium held responses.

The anti-predator behaviors of numerous Ostariophysan species have been documented, although the diversity of minnows in North America, especially in the South Eastern United States, leads to a variety of different responses. Northern Redbelly Dace (*Chrosomus eos*) are a well-studied North American minnow example (Dupuch et al. 2004, Wisenden and Barbour 2005). Northern Redbelly Dace (NRBD) (family Leucisidae) are often found in lakes and ponds in the northern United States. NRBD respond to conspecific skin extract by moving closer to the substrate and farther away from the conspecific skin extract, often avoiding a 2-8 meter area where the skin extract has been released in the natural environment (Wisenden and Barbour 2005). Furthermore, NRBD show increased in-group cohesion, and exhibit dashing and freezing behaviors in response to conspecific skin extract in laboratory experiments (Dupuch et al. 2004). Southern Redbelly Dace (SRBD, *C. erythrogaster*), are a sister species to the Northern Redbelly dace. They are exclusively small-stream (2^{nd} and 3^{rd} order) dwelling minnows found in the Ohio, Missouri, and Mississippi River drainages and are locally abundant in central Tennessee. They have been documented to exhibit some degree of anti-predator behavior in response to conspecific skin extract (Verheijen 1963, Pfeiffer 1977); observed behaviors were similar to other minnows such as looking for shelter, increased shoal cohesion, and agitated swimming

(Verheijen 1963). While the fright response of NRBD has been thoroughly studied in the natural environment, the difference in habitats between NRBD and SRBD may lead to the species performing different anti-predator behaviors.

In this paper we set out to determine the role of chondroitin as an alarm substance for SRBD, and compare the behaviors seen in a laboratory setting to those in the natural environment. We hypothesize: *(1)* that chondroitin would elicit similar anti-predator responses to that of conspecific skin extract; and *(2)* That wild SRBD respond to conspecific skin extract with the same anti-predator behaviors detected in aquarium-held experiments. We predicted that chondroitin would lead to an increase in time spent *Freezing, Darting,* and *Burrowing*, as well as increased group cohesion. We also predicted that wild fish would increase time spent *Freezing, Darting,* and *Burrowing* when exposed to conspecific skin extract in their natural environment.

Materials and Methods

Stimulus preparation and titration

To characterize the fright response of SRBD and to compare the response between skin extract and chondroitin, we created two types of stimuli: skin extract and chondroitin. We created skin extract stimulus using five SRBD individuals, following the protocols of Smith (1989), Cashner (2004), and Stabell (2010). We homogenized 0.45 g of skin in 20 mL of room temperature spring water in a 50 mL flask. We then filtered the mixture over batting to remove scales and other large fragments, and obtained a concentrated volume of 35 mL. We added 180 mL of spring water to create a final diluted volume of 215 mL.

The high concentration chondroitin stimulus was made from 0.7 g chondroitin sulfate sodium salt from shark cartilage (Sigma C4383) dissolved in 50 mL room temperature spring water, creating an equivalent concentration to that used by Mathuru et al. (2012). A second,

lower concentration, chondroitin stimulus was also made following the protocol from the 2017 United States Pharmacopeia. We used three standards of shark chondroitin diluent (2.5 mg/ 5 mL, 5 mg/5 mL, 7.5 mg/5 mL), and used 5 mL of our diluted skin extract solution as our unknown. Peak absorbance was measured with a photoelectric probe and the three standards ranged from 6.08×10^{-2} to 1.40 absorbance units. We recorded a peak absorbance of 3.6850×10^{-2} for our skin extract, which was lower than our lowest standard. Therefore, we chose a concentration half of the lowest standard, 1.25 mg of chondroitin, as our lower concentration. Based on these results, we dissolved 0.0125 g chondroitin sulfate sodium salt from shark cartilage (Sigma C4383) in 50 mL of room temperature spring water to create a lower concentration chondroitin solution. All three stimuli (skin extract, 0.7 g chondroitin, 0.0125 g chondroitin) were divided into aliquots and frozen for future use.

Collection and Housing

Fish were collected from an unnamed tributary of Millers Creek (Cumberland River system) in Cheatham County, TN (TWRA Permit # 1779) using a small seine, and transported to the housing facility in aerated buckets. The housing facility was a controlled environment at Austin Peay State University, which consisted of a 10-gallon acclimation tank and six 10-gallon experimental tanks. Aquaria were set up with sponge filters with cotton batting, gravel, and dechlorinated tap-water. During all acclimation and testing periods, the individuals were subjected to a 12 hours light/dark cycle and water temperature of approximately 24º C. The fish were held in the acclimation tank for at least a week or until they were feeding regularly and no longer exhibited high stress behaviors such as increased opercular movement frequency, excessive darting, or reduced swimming activity.

Post-acclimation, fish were transferred to clean 10-gallon experimental tanks. Three fish were assigned to each tank using a random number generator, and each tank was randomly assigned a stimulus (conspecific skin extract, 0.07 mg chondroitin, 0.0125 mg chondroitin). Because they are a shoaling species, fish were put into groups of three to accelerate habituation and reduce the stress that they would be under if housed individually (Abrahams and Colgan 1985). Furthermore, we have found that housing SRBD individually increases stress, morbidity, and attempts to escape the aquarium (*pers. obs*). Fish were allowed a three-day acclimation period to the new tanks before testing began. Fifty-four individuals were used during all aquarium trials, and each was used only once.

Aquarium-held Response to Skin Extract and Chondroitin

To determine if chondroitin acts as an effective alarm substance for SRBD we compared the average time spent performing three target behaviors pre and post stimulus introduction across the three stimuli (conspecific skin extract, 0.07 g chondroitin, 0.0125 g chondroitin). We focused on three behaviors that are indicative of an anti-predator response in many minnows: *Darting* (rapid movements in which the fish is swimming at speeds at least two times their standard swimming speed), *Burrowing* (the fish digging it's body into the gravel substrate), and *Freezing* (periods of fully stopped movement for five seconds or longer). We also concentrated on changes in shoal cohesion. We used a two way repeated measures ANOVA along with a post-hoc pairwise Tukey's HSD to assess differences in our focal behaviors amongst the three stimuli pre and post stimulus introduction.

Black paper was placed between aquaria to block visual cues among experimental tanks, and each stimulus type was tested using six replicates. The stimulus was introduced to each tank using a 5 mL syringe attached to 3 cm of fresh aquarium tubing on the side of the tank with the

aerator. For each replicate, we filmed the fish for three minutes, introduced 5 mL of skin extract solution, and continued filming for another three minutes to compare behaviors pre and post stimulus introduction.

We used VidSync v 1.661 software to determine time spent *Darting* and to calculate average distance between fish in order to investigate changes in shoal cohesion. Time spent *Burrowing* and *Freezing* were assessed visually using raw footage. All video footage was calibrated following instructions from VidSync v. 1.661 (Neuswanger et al., 2016). Distortion correction chessboards and calibration frames were created following the design on the VidSync website (http://www.vidsync.org/Hardware), printed on clear vinyl, and attached to 20 by 31 cm Plexiglas boards. The correction chessboard was placed inside the tank against the front pane to correct radial distortion, while the correction frames were placed inside the tank against the front and back panes, 26.67 cm apart. The calibration boards were recorded for each tank and then removed. A 30-minute window occurred between calibration and the "pre stimulus introduction" recording period. Distortion correction and 3D calibrations were performed in the software using default instructions from the VidSync website before analysis began.

Field Experiments

Field experiments were conducted at Dry Fork Creek in Davidson County, TN (36.2636 N, -87.8548 W), a tributary of Marrowbone Creek and part of the Cumberland River watershed. Water temperature was consistently 16^{O} C throughout the May and June testing period, and testing occurred on two days, four weeks apart. The first day consisted of two replicates with both taking place in runs. The second consisted of three replicates, two in runs and one in a pool. No localities were repeated. Trials were filmed underwater with a GoPro Hero8 anchored near the substrate using stream rocks and filming downstream. Cameras were placed in localities

where large shoals of SRBD were identified via bank observation 20 minutes prior to testing. Recording began when the video camera was placed in the stream to avoid frequent disturbance to the focal area. Fresh aquarium tubing, 0.9 m in length, was secured to the bottom of the stream, with the end positioned in front of the camera lens. Two pieces of aquarium tubing were used, one for each stimulus (conspecific skin extract or water control), in order to avoid mixing stimuli. Calibration boards were used and recorded for future video correction. The boards were placed in the stream with the first board 30.5 cm in front of the camera the second board 30.5 cm behind that. After manipulating the calibration equipment in the stream, we waited one minute, or until fish exhibited acclamation, whichever came first, to begin observations. We considered fish acclimated when the shoal returned to the area.

We used Bright Dyes fluorescent green tablets to determine flow rate, and observed that a stimulus enters and dissipates from the area within one minute in fast moving streams and five minutes in the pool. We recorded the field of view for three minutes pre and three minutes post stimulus introduction, but following the results of the flow assessment, we only used the first one-minute of pre and post stimulus introduction in analysis for the run habitats. Further, to remove the confounding variable of different habitats and flow rates, only the four run locations were used for data analysis.

We introduced the stimulus (5 mL of skin extract or 5 mL of distilled water) via syringe through the in-stream tubing, and then blew through the tubing to ensure the entire stimulus entered the stream. Each location was exposed to both skin extract and water, and the order of exposure was determined by coin flip, with a three-minute acclimation period between treatment observations. Video calibration and defined behaviors were the same for both the field and the lab components, with the addition of "average distance of fish from the stimulus introduction

tube". In addition to video footage, we used *ad libitum* sampling to make visual observations from above the water. We characterized the fright response of wild SRBD by calculating average time spent performing each of our focal behaviors. We used a two way repeated measures ANOVA along with a post-hoc Tukey's HSD to assess differences in our focal behaviors among the two stimuli (conspecific skin extract or water) pre and post stimulus introduction. We also used a two way repeated measures ANOVA to evaluate the effect of stimuli and time on number of fish present, and a two-way repeated measures ANOVA to evaluate the effect of stimuli and time on distance of fish from tubing. All statistical analyses were performed in R Studio (R Core Team 2017).

Results

Comparison of Responses between Chondroitin levels and skin extract

Fish exposed to conspecific skin extract spent significantly more time *Darting* than either chondroitin stimulus ($F_{2, 32} = 5.01$, $p = 0.0128$) (Fig 1A). The effect of time (pre or post) was also significantly different among treatments ($F_{1, 32} = 5.01$, $p = 0.027$). This was also the case for *Burrowing*. Fish exposed to conspecific skin extract spent significantly more time *Burrowing* than either chondroitin stimulus ($F_{2, 32} = 3.31$, $p = 0.049$) (Fig 1B). Time spent *Freezing* did not differ among treatments ($F_{2, 32} = 1.104$, $p = 0.344$) (Fig 1C), nor did average distance between bodies ($F_{2, 32} = 0.22$, $p = 0.803$).

Field Response

Fish did not perform any *Burrowing* or *Freezing* behaviors in the natural environment. We did detect a trend that fish exposed to conspecific skin extract spent more time *Darting* post introduction than those exposed to the control ($F_{1, 12} = 4.65$, $p = 0.052$) (Fig 2). We used distance from tubing as a proxy for group cohesion in the field. The effect of time (pre or post) on

distance from tubing was not significant ($F_{1, 12}$= 0.038, p = 0.848), nor was the effect of stimulus (control or conspecific skin extract) ($F_{1, 12}$ = 0.843, p = 0.377) (Fig 3A). The effect of stimulus and time were also analyzed with regards to number of fish in view of the camera. The effect of time was not significant ($F_{1, 12}$= 0.907, p = 0.360), nor was the effect of stimulus ($F_{1, 12}$ = 0.012, p = 0.914) (Fig 3B).

We catalogued our bank observations in an ethogram (Table 1) to capture behaviors that may not be visible from the underwater camera perspective. Before exposure to either stimulus, fish were foraging in a shoal, moving in a figure-eight pattern. Upon introduction of conspecific skin extract into the stream, fish scattered with no specific direction. The scatter was up to a three-meter distance from injection site, and the shoal lost it's foraging organization. This behavior was also seen when we entered the stream to set-up equipment, but was never seen when the control stimulus was introduced. Juvenile fish, estimated based on body length, which were roughly 50% smaller than adults, stayed within one-meter of the introduction site longer than larger (adult) fish. Fish returned to foraging behavior in a figure-eight pattern within thirty seconds for runs and one minute in the pool site.

Discussion

Our data suggests that chondroitin does not elicit anti-predator responses in lab held SRBD. While variation in anti-predator behaviors across species and populations may result from differences in stimulus composition and concentration, we demonstrated that SRBD did not respond to either high or low concentrations of chondroitin. Southern Redbelly Dace only increase *Darting* and *Burrowing* activity when exposed to a conspecific skin extract; these behaviors are in line with those seen by von Frish in European Minnows (1939), and Smith's (1992) account of the Ostariophysan anti-predator behaviors. Increased darting is also a behavior

similar to the agitated swimming described by Verheijen (1963) for the species. Darting behaviors likely confuse predators but also alert conspecifics that there is a threat (Dupuch et al., 2004). Vereheijen (1963) previously documented increased shoal cohesion; although we did not detect a significant response in shoaling behavior, this may be a result of small sample size (six replicates).

We did not test chondroitin in natural environments because we could not determine that this chemical to elicits anti-predator behaviors in a laboratory setting. It may be proposed that species have different levels of chondroitin in their skin, influencing species-specific response to the chemical. Chondroitin may elicit anti-predator responses in non-Ostariophysan fish such as the Northern Studfish (Farnsley et al. 2016) or may be species specific such as for Zebrafish (Mathuru et al. 2012), but is not effective for the SRBD.

Wild SRBD do not respond to conspecific skin extract the exact same way as in aquarium-based experiments. There were no instances of *Freezing* or *Burrowing* seen in a native stream; instead, fish responded to conspecific skin extract with a *Scatter* behavior not noted in aquarium experiments of this type. This *Scatter* behavior only occurred under putatively predator-simulated conditions (humans entering the stream and release of conspecific skin extract into the water). This may be a reflection of the *Darting* behavior that is seen in aquaria, and should be looked into further. In aquaria *Darting* behaviors may persist longer than in natural environments because there is no flow through of water to dissipate the stimulus and nowhere for the fish to escape. In the wild, *Scatter* may take less time than *Darting* due to the larger area of movement, places to hide, and flow of the water through the natural habitat. It likely serves as a way to confuse predators just as *Darting* does (Dupuch et al. 2004).

We suggest that flow rate and hydrology play an important role in cue detection as it does in other fields (Song et al. 2017). We based our methodology and observation period length on visual observation that the stimulus can enter and dissipate within one minute in fast moving riffles, and dissipates within 5 minutes in pools after first spreading vertically. This is in line with a critique that 30-minute observations periods are too long and that most of the behavior may fall within the first 2-3 minutes post exposure (Friesen and Chivers 2005). We found that in fast moving riffles the behavior may take place within the first thirty-seconds post exposure. Further, we noted that fish move in and out of view throughout the data-recording period, therefore, using the number of post-exposure fish in an area as a metric may not be as informative in this environment based on camera footage alone.

When fish skin is damaged, alarm cues are released into the water, and the ability to detect and respond to this cue is important for survival. SRBD perform anti-predator behaviors in response to conspecific skin extract in both laboratory and field settings, although the behaviors differ between settings. Size of the area exposed and flow play an important role in detection of behaviors and their duration. This may be the key to understanding the difference between behaviors observed in aquaria versus natural environments. Additionally, although multiple chemical compounds have been proposed as a universal alarm compound (Pfeiffer et al. 1985, Brown et al. 2000, Mathuru et al. 2012), the chemical component that elicits the response for this species has yet to be uncovered.

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Table 1: A table defining the behavioral states recorded in the laboratory and field studies of anti-predator responses of *C. erythrogaster*.

Category	Behavior	Description	Stimulus	# Tanks or sites Performing Behavior	Average duration (s)
Field	Freezing	Periods of fully stopped movement for five seconds or longer, often seen after erratic behavior and seen when introduced to all three stimuli	Skin extract	6	8.28 s
			High	1	0.27 s
			Low	0	0.00 s
	Darting	Visually rapid movements in which the fish is swimming at speeds at least two times their standard swimming speed	Skin extract	6	15.27 s
			High	4	1.00 s
			Low	1	0.27 s
	Burrowing	Digging self into the gravel substrate using side of the body, following the introduction of an alarm substance. Seen in every fish post conspecific skin extract introduction, but not seen upon introduction of either chondroitin concentration with the exception of one outlier	Skin extract	5	65.44 s
			High	4	46.00 s
			Low	4	23.50 s
Laboratory	Foraging	Organized movement of five or more fish in a figure eight pattern over a three meter distance in search of food, seen before a stimulus introduction and within a minute post introduction	Skin extract	5	N/A
			Control	5	N/A
	Scatter	Burst of fast movement in no organized pattern or direction upon introduction of stimulus into the stream. Also seen when humans entered the stream to manipulate equipment	Skin extract	5	N/A
			Control	5	N/A

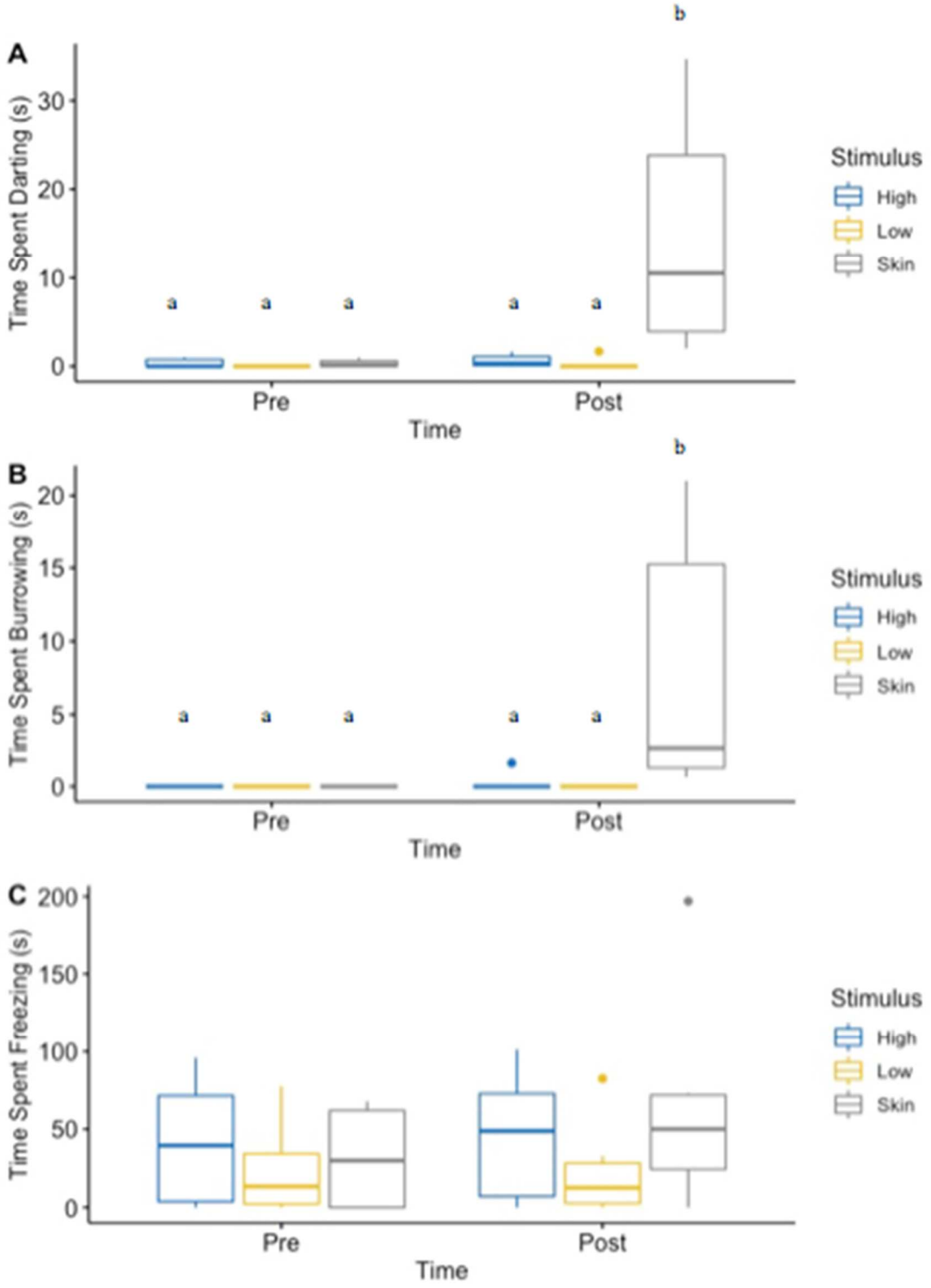

Figure 1. Boxplot showing the distribution (median and interquartile ranges) of time spent (A) *Darting* (B) *Burrowing* (C) *Freezing* pre and post introduction stimulus (high chondroitin concentration, low chondroitin concentration, or conspecific skin extract). Points above and below the whiskers indicate outliers. For each behavior, boxes marked with different letters are significantly different (Tukey's HSD post hoc comparison, $p < 0.05$).

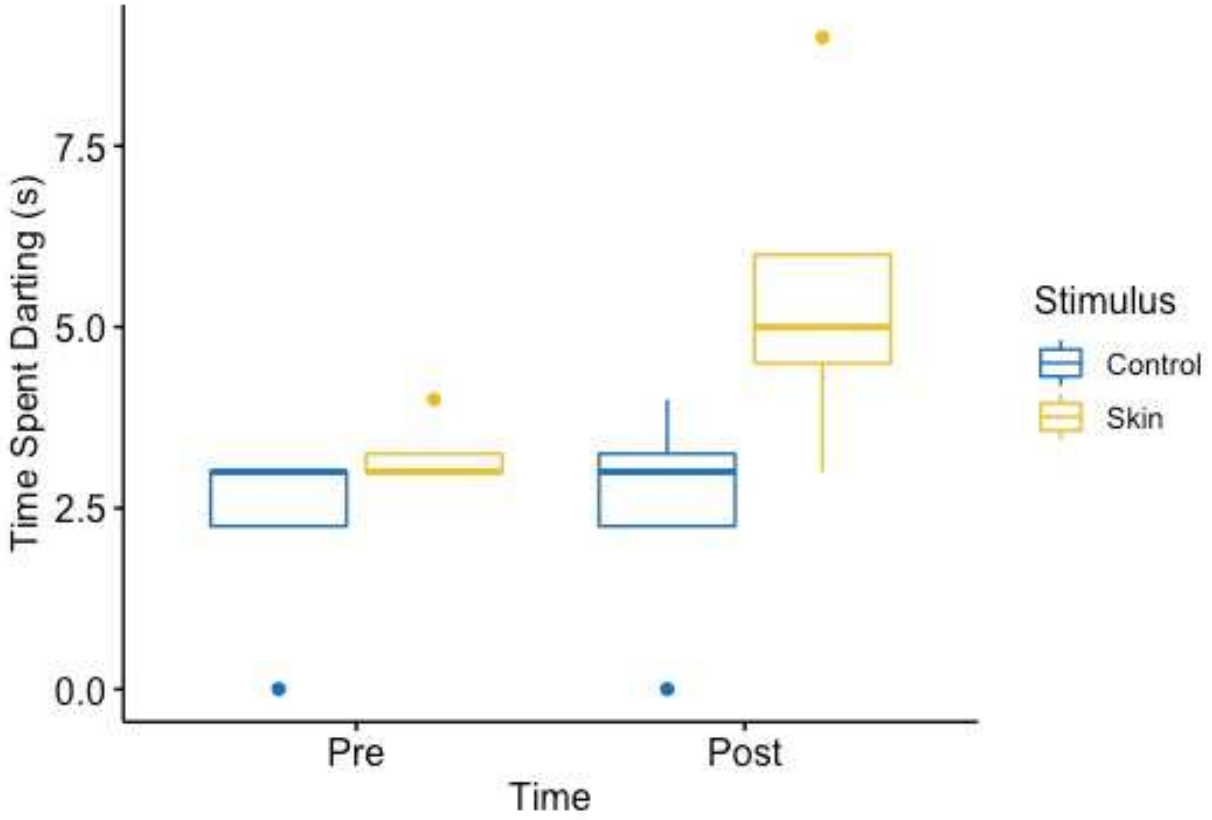

Figure 2. Boxplot showing the distribution (median and interquartile ranges) of time spent *Darting* pre and post introduction stimulus (control or conspecific skin extract) in the field. Points above and below the whiskers indicate outliers (ANOVA $p = 0.052$).

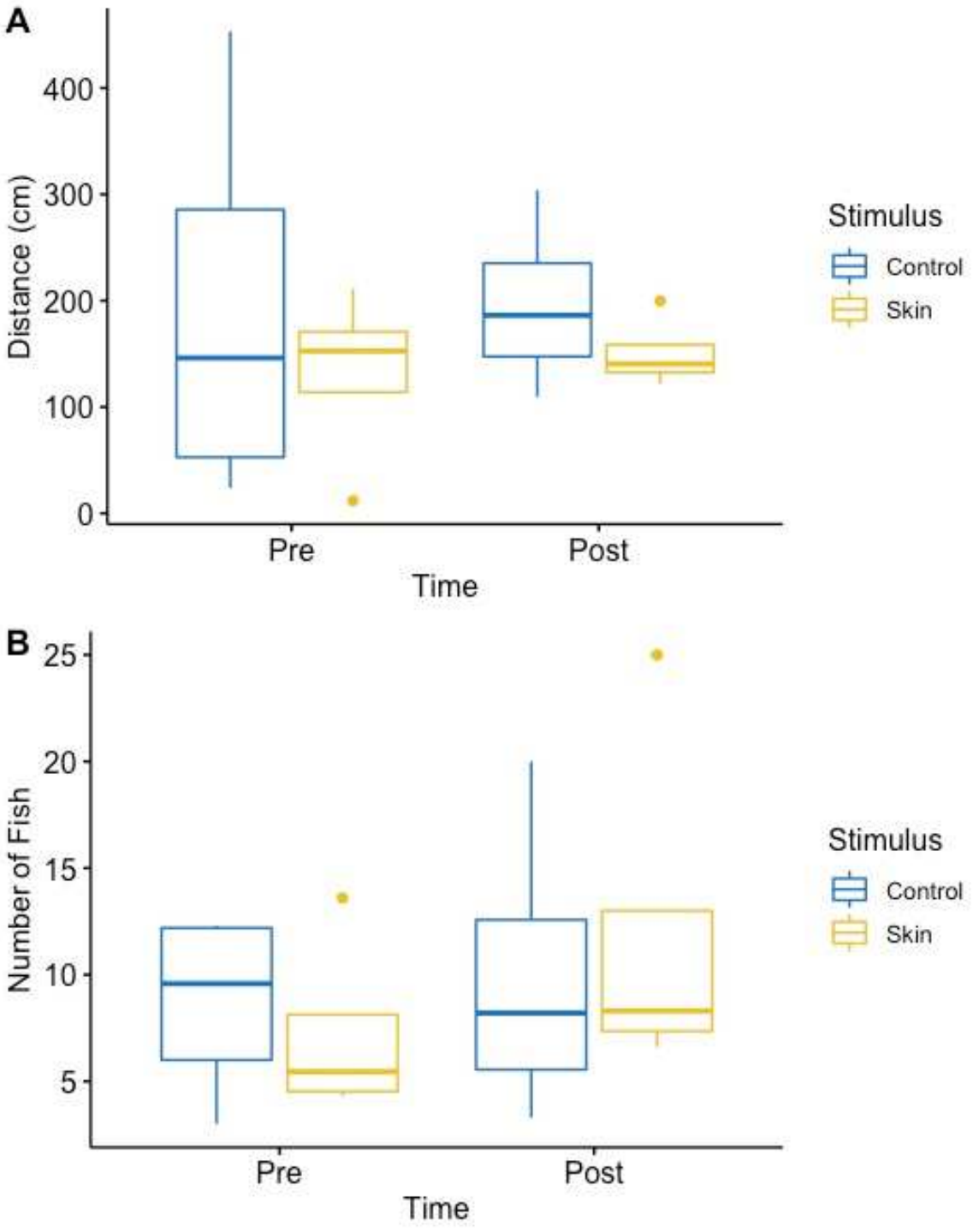

Figure 3. Boxplot showing the distribution (median and interquartile ranges) of (A) distance from fish to tubing (B) number of fish in view of the camera pre and post introduction stimulus (control or conspecific skin extract) in the field. Points above and below the whiskers indicate outliers (ANOVA $p = 0.377$, $p = 0.914$).