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NOTE

The effect of a single prerelease exposure to conspecific alarm cue on poststocking survival in three strains of rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Significant resources go toward rearing and stocking fish globally, yet poststocking survival is often low, largely due to high predation rates on hatchery-reared fish. Antipredator behavior has been enhanced in many species through exposure to chemical cues that simulate predation events, but the implementation of such protocols may be logistically challenging. It has been suggested that a single exposure of hatchery fish to chemical cues while en route to stocking locations may be sufficient to enhance antipredator behavior and improve survival. We tested whether a one-time exposure to conspecific alarm cues while en route to a stocking site increased poststocking survival of three strains of rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)). We found no difference in mortality rates between strains or between treatment fish (exposed to alarm cues) and control fish (not exposed to alarm cues), suggesting that this quick and easy protocol was insufficient and that more complex techniques should be explored to increase poststocking survival.

Key words: alarm cue, hatchery, *Oncorhynchus mykiss*, rainbow trout, survival, strain.

Résumé : D'importantes ressources soient affectées à l'élevage et l'ensemencement de poissons à l'échelle planétaire, mais la survie après l'ensemencement est souvent faible, principalement en raison de taux de prédation élevés des poissons élevés en alevinière. Le comportement antiprédation a été rehaussé chez de nombreuses espèces par l'exposition à des signaux chimiques qui simulent des évènements de prédation, mais la logistique de l'application de tels protocoles peut s'avérer ardue. Il a été suggéré qu'une seule exposition des poissons d'alevinière à des signaux chimiques durant leur déplacement vers le lieu d'ensemencement pourrait suffire pour rehausser leur comportement antiprédation et améliorer leur survie. Nous avons vérifié si une seule exposition à des signaux d'alarme de conspécifiques durant le déplacement vers un site d'ensemencement rehaussait la survie après l'ensemencement de trois lignées de truites arc-en-ciel (*Oncorhynchus mykiss* (Walbaum, 1792)). Nous n'avons constaté aucune variation des taux de mortalité entre les lignées, ni entre les poissons ayant subi le traitement (exposés aux signaux d'alarme) et des poissons témoins (non exposés aux signaux d'alarme), ce qui donne à penser que ce protocole rapide et facile n'était pas suffisant et que des méthodes plus complexes pour accroître la survie après l'ensemencement devraient être explorées. [Traduit par la Rédaction]

Mots-clés : signal d'alarme, alevinière, *Oncorhynchus mykiss*, truite arc-en-ciel, survie, lignée.

Introduction

Significant resources are dedicated worldwide to produce fish for stocking. Fish are stocked to maintain and bolster fisheries for recreation, reintroduction, and conservation purposes; however, the poststocking survival of hatchery-reared fish tends to be low (McNeil 1991; Brown and Laland 2001). Researchers have successfully increased poststocking survival through a variety of manipulations within the hatchery environment, such as altering rearing density (Brockmark and Johnsson 2010), habitat enrichment (Berejikian et al. 1999; Brown et al. 2003), and by providing experiences to increase antipredator behavior (Mathis and Smith 1993; Berejikian et al. 1999; Mirza and Chivers 2000; Kydd 2014). Efforts to increase antipredator behavior are particularly valuable because predation is the largest source of mortality for stocked fish (Olla et al. 1998; Brown and Laland 2001; Jackson and Brown 2011). Despite some evidence of success (for a review see Hutchison et al. 2012*a*), the most effective and efficient way to increase survival through predator experience remains to be determined.

Chemical cues have been used to alter fish behavior while avoiding complications implicit in exposing fish to actual predators (e.g., D'Anna et al. 2012). Conspecific alarm cues can be paired with predator kairomones to allow fish to recognize a particular odor as a threat through associative learning (Griffin 2004). Fish have also been shown to display enhanced antipredator behavior for some time following an exposure to alarm cues alone (Mirza and Chivers 2003; Kopack et al. 2015). If fish are exposed to alarm cues immediately prior to stocking, carryover effects from this increase in antipredator behavior may allow fish to survive the first few encounters with predators, when most mortality occurs (Hutchison et al. 2012*a*; Olson et al. 2012). Additionally, further learning can take place during those first few encounters to reinforce these behaviors (Hutchison et al. 2012*a*).

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The use of chemical cues to increase antipredator behavior in hatchery fish before stocking seems promising, but can be logistically challenging, especially at large scales. Many studies have used multiple exposures, different combinations and concentrations of cues, and have exposed fish individually or in small batches (e.g., Mirza and Chivers 2003; Ferrari and Chivers 2006; Olson et al. 2012), but these approaches may be difficult to successfully implement in production-level hatchery settings. However, increased antipredator behavior has been shown when exposing individuals or groups of individuals, only once to chemical cues of predation (in Arctic char (*Salvelinus alpinus* (L., 1758)): Vilhunen 2006; in fathead minnows (*Pimephales promelas* Rafinesque, 1820): Olson et al. 2012; in rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792): Kopack et al. 2015). Furthermore, Olson et al. (2012) suggested that increased poststocking survival might be achieved by simply adding cues to the holding tanks of transport vehicles while en route to the stocking site, though it had not been field tested prior to this experiment.

We designed an experiment to test whether a single exposure to chemical alarm cues while en route to the stocking site might be sufficient to increase survival post stocking. We also wanted to compare the relative importance of fish strain (across a gradient of domestication levels) on survival following a single exposure to chemical cues. We exposed half of three different strains of rainbow trout (the German rainbow trout (GR), a cross between the GR and the Snake River cutthroat trout (*Oncorhynchus clarkii* (Richardson, 1836)); HN2), and a cross between the GR and a Colorado River rainbow trout (HXC)) to conspecific alarm cues in holding tanks during a 1.5 h transit to the stocking site. Though the GR strain has poor survival in the wild due to its history of domestication (Fetherman and Schisler 2013), previous work has shown that the GR fish exhibit antipredator behavior in response to conspecific alarm cues (Kopack et al. 2015). Although the GR strain is capable of responding appropriately to chemical cues of predation (Kopack et al. 2015), this strain has been reared solely in the hatchery environment for more than 100 years, which may have reduced its natural antipredator behavior (Olla et al. 1998). We therefore expected that GRs crossed with less-domesticated strains would have increased poststocking survival relative to the pure GR strain. We compared mortality rates of unexposed control fish to exposed treatment fish from all three strains through monthly captures, predicting that exposed fish would have increased poststocking survival relative to the unexposed fish.

Materials and methods

Colorado Parks and Wildlife (CPW) stocks a variety of strains and crosses of fingerling rainbow trout into Parvin Lake (Red Feather Lakes, Colorado) each year to experimentally compare their poststocking survival, susceptibility to whirling disease (*Myxobolus cerebralis* Hofer, 1903), and growth rates. In the spring of 2014, we exposed rainbow trout to conspecific alarm cues while they were in the transport vehicle en route to Parvin Lake. All experimental fish were reared at the CPW Bellvue Fish Research Hatchery in Bellvue, Colorado. We used 1000 fish from each of three strains. Each strain was previously batch marked with coded wire tags in the nose using a Mark IV automated injector (Northwest Marine Technology, Inc., Shaw Island, Washington). Three days prior to stocking, we randomly transferred fish into two separate raceways (500 fish of each of the three strains in each raceway) for the treatment and control groups (1500 fish total in each group). Using a common method in fisheries for marking fish (Reimchen and Temple 2004), the adipose fins of fish in the treatment group was clipped to distinguish between the two groups. To provide a similar experience for each individual, we anesthetized fish in both groups using AQUI-S 20E (INAD study number 11-741-14-138H), and handled all fish as we transferred them to separate raceways, though only fish exposed to alarm cue had their adipose fins removed.

Table 1. Number of treatment and control rainbow trout (*Oncorhynchus mykiss*) captured by strain, total, and percentage of stocked treatment and control fish captured over the course of the experiment.

	Strain				
Status	GR	HN2	HXC	Total	Percent captured
Treatment	20	35	21	76	5.1
Control	22	41	21	84	5.6
Total	42	76	42	160	5.3
Percent captured	4.2	7.6	4.2	5.3	

Note: Percentages were calculated based on number of treatment or control fish stocked (1500), number stocked per strain (1000), and total number of fish stocked (3000).

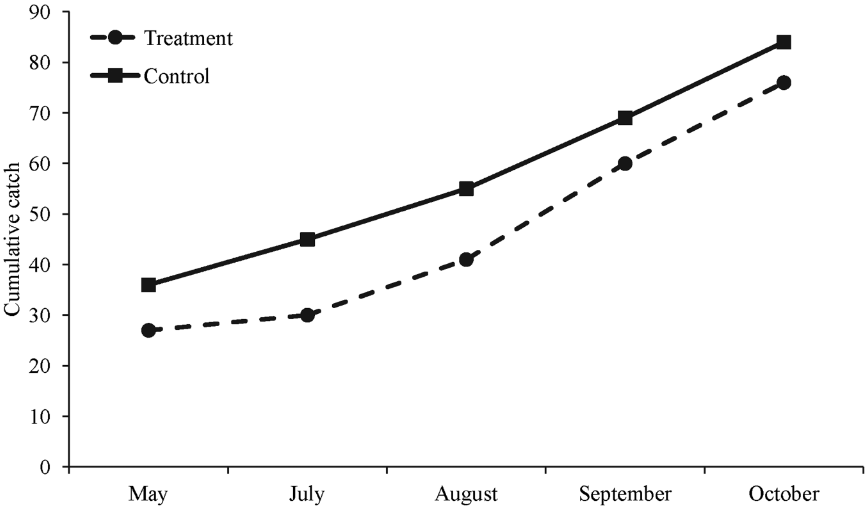
Table 2. Linearized catch curve-predicted instantaneous mortality rates and interval morality rates of three strains of rainbow trout (*Oncorhynchus mykiss*) exposed (treatment) and not exposed (control) to conspecific alarm cue en route to stocking in Parvin Lake, Colorado.

	Instantaneous mortality rate		Interval mortality rate	
Strain	Treatment	Control	Treatment	Control
GR	0.009	0.012	0.821	0.882
HN2	0.001	0.003	0.215	0.352
HXC	Inestimable	0.002	Inestimable	0.329

Prior to transport to the stocking site, 0.30 L of conspecific alarm cue was added to the 530 L tank holding the 1500 fin-clipped rainbow trout, whereas 0.30 L of water control was added to the 530 L tank holding the 1500 unclipped rainbow trout (concentration calculated from Kopack et al. 2015). The alarm cue was made by lacerating the epidermis of sacrificed rainbow trout in a crosshatch pattern with a scalpel, rinsing each fish with 30 mL of distilled water, and straining the liquid through cheese cloth before freezing at −62 °C for 1 week prior to the experiment (following Kopack et al. 2015). The travel time, or cue exposure time, was 1.5 h. Using chutes running from the truck to the lake, we first stocked the 1500 fish that were not exposed to alarm cue and allowed them to disperse for approximately 15 min. Then fish that were exposed to alarm cue were stocked. This was done to minimize interactions between treatment and control individuals and to reduce the potential of exposing control fish to residual alarm cue from the treatment tank. All fish were cared for and handled in accordance with the *Guide to the Care and Use of Experimental Animals*; their use for the purposes described herein were reviewed and approved by Colorado Parks and Wildlife prior to experimentation.

Rainbow trout were sampled each month for six consecutive months (May–October 2014) using a boat-mounted electrofishing unit (Fetherman and Schisler 2013). All fish collected during the sampling efforts were examined for adipose clips to obtain a count of treatment and control fish captured. In addition, 35 (±18) rainbow trout were sacrificed at each sampling occasion, with the exception of June 2014, so that coded wire tags could be removed and used to identify individual fish to strain. We chose to lethally sample this small number of trout each month (cumulatively 5.3% of the total stocked; Table 1) to avoid influencing mortality rates by treatment and strain when sampling. The numbers of treatment and control fish were counted and compared to determine if exposure to alarm cue affected numbers of fish captured. Instantaneous mortality rates (death rate at any given point in time) and experiment interval mortality rates (death rate at the end of the 6 month experimental interval) were calculated from linearized catch curve data using SAS Proc REG and compared using SAS Proc GLM (SAS Institute, Inc. 2015) using methods of Miranda and Bettoli (2007). Mortality rates were calculated and compared among treatment and control individuals within a strain, among strains (total, regardless of treatment or

Fig. 1. Cumulative catch curves showing the number of treatment and control rainbow trout (*Oncorhynchus mykiss*) that were sampled each month from May through October 2014 (excluding June). The values are cumulative so that numbers from October represent the total number of fish sampled.



control status), and among treatment and control individuals (total, regardless of strain).

Results

There was no significant difference in instantaneous mortality rates among the strains ($F_{[2,7]} = 0.36$, $p = 0.712$), or between treatment and control fish within any of the strains for which they were estimable (GR: $F_{[1,5]} = 0.08$, $p = 0.787$; HN2: $F_{[1,6]} = 0.03$, $p = 0.878$; HXC: inestimable; Table 2). We therefore pooled data across strains to compare treated and control fish. The cumulative catch curves suggest that there was little effect of exposure to alarm cue on total numbers of fish captured over the course of the experiment (Fig. 1). This was supported by the comparison of the instantaneous and interval mortality rates, which also did not show a difference between treatment (0.003 and 0.395, respectively) and control (0.004 and 0.506, respectively) fish ($F_{[1,6]} = 0.05$, $p = 0.836$). Inconsistent with our expectations, higher numbers of control fish were captured in the first sampling occasion compared with treatment fish across all strains.

Discussion

This study was designed to test whether a single exposure to alarm cues en route to a stocking site could increase poststocking survival of three different strains of rainbow trout. Although fish (including rainbow trout) have displayed increased antipredator behavior following exposure to alarm cues (Mirza and Chivers 2003; Kopack et al. 2015), we found no support that exposed fish had higher survival than control fish over the course of a 6-month period in a Colorado reservoir. It is possible that a difference in survival may have been detected if we sampled for longer than 6 months, but this seems unlikely because most mortality occurs shortly after stocking (Hutchison et al. 2012*b*; Olson et al. 2012). Furthermore, we expected exposure to alarm cue to have short-term effects, perhaps allowing exposed fish to survive their first few encounters with a predator (Mirza and Chivers 2003; Hutchison et al. 2012*b*; Kopack et al. 2015). If so, a difference in survival would have been detected in the first month of sampling, although more frequent sampling combined with higher numbers of fish may have increased our ability to detect that difference. It is also possible that the fin clipping we used to identify treated fish reduced their survival by reducing their burst swimming capabilities. However, the adipose fin is likely more important for stabilization in high-flowing water (Reimchen and Temple 2004; Buckland-Nicks et al. 2012), and our experiment was conducted in a lentic system.

It is important to note that our methods did not include an associative learning treatment, where alarm cues are paired with predator kairomones to train fish to associate a particular predator odor with danger, as was suggested by Olson et al. (2012). Instead, we compared unexposed fish to fish that were exposed to alarm cues alone for several reasons. First, our previous work found that alarm cues alone increased antipredator behavior in rainbow trout, without any additional benefit of pairing it with brown trout (*Salmo trutta* L., 1758) predator kairomones (Kopack et al. 2015). Second, we wanted to test the simplest protocol in terms of time, money (materials and labor), and hatchery feasibility. Third, though there are no restrictions on using conspecific alarm cues, it is often difficult to bring new species of predatory fish into a hatchery system due to limited availability, concern over disease transmission, and hatchery regulations. Finally, the choice of predatory species may be complicated by the fact that most prey fish will encounter a suite of predators and exposure to a single predator species may not aid in the avoidance of other predators (Blumstein and Daniel 2002). However, it is possible that an associative learning treatment would have been more effective than alarm cues alone. Furthermore, though logistically more difficult, repeated exposures to the chemical cues of predation might enhance antipredator behavior and survival (Kydd, 2014). Additionally, the duration of exposure to predator cues should be explored, because it could affect the likelihood of habituation. In our study, habituation to alarm cues could have occurred during the 1.5 h transport, such that fish no longer exhibited antipredator behavior by the time they were released. Temporal thresholds of habituation to cues of predation have not been established but should be explored because this information will allow managers to optimize training protocols.

Though we found no significant differences in poststocking survival of treatment versus control fish, the pattern of fish captured from each strain in this experiment is similar to previous evaluation years, with a higher number of HN2s captured cumulatively in relation to either the HXC or the GR (Fetherman and Schisler 2013). Furthermore, though not a significant result, we found that the two less-domesticated strains (HN2 and HXC) had lower interval mortality rates than that of the pure GR (with 53.0% and 55.3% better survival, respectively) within the control treatment. Given the GR's long history and strong artificial selection in a hatchery setting (Fetherman et al. 2012), this finding suggests that crosses with wild rainbow trout populations may alter the genetics of hatchery fish to create more natural phenotypes. This manipulation of the genetic background may have a larger influence on poststocking survival than environmental manipulations, e.g., predator conditioning. Because our previous work has shown that the GR strain is capable of appropriately responding to alarm cues (Kopack et al. 2015), future work should include the role of genetic diversity and domestication on survival of hatchery fish.

This work highlights the importance of validating protocols that show promise in the laboratory by performing survival experiments in the wild. In a previous laboratory study, rainbow trout displayed significant increases in antipredator behavior in response to alarm cues (Kopack et al. 2015), but this did not translate to increases in survival in the wild in this study. However, a challenge associated with studies in the wild is that exposure to predator cues may reduce capture probability. For example, predator cues may induce behavioral changes, such as reduced activity and exploratory behavior (Kopack et al. 2015), that prevent conditioned fish from being captured. A possible solution to detect differences in survival by treatment might be to enclose both predators and prey in net pens within lakes, which would allow a 100% capture probability for all treatments. Experiments in the wild are uncommon, but some have shown increased poststocking survival rates for conditioned fish (using live predators, predator models, and refuge in white seabream (*Diplodus sargus* (L., 1978)): D'Anna et al. 2012; using live predators, predator models, and chemical cues of predation in Murray cod (*Maccullochella peelii* (Mitchell, 1838)): Hutchison et al. 2012*b*). These findings coupled with the null results from the current study highlight the importance of evaluating methods to enhance survival in the laboratory and the wild, as well as verifying them for species of interest. Future research should use both laboratory and natural settings to optimize species-specific protocols to increase poststocking survival if we hope to improve hatchery practices and conservation efforts around the world.

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