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Behavioral responses of a highly domesticated, predator naïve rainbow trout to chemical cues of predation

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ABSTRACT

Fisheries management agencies allocate significant proportions of available resources to rear fish for stocking in lakes, rivers, and reservoirs. However, domesticated fish reared in a hatchery environment may fail to exhibit normal antipredator behavior and can have relatively low survival when released into natural habitats. Exposing hatchery fish to natural predator cues can provide information about their capacity to exhibit appropriate behavioral responses and has the potential to enhance antipredator behavior and subsequent survival in the wild. We investigated immediate behavioral responses to an acute exposure to chemical cues of predation in highly domesticated, hatchery-reared rainbow trout *Oncorhynchus mykiss*. We used a frequently stocked and economically important strain of rainbow trout, the German Rainbow (GR), which is resistant to whirling disease but particularly susceptible to predation. We exposed individual rainbow trout to alarm cues from conspecifics, kairomones from brown trout *Salmo trutta* predators, and a combination of the two cues. Fish exposed to these cues exhibited changes in behavior expected to reduce predation risk, including a reduction in time spent actively swimming and exploring, and an increase in time spent frozen. Thus, these highly domesticated, hatchery-reared fish retain the innate ability to express appropriate responses to the threat of predation. Future research should investigate whether repeated exposure to predation cues in a hatchery setting could translate to long-term enhancement of antipredator behavior and increased survival rates, as this would provide a rapid, simple and low cost way to increase the efficiency of stocking programs for recreational purposes and, more importantly, native fish restoration and conservation.

1. Introduction

In the US, fisheries management agencies stock millions of fish annually in rivers, lakes, and reservoirs. Because these efforts are costly (Johnson and Martinez, 2000), an increase in efficiency of rearing or stocking practices has the potential to benefit hatchery and management programs. Specifically, techniques that increase survival of hatchery fish post-stocking can increase the number of fish available for anglers while reducing the number of fish required for rearing and stocking (Olson et al., 2012; Vilhunen, 2006). In addition, invaluable fish restoration efforts can be hindered when piscivores (introduced and native) consume stocked native fish (Karam and Marsh, 2010). Thus, techniques that increase post-stocking survival may be beneficial for native fish restoration and conservation efforts (e.g., Hutchison et al., 2012).

Stocked fish tend to have relatively low survival rates, especially immediately after they are stocked, presumably in part because naïve hatchery fish do not exhibit natural behavior important for survival in the wild, including appropriate foraging and antipredator behavior (Brown and Laland, 2001; Brown et al., 2003; Suboski and Templeton, 1989). Poor post-stocking survival associated with reduced antipredator behavior may be due to hatchery-selection, differential experience with ecologically relevant cues, or a combination of the two (Jackson and Brown, 2011). Many animals, including fish, exhibit innate antipredator behavior (Ferrari et al., 2010b). However, experience with predators and their cues can allow for higher potential survival after stocking through

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enhancement of antipredator behavior (Brown and Smith, 1998; Hawkins et al., 2008; Vilhunen, 2006).

There are many ways to evaluate and enhance ecologically relevant behavior in hatchery fish, including providing access to habitat structure (Braithwaite and Salvanes, 2005) and direct exposure to predators or to predator models (Brown and Magnavacca, 2003), but experience with chemicals of predation has shown particular promise (Vilhunen, 2006). Chemical cues that elicit antipredator behavior include both conspecific alarm cues (Ferrari et al., 2010b) and predator produced kairomones (Hawkins et al., 2008). Exposing hatchery-reared fish to these chemical cues prior to release can reveal their ability to exhibit appropriate responses and has the potential to enhance antipredator behavior (Chivers et al., 2002 *in* fathead minnow *Pimephales promelas*; D'Anna et al., 2012 *in* white seabream *Diplodus sargus sargus*; Hutchison et al., 2012 *in* Murray cod *Maccullochella peelii*; Mirza and Chivers, 2000 *in* brown trout *Salmo trutta*). Pairing conspecific alarm cues with predator kairomones may allow fish to more effectively associate the predator kairomone with danger (Berejikian et al., 2003; Brown et al., 2013; Ferrari et al., 2005). The coupling of these cues has also shown to have additive effects resulting in a stronger behavioral response when compared to the use of alarm cue or predator cue alone (Brown and Smith, 1998). These techniques may be particularly useful for domesticated fish strains since responses to predators decline with each generation in captivity (Brown and Laland, 2001; Jackson and Brown, 2011). Both laboratory and field experiments have shown that experience recognizing predators leads to increased survival during predator encounters highlighting the importance of providing appropriate experience with predators to hatchery-reared fish (Brown et al., 2013; Brown and Laland, 2001; Chivers et al., 2002; Mirza and Chivers, 2000). Fish exposed to these cues exhibit an increase in natural antipredator behavior, including spending more time in a frozen posture or hidden under cover and less time foraging or exploring (Brown et al., 2003; Brown and Laland, 2001; Brown and Smith, 1998). These differences in behavior can result in decreased vulnerability because of decreased conspicuousness (Mesa et al., 1994).

The German Rainbow (GR) is a hatchery-derived rainbow trout *Oncorhynchus mykiss* that has developed resistance to *Myxobolus cerebralis*, the parasite responsible for salmonid whirling disease, as a result of decades of exposure to the parasite in a German hatchery (Hedrick et al., 2003). This strain is fast-growing, matures early (Schisler and Fetherman, 2009), and has a low parasite burden following exposure to *M. cerebralis* (Fetherman et al., 2012; Hedrick et al., 2003; Schisler et al., 2006), making it desirable in rearing and stocking programs. However, due to its history of domestication, survival in the wild has been low (Fetherman and Schisler, 2013), and it is particularly susceptible to predation post stocking relative to other strains (unpublished data). Given the GR's relatively long history of domestication, it was unknown if artificial selection could have led to the loss of chemosensory risk assessment in this particular strain, or if they still possessed the innate ability to respond to chemical cues of predation. We performed two experiments, the first designed to determine whether alarm cues, predator cues from a brown trout predator, or a combination of these cues could be used to elicit antipredator behavior in GR fish. If hatchery-reared GR fish are capable of responding to predator cues, we predicted that fish exposed to either or both of these cues would exhibit greater antipredator behavior compared to control fish. Specifically, we expected fish exposed to these cues to spend less time actively swimming (following Ferrari et al., 2010a) and exploring (following Brown and Smith, 1998) and more time in a frozen posture (following Brown and Smith, 1998), near the bottom of the water column (following Berejikian et al., 1999), and in the shade. Further, we expected the combination of the two cues to have a greater influence on behavior than either cue alone due to their potential additive effects. In the second experiment, GR fish from the first experiment were immediately exposed to a brown trout predator. If prior exposure to predator cues enhances subsequent antipredator behavior, we expected fish previously exposed to chemical cues of predation to be more likely to use a protective structure compared to control fish. The ability of this highly domesticated strain to respond to chemical cues of predation and the short term persistence of antipredator behavior post exposure to these cues, during a live predator encounter, would provide additional support to suggest that the GR may benefit from training.

2. Materials and methods

2.1. Fish husbandry

We obtained 70 GR fingerlings from the Colorado Parks and Wildlife (CPW) Bellvue Fish Research Hatchery (Bellvue, CO) that ranged in size from 54 to 88 mm total length (TL). They were held in a 76 L recirculating tank in the CPW Toxicology Laboratory (Fort Collins, CO), maintained at 10.5 ± 1 °C, and fed trout starter feed (Rangen Inc.^{TM}, Idaho) at 1% of the total mass of fish in the tank, once daily.

Eighteen brown trout, each approximately 140 mm TL, were acquired from the CPW Bellvue-Watson Fish Hatchery (Bellvue, CO) for use in the predator encounter trials. Brown trout predators were selected because they are the most common predator of rainbow trout in the state of Colorado, and they were free of disease, readily available, and easy to maintain. The brown trout were housed in three, 133 L recirculating tanks that were subdivided into six sections by transparent, porous dividers and maintained at 10.5 ± 1 °C. Each tank and corresponding brown trout was assigned a number that was used to systematically choose a predator for encounter trials so that each predator was used only once.

To ensure that brown trout would exhibit natural predator behavior, we provided them with experience feeding on live prey prior to the study. Each fish was allowed three opportunities to eat live fingerling rainbow trout within a five-day period when their normal food, commercial pellets, was withheld. Only brown trout ($n = 16$) that aggressively consumed live prey were used in this experiment, resulting in the exclusion of two fish. Food (commercial pellets and live prey) was withheld from experimental brown trout for five days prior to the predator encounter trials to ensure active foraging during the experiment.

2.2. Chemical cue extraction

Alarm cue was extracted from the epidermal tissue of six recently decapitated GR rainbow trout 80 to 120 mm TL by scoring both sides of the fish in vertical, horizontal and diagonal directions with a razor blade. The skin was then washed with 30 mL of deionized water, and the liquid was strained through cheesecloth (following Nordell, 1998). Alarm cue was immediately frozen and stored at −20 °C.

Predator cue was collected from a single brown trout. Prior to this process, food was withheld from the brown trout ≥120 h to ensure no alarm cue was present due to gut retention of commercial feed. Predator cue was then collected by transferring and holding a brown trout in a 9.5 L non-circulating tank (width, length, and depth of 15, 30, and 20 cm, respectively) in a cold-water bath for 24 h. After 24 h, the brown trout was removed, and water from the tank was collected in 50 mL containers and stored in a freezer at −20 °C (following Brown and Smith, 1998).

2.3. Behavior trials

Rainbow trout were marked using Visual Implant Elastomer (VIE) tags (Northwest Marine Technology Inc., Shaw Island, WA). Each of 64 experimental fish was randomly assigned a color marking corresponding to each of the four treatments (alarm cue = red, alarm and predator cue combined = yellow, predator cue = blue, control = no mark) resulting in 16 fish per treatment. The fish were sedated using tricaine methanesulfonate (MS-222; Argent Laboratory, Redmond, WA), and VIE tags were injected under the skin on both sides of the dorsal fin using a hypodermic needle. Control fish were also sedated and a hypodermic needle was placed under the skin, but no elastomer was injected. Fish were resuscitated in aerated water and returned to the holding tank until they were used in the experiment.

Prior to each behavioral trial, four 9.5 L tanks (width, length, and depth of 15, 30, and 20 cm, respectively) were set up in a row and surrounded on all sides by a blind so that the fish could not see each other. Tanks were illuminated from above by two 60-W full spectrum bulbs, each centered over two tanks. To provide a darker section of the tank where fish could seek cover, a 5 cm shadow was created across the front of each tank by taping black cardboard to either side of the blind at an angle so it could not be seen by the fish.

Air tubes used to deliver treatment cues were taped along with an air stone to one corner of each tank (following Brown and Smith, 1998). The placement of the cue line and air stone was randomly chosen by assigning a number (1–4) to each corner of the tank (back right, back left, front right, and front left) and rolling dice for every trial. The tanks were then filled to approximately 4 cm away from the rim with 10.5 ± 1 °C water that lacked fish chemical cues.

On the day of each trial, alarm and predator cues were allowed to thaw at room temperature (20 °C). After thawing, 10 mL of liquid was loaded into syringes for each of the four treatments: 5 mL of alarm cue and 5 mL of deionized water for the alarm cue treatment, 5 mL of predator cue and 5 mL of deionized water for the predator cue treatment, 5 mL of alarm cue and 5 mL of predator cue for the combination treatment, and 10 mL of deionized water for the control treatment. Deionized water was selected as a control to ensure results would be comparable to other studies that used this approach (Berejikian et al., 2003; Ferrari et al., 2005; Vilhunen, 2006). Although some studies have evaluated fish behavioral responses to non-predatory fish odors as controls (e.g., Brown and Magnavacca, 2003; Chivers et al., 2002; Hawkins et al., 2008), these studies found a general lack of response to these odors, and this approach would have required introducing novel species (and potentially diseases) to the laboratory environment. All syringes had color-coded tape that corresponded to the treatment to prevent cross contamination of the delivery lines and tanks.

The four chemical treatments were randomly assigned to each of the four tanks (position 1–4 in the row of tanks) for the first test group, but were then systematically assigned for subsequent groups so that each position received all four treatments before repeating. Four rainbow trout with marks representing the four treatments were netted from the holding tank and measured (TL). Each fish was placed in its respective tank, covered by the blind, and allowed to acclimate for 60 min in fresh water aerated by an air stone.

Trials were recorded using video cameras, two mounted above the tanks for a top view and two mounted on the side for a side view. This allowed fish behavior to be extracted from video recordings and minimized disturbance during the trial. To begin the trial, air stones were turned off and behavior was recorded for 5 min, representing the pre-exposure period. After the initial five-minute period, the appropriate chemical treatment was added to the water and air stones were turned on for 1 min to allow water to circulate. Following the one-minute circulation period, the air stones were turned off and behavior was again recorded for five more minutes, representing the post-exposure period. Upon completion of the trial, cue lines were flushed with deionized water and tanks were removed and cleaned by soaking for 10 min in an iodine solution.

Video recordings were later viewed and scored for five different behavior metrics that were expected to reflect variation in antipredator behavior. An estimate of exploratory behavior was calculated by dividing the bottom of the tank into six equal sized squares (blocks) and counting the total number of blocks each fish entered, ranging from 1 to 6 (a fish was only considered to have entered the box if more than half of its TL crossed the line from one square into another). We also extracted the proportion of time spent actively swimming (defined by the body of the fish oscillating back and forth, or from side to side, and movement in a three dimensional space) and the proportion of time spent frozen (defined as no movement of the fins or body in any direction in a three dimensional space). These two behavioral states were mutually exclusive, but did not comprise 100% of the time budget since another state (swimming in place, where the caudal and pectoral fins move but the fish covers no ground) was identified but not analyzed. The final two behavioral states, proportional use of the top half of the water column and the proportion of time spent in the shade (specific metrics were used to define these two behaviors by having more than half of the TL in or out of shadow and, above or below the middle of the tank), were not mutually exclusive.

2.4. Predator encounter trials

Less than 5 min the end of the 5 min exposure to cues, fish were introduced into the predator trial. A white, circular, plastic 95 L tank (55 cm diameter and 41 cm depth) was used for the predator encounter tank. The tank was filled with 57 L of water free of chemical cues (10.5 ± 1 °C) and outfitted with an air stone. We placed a protective cage structure (width, length, and depth of 20, 18, and 40 cm, respectively) in the center of the tank that had holes small enough for the rainbow trout fingerlings to pass through but not the brown trout predator.

A brown trout was systematically chosen, based on a previously assigned number so that each predator was used once, placed in the encounter tank, and allowed to acclimate for 60 min with the air stone on. The air stone in the encounter tank was then turned off, and the four test rainbow trout were moved immediately from their individual tanks to the common predator encounter tank. All four fish were simultaneously released from a single net, outside of the protective cage structure, on the opposite side of the encounter tank from the predator, and we recorded behavior for 60 min. Although we were interested in whether exposure to cues made fish less susceptible to predation, no rainbow trout were consumed by the brown trout during any of the predation trials. Instead, we used these trials to examine the use of protective structure by rainbow trout as a measure of antipredator behavior. When a fish entered or exited the structure, its tag was identified by an observer using a UV flashlight, and the time was recorded. A video camera mounted above the encounter tank also recorded movements, and the video footage, combined with the fish identification, was used to calculate the proportion of time out of 60 min that each fish spent in cover (inside the protective structure). The observation tank was rinsed and refilled between trials.

2.5. Statistical analyses

To compare behavior before and after the introductions of chemical cues and across treatments, we ran a repeated measures

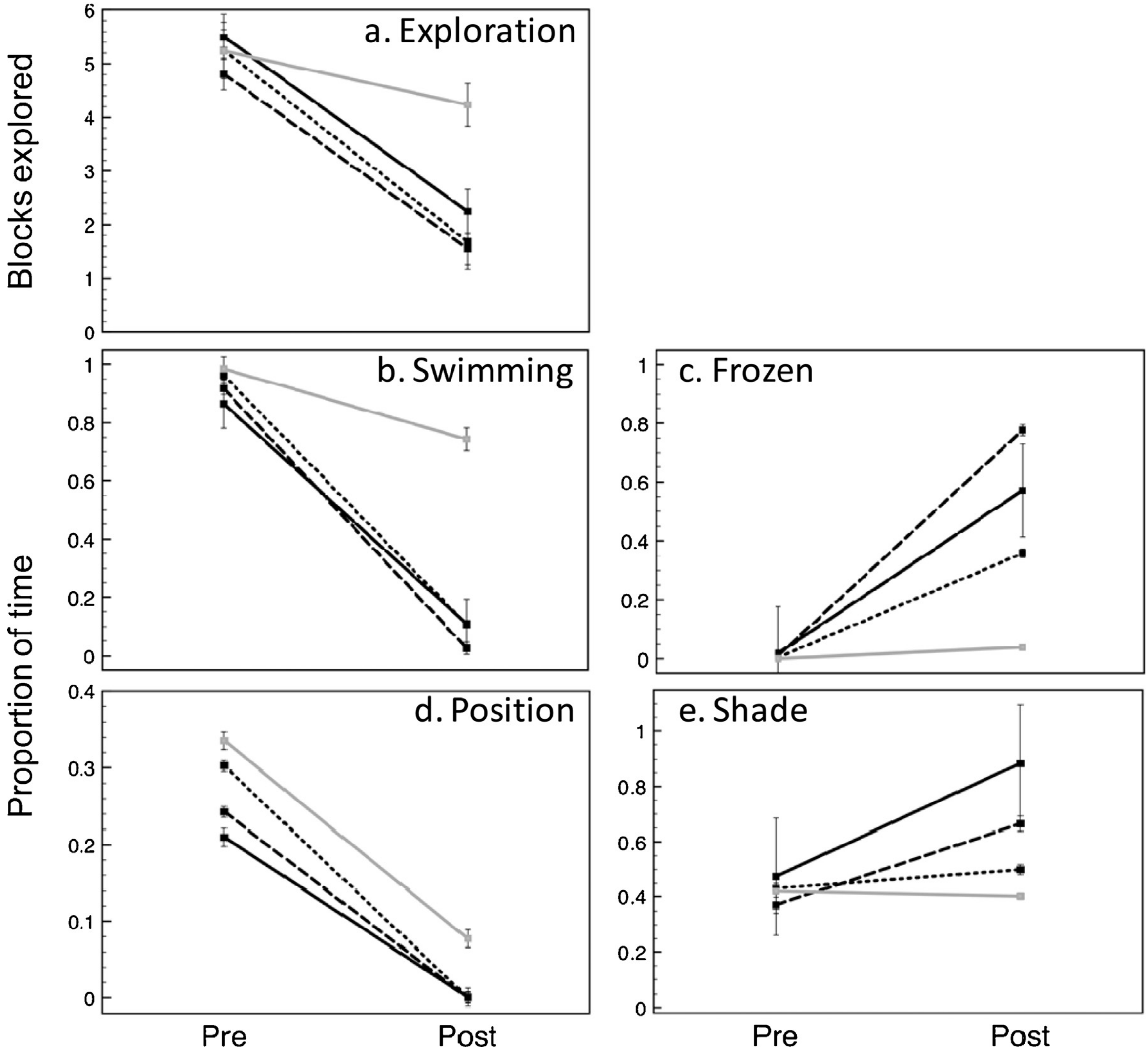

Fig. 1. Change in (a) the number of blocks explored, (b) proportion of time spent actively swimming, (c) proportion of time spent frozen, (d) proportion of time spent in the top half of the water column, and (e) proportion of time spent in the shade between the pre-exposure and post-exposure time periods for the four treatments: control (gray solid line), predator cue (small-dashed black line), alarm cue (long-dashed black line), and the predator and alarm cues combined (solid black line). Note that the axes are not identical in all cases.
Error bars represent standard error around the mean.

analysis of variance (RM ANOVA) for each behavior (five analyses total; $n = 64$ in each analysis) using SAS proc Mixed (SAS Institute Inc., 2013). The five behavior metrics evaluated were: (1) number of blocks covered (exploratory behavior), (2) proportion of time spent actively swimming (activity level), (3) proportion of time spent frozen, (4) proportion of time spent in the top half of the water column, and (5) proportion of time spent in the shade. The four treatments evaluated were: (1) alarm cue only (A), (2) alarm and predator cue (AP), (3) predator cue only (P), and (4) control (C). All proportions were normalized using an arcsine square-root transformation prior to analysis. Treatment, exposure period (pre and post), the interaction between treatment and period, and fish TL were included as fixed effects, with individual included as a random effect, for all analyses. Values were reported from the Type III sum of squares. If significant fixed effects ($P \leq 0.05$) were identified for a given model, the least squares means method was used to determine significant differences in behavior among treatments and periods.

The models described above allowed us to compare the change in magnitude of each behavior as a result of each treatment, but because of correlations among some of our variables, we also wanted to compare a composite variable summarizing overall behavioral changes across treatments. We therefore performed a principle components analysis (PCA) that included each of the five behaviors after first subtracting the post-exposure score from the pre-exposure score for each individual. We then performed an ANOVA comparing PC1 across treatments.

To determine if treatment influenced use of the protective structure in the predator encounter trials, the proportion of the total trial time spent in cover was compared in one single factor ANCOVA ($n = 64$). Based on the results of the previous analysis showing similar behavioral changes in PC1 for all three chemical cue treatments (A, AP, and P), in this analysis we combined the three treatments into a single group (treatment) and compared the proportion of time spent in cover with that of the control fish. Proportions were arcsine square-root transformed prior to analysis. To identify and account for possible differences between the control and treatment groups in the amount of time it took to first discover the protective structure, the amount of time between the beginning of the trial and first entry into the structure was calculated for each fish. This value was used as a covariate in the analysis, since it would affect the proportion of the total trial time spent in cover. A Bonferroni adjustment was used to compare the two groups, and values were reported from the Type III sum of squares.

3. Results

3.1. Behavior trials

Exposure to chemical cues resulted in a significant change in many of the behavior metrics measured. There was a significant interaction between treatment and exposure period for exploratory behavior ($F_{3,97} = 6.52$, $P < 0.01$). Exploratory behavior, measured by the number of blocks covered, was reduced as a result of exposure to a cue ($P < 0.01$). Though fish in the control treatment also exhibited a reduction in exploratory behavior over time ($P = 0.03$), the reduction was significantly greater in fish exposed to a cue in comparison to the control fish in the post-exposure time period ($P < 0.01$; Fig. 1a). The interaction between treatment and exposure period was also significant for activity level ($F_{3,60} = 5.89$, $P = 0.01$). Fish exposed to a chemical cue exhibited a reduction in activity level, i.e., the proportion of time spent actively swimming, from the pre-exposure to post-exposure time periods ($P < 0.01$; Fig. 1b). Control fish also exhibited a reduction in activity level ($P < 0.01$), suggesting that some of this effect could be related to the amount of time spent in the experimental tank. However, activity level in the post-exposure time period was significantly lower in fish exposed to a chemical cue compared to the control fish ($P < 0.02$). In addition, activity level of fish exposed to the alarm cue was significantly lower in the post-exposure time period than fish exposed to the predator cue ($P = 0.02$; Fig. 1b). A significant interaction between treatment and exposure period ($F_{3,60} = 4.35$, $P < 0.01$) revealed that the proportion of time spent frozen increased significantly for fish exposed to a chemical cue ($P < 0.01$) but did not increase for control fish ($P = 0.28$; Fig. 1c). In addition, fish exposed to the alarm cue spent significantly more time frozen in the post-exposure time period than did fish exposed to the predator cue ($P < 0.01$; Fig. 1c). Fish in all four treatments, including the control group, exhibited a significant and similar reduction in the proportion of time spent in the top half of the tank from the pre-exposure to post-exposure time periods (Fig. 1d), as indicated by a significant period effect ($F_{1,60} = 200.23$, $P < 0.01$) and lack of significant interaction ($F_{3,60} = 2.08$, $P = 0.11$), suggesting that fish tended to settle to the bottom the longer that they were held in the tank regardless of exposure to chemical cues. Similarly, a significant period effect ($F_{1,60} = 12.26$, $P < 0.01$) and lack of significant interaction ($F_{3,60} = 1.14$, $P = 0.34$) suggested that, on average, proportion of time spent in the shaded portion of the tank increased over time, but exposure to a cue did not result in differences in shade use among the treatments in the pre- and post-exposure time periods (Fig. 1e).

Changes in behavior from the pre-exposure to post-exposure periods were correlated for several of our behavioral metrics (time active, time frozen, and area explored; Supplementary Table 1). Hence, we used PCA to create a composite variable characterizing this overall change in behavior that could be compared across treatments. Principle component 1, which explained 51% of the variance in the model, was comprised of three highly correlated changes in behavior: change in time spent active (0.89), change in time spent frozen (−0.75) and change in blocks explored (0.85; Supplementary Table 2). Larger absolute values for PC1 indicated a greater increase in antipredator behavior in the post-exposure period relative to the pre-exposure period (e.g., spending more time frozen, less time active and exploring fewer blocks). Comparing PC1 across treatments in an ANOVA showed a smaller change in antipredator behavior for the control group than the three treatment groups ($F_{1,60} = 7.52$, $P < 0.01$), but there was no difference among these three treatments according to a Tukey's HSD post hoc analysis (Fig. 2).

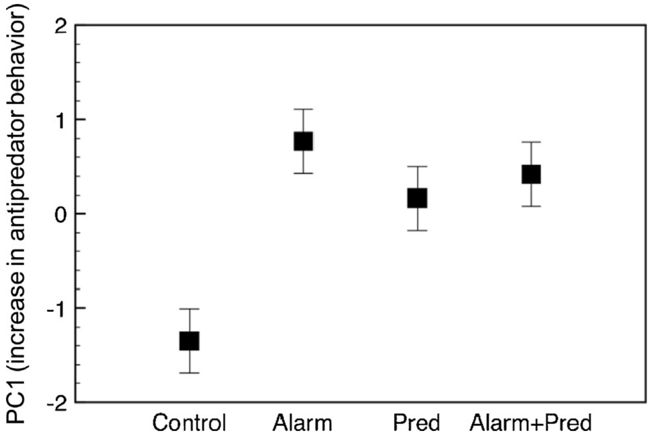

Fig. 2. PC1 values reflecting change in overall activity level for the four treatments (control [Control], alarm cue [Alarm], predator cue [Pred], and alarm and predator cues combined [Alarm + Pred]). Error bars represent standard error around the mean.

3.2. Predator encounter trials

Exposure to a chemical cue (treatment) did not have an effect on the proportion of the total time that a fish spent in cover isolated from the brown trout in the predator trials (ANCOVA; $F_{1,63} = 1.97$, $P = 0.17$). Although not significant, a trend in the data was apparent, with fish exposed to a chemical cue spending a larger proportion of time in cover than control fish (0.66 ± 0.03 and 0.36 ± 0.02, respectively). On average, it took 206 s for fish to enter cover, and the amount of time to enter the cover did not differ among treatments and control ($F_{22,63} = 0.89$, $P = 0.61$).

4. Discussion

We investigated behavioral responses to chemical cues associated with predation in a highly domesticated and predator-naïve strain of rainbow trout (GR strain) that is utilized for stock development in Colorado due to its resistance to whirling disease. In support of our predictions, we found significant increases in antipredator behavior upon their first exposure to alarm cue, predator cue, and a combined alarm and predator cue. In response to all cues, we observed shifts in three different behavior metrics, including a reduction in active swimming, a reduction in exploratory behavior, and an increase in time spent frozen. None of the cues had significant effects (compared to the control) on the time spent in the top half of the water column or time spent in the shade. Although we did not detect treatment effects on behavior during the predator encounter trials, our observations suggest that these techniques may warrant further investigation.

The finding that all three chemical cue treatments reduced exploratory behavior and time spent actively swimming while increasing time spent frozen supports our prediction that chemical cues would elicit antipredator behavior. Though all chemical cue treatments induced greater shifts than the control in these behavioral metrics, responses did not differ among the three chemical cue treatments. We expected the combined cue to elicit a larger response than either the alarm or predator cue alone given its greater quantity of chemical cue overall (10 mL compared to 5 mL) and previous findings that the magnitude of response increases with cue concentration (Mirza and Chivers, 2003). However, we used cue concentrations well above the threshold expected to elicit a response in rainbow trout (Mirza and Chivers, 2003). Thus, it is possible that we did not detect this difference because 5 mL of either cue elicited a maximum response, such that an additive effect of combining these cues would not be expected. It is also important to note that the response we detected to the brown trout predator cue may not be widely generalized to all predator species.

Our findings from the two other behavioral metrics, the use of water column and the shade, did not match our predictions. We expected predator cues to induce the GR to spend more time in the shade and at the bottom of the tank, simulating their natural use of protective cover and structure (e.g., rocks and vegetation on the substrate) in order to avoid fish predators (Tabor and Wurtsbaugh, 1991). Fish in all treatments reduced their time at the top half of the water column from more than 20% pre-exposure to almost 0% post-exposure. However, the control group showed a similar decrease, suggesting this pattern involved a temporal shift independent of the addition of chemical cues. This shift over the course of the trial may be explained by the food searching behavior of hatchery fish, where they first explore the surface for food pellets before moving to the substrate as floating pellets sink. It is unlikely that this response was induced by the disturbance associated with the addition of water (regardless of the presence of chemical cues), since water was added from a tube at the bottom of the tank and did not agitate the surface. Though we divided the use of the water column coarsely (top half vs. bottom half), future experiments might detect differences among treatments with more fine-scale subdivisions.

We expected the differences in behavior observed immediately after chemical cue exposure would result in differential performance in our subsequent predator encounter trials. However, we did not detect significant differences in the use of protective cover in these trials because of high within-treatment variation. The high level of observed variation could be related to the complexity of factors affecting behavior in these trials (e.g., differences in the rate of finding the structure, predator–prey interactions, conspecific interactions, etc.). Despite the lack of statistical significance, some interesting patterns emerged from the predator encounter trials that might be considered biologically important. Fish exposed to a chemical cue spent 30% more time in the cover than did the control fish. This increase in antipredator behavior immediately following an acute exposure to a cue of any kind could affect short-term survival. It would be interesting to determine whether fish exposed to predator cues immediately before release into natural systems might be more likely to survive early encounters with novel predators. The ability of these highly domesticated fish to respond to predator cues and any potential effects on survival have important economic implications, particularly when considering the decision to stock thousands of fish. For example, in a hatchery setting in Colorado, it costs $1.27 to raise a rainbow trout to a size often considered vulnerable to anglers (254 mm; Johnson and Martinez, 2000). Thus, if exposure to predator cues results in acute behavioral responses, and if these can be utilized to enhance survival immediately after stocking, the realized savings might be significant when multiplied by the large numbers of fish produced and stocked annually.

Even with the growing body of evidence indicating that predator training can alter behavior in ways that might increase survival, large-scale experiments in natural settings are lacking (Brown et al., 2013). Of the few attempts, there has been limited success at documenting an increase in survival in the wild as a result of training. However, when successful, survival rates have been shown to increase by 50% (D'Anna et al., 2012) and as high as fourfold (Hutchison et al., 2012) in trained fish. Importantly, training has been shown to improve survival in Murray cod, which is the subject of restoration and conservation efforts (Hutchison et al., 2012). This highlights the potential benefit of applying these techniques to maximize the effectiveness of other native fish restoration and conservation efforts that incorporate stocking. This experiment shows that natural antipredator behavior can be elicited by exposure to chemical cues of predation, even in a genetically bottlenecked population (in this case due to long-term captive breeding), and thus could potentially be applied to other small or declining populations (Vilhunen, 2006).

Based on the results of this study, exposure to alarm cue alone may elicit short-term antipredator responses similar to exposures to alarm and predator cues combined, and would also be the easiest way to provide experience with predation cues. Extracting and adding conspecific alarm cue is relatively easy in hatchery settings, especially if added in batches in the hatchery or to holding tanks of transportation vehicles while in route to the stocking destination, as suggested by Olson et al. (2012). Further, there is a reduced risk of disease associated with cues from conspecifics reared in the same hatchery rather than predator cues that require biological material from species raised outside of a hatchery. Additionally, the use of conspecific cues avoids the problem of selecting a particular predator species, as antipredator responses may vary across predator types, and it may not be possible to expose hatchery fish to cues from all potential predators. Further, it is unclear whether individual predator recognition is required to increase post-stocking survival (Blumstein, 2006). Our results support continued efforts to evaluate techniques that prepare hatchery-reared fish for introduction to the wild. Future efforts should focus on how modifications in behavior through training (chemical, physical, or otherwise) might translate to long-term post-stocking survival. One must also consider the costs and resources required to conduct effective fish training. If these costs are outweighed by the benefits of realized increases in survival of stocked fish, then hatcheries and managers may benefit from applying these types of approaches to increase efficiency.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2015.04.005

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