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Copper barriers can cause behavioral artifacts in experiments with marine snails

Alizia J. Barnes, Jennifer M. Hill*

Louisiana Tech University, PO Box 3179, Ruston, LA 71272, USA

ABSTRACT: Many gastropod species will not cross copper barriers; thus, copper has been used to manipulate their movements and minimize caging artifacts. However, as the reason for copper barrier avoidance is unknown, it is difficult to predict experimental artifacts that might result from its use. Copper can be toxic to gastropods, resulting in behavioral alterations or death, but there is little evidence copper barriers can cause these effects. In this study, we assessed whether copper mobilizes from copper barriers in water and alters gastropod behavior, using periwinkle snails *Littoraria irrorata.* Snails were placed in containers with and without copper tape and in copper solutions (0, 0.1, 0.2, 1.0, and 2.0 ppm) created by soaking copper tape in seawater. We measured the number of snails that climbed out of the water and the ability of snails to emerge and right themselves. Snails in 1.0, 2.0 ppm, and copper tape treatments were immobilized and did not climb out of the water or emerge from their shells. Snails in lower concentrations were more likely to climb out of the water. Low copper concentrations also reduced the number of snails emerging and righting, but those that did emerge did so in less time. When exposed snails were moved to clean water, snails previously immobilized by copper were quicker to crawl out of the water. Our results demonstrate that copper can mobilize from copper barriers resulting in alterations to snail behavior. To avoid experimental artifacts, we suggest the use of copper barriers be avoided in future experiments.

KEY WORDS: Heavy metals · Cageless methods · Periwinkles · Gastropod

1. INTRODUCTION

To perform experiments in marine and freshwater communities, ecologists typically manipulate organisms by either constructing communities of animals and plants in mesocosms (e.g. aquariums, buckets, etc.) or by excluding or enclosing animals in the field using cages. These differing manipulations can create a variety of experimental artifacts in which the manipulation of animals and their habitats results in undesired effects that can influence experimental outcomes (Virnstein 1977, Connell 1997, Hindell et al. 2001, Felsing et al. 2005). Cages that incorporate the natural variability of field environments can cause shading and housing effects and dampen fluid velocities (Cubit 1984, Miller & Gaylord 2007). In contrast, constructing communities in mesocosms can mimic ecological communities without the environmental variability that affects experimental repeatability. However, mesocosms are impacted by scaling distortions, and mesocosm walls can alter the behavior and movements of organisms (Petersen et al. 1999, Petersen & Hastings 2001). The impact of each artifact on experimental conclusions is likely influenced by how well organisms respond to experimental manipulations. Some species behave similarly in mesocosm and field conditions, while other organisms defy a variety of experimental manipulations much to the anguish of the scientist.

Many gastropod species, such as snails and limpets, are often uncooperative with both field and lab manipulations. Their small size often permits them to

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*Corresponding author: jmhill@latech.edu move freely in and out of field cages and their mobility both in and out of the water allows them to climb over cage walls and out of tanks and buckets meant to confine them. Consequently, keeping gastropods confined to mesocosms and cages in experiments can require small mesh sizes or covering mesocosms, which can create a wide variety of experimental artifacts (Cubit 1984). To avoid these artifacts, copper barriers have been used in a variety of forms to limit gastropod mobility, as many gastropods will not cross copper barriers (Sousa 1979, Cubit 1984). Classically, and most commonly, copper-based anti-fouling paints have been used in rocky intertidal studies to confine limpets (Cubit 1984, Paine 1984, Johnson 1992, Range et al. 2008). To a lesser extent, copper foil, sheets, wires, and tape have also been used to limit gastropod movements in both lab and field experiments (Table 1).

The mechanism that causes gastropods to avoid copper is largely unknown (Cubit 1984), but hypotheses include that copper may generate some aversive electrically conductive reaction or that it is toxic (Sousa 1979, Johnson 1992). Without knowing the mechanism, it is difficult to predict the experimental artifacts that copper might induce (Johnson 1992, Range et al. 2008). If copper is simply aversive, we might expect similar artifacts to other caging methods (Johnson 1992). However, if copper is toxic, it may cause a variety of artifacts depending on the organism's sensitivity to copper, copper's mode of toxicity, and its ability to leach and/or mobilize in water (Johnson 1992). The combination of these factors may make toxic copper artifacts difficult to detect or assess using artifact controls (Johnson 1992). Further experimental complications may result if copper causes sublethal changes in gastropod behavior that may alter the outcomes of species interactions within experiments.

Copper is generally toxic to gastropods, but lethal concentrations are dependent upon species, time of exposure, and water conditions (Betzer & Yevich 1975, Nebeker et al. 1986, Rogevich et al. 2008). Evidence indicates that snail exposure to copper contributes to snail mortality (Rogevich et al. 2008, Brix et al. 2011, Besser et al. 2016, Holan et al. 2017). There is also evidence that copper can cause sublethal behavioral effects, especially in laboratory environments. For instance, exposure to copper can reduce snail grazing rates (Elfwing & Tedengren 2002, Das & Khangarot 2011) and reduce snail mobility (Holan et al. 2017, Osborne et al. 2020). Copper is highly mobile and can leach into water from anti-fouling paints and treated lumber (as reviewed in Weis & Weis 1996, Neira et al. 2014). However, there is little evidence that copper barrier methods such as tape, foil, or wire may leach copper into the surrounding water and cause impacts to animal behavior (but see Cartwright et al. 2006 and Corte et al. 2017 for results on copper-based anti-fouling paint).

Most recently, copper tape has been used in mesocosm studies with periwinkle snails *Littoraria irrorata*. Periwinkle snails are important grazers and fungal farmers in salt marsh habitats along the Gulf and Atlantic coasts of the USA that have received significant attention for their role as mesograzers in marsh trophic cascades (Silliman & Zieman 2001, Silliman & Bertness 2002, Silliman et al. 2004, Kimbro 2012, Soomdat et al. 2014). These trophic cascades have important implications for the productivity of estuarine marshes that are already declining due to a variety of anthropogenic threats (reviewed by Kennish 2002). Consequently, the desire to manipulate these communities in mesocosms to determine how environmental mechanisms affect trophic cascades has increased (Moore et al. 2020). Yet in laboratory environments, periwinkles generally spend more time

Table 1. Methodologies for the use of copper tape, wire, foil, and anti-fouling paint

Method	Lab or Field	Publication(s)
Copper wire used to prevent snails from accessing prey	Field	Gosselin & Chia (1995)
Copper strips used to contain gastropods	Field	Cubit (1984), Menge et al. (1993)
Copper ring used to manipulate limpets	Field	Harley (2002)
Copper sheets used to exclude limpets	Field	Steneck & Dethier (1994), Kordas et al. (2017)
Copper foil strips used to manipulate snails	Lab	Tepler et al. (2011)
Copper tape used to prevent wall climbing by snails	Lab	Kimbro (2012), Soomdat et al. (2014)
Copper based antifouling paint used to manipulate limpets	Field	Cubit (1984), Paine (1984), Steneck & Dethier (1994), Range et al. (2008), Guerry & Menge (2017), Lathlean et al. (2017)

climbing mesocosm walls than climbing on marsh plants, which has necessitated the use of copper tape inside of the mesocosms to prevent this behavior (Kimbro 2012, Soomdat et al. 2014). While investigating potential setups for a periwinkle experiment, we replicated the use of copper tape in glass bowls to limit the periwinkle's affinity for walls. Unexpectedly, we observed that snails in copper tape-lined containers were generally immobile. However, when snails were relocated to containers without copper tape, activity in these snails resumed. Based on these observations, we hypothesized that copper was leaching from the tape and impacting snail behavior and mobility, which could result in experimental artifacts when copper tape is used. No previous studies have suggested copper may leach from copper tape, nor has copper toxicity been assessed in *L. irrorata.* To test our *a priori* hypothesis, we designed an experiment based on our previous observations to examine periwinkle mobility and behavior in response to copper tape and copper solutions created by soaking copper tape in seawater.

2. MATERIALS AND METHODS

Periwinkle snails *Littoraria irrorata* were collected by hand from marshes in Lake Barre and other areas within Terrebonne Bay in coastal Louisiana, USA. Snails were transported to Louisiana Tech University (Ruston, LA), where they were maintained in tanks using filtered recirculated artificial seawater (Instant Ocean, 10 ppt). Snails were maintained on a diet of frozen cordgrass *Spartina alterniflora* and were held for several months due to their use in multiple experiments. However, no snails were used more than once. As snails were held for several months, it is possible snails may have been more sensitive to copper due to declining condition in the lab. However, we saw no evidence snails were in poor condition prior to the experiment. No snail mortality occurred during our experiment and all snails in controls and copper treatments were subject to the same laboratory conditions.

2.1. Experimental setup

To examine if copper tape could impact periwinkle behavior, we exposed periwinkles to differing concentrations of a copper tape solution. A single copper tape stock solution was created by soaking copper tape (7.9 m length × 2.5 cm width) in 10 l of seawater (10 ppt) for 48 h to create a 4 ppm copper stock solution. This stock solution was then diluted with 10 ppt seawater to create 5 copper treatments: 2, 1, 0.2, and 0.1 ppm. We chose these concentrations based on preliminary assays that demonstrated a band of copper tape lining the inside of an experimental bowl would yield a copper concentration of approximately 2 ppm when soaked for 48 h. Thus, our highest concentration of copper solution would yield a similar concentration to lining our laboratory containers with copper tape. All concentrations were confirmed using a high range copper colorimeter (±0.05 ppm; Hanna Instruments).

Glass bowls (10 cm height × 20 cm diameter; 2 l) were filled with 1.3 l of seawater from 5 treatments: 2, 1, 0.2, 0.1, 0 ppm (n = 6 per treatment; 30 bowls with copper solutions). These treatments were compared with a copper tape treatment with a limited number of replicates (n = 3) in which the inner base was lined with copper tape and filled with 1.3 l of 10 ppt seawater (33 bowls total). This limited number of replicates was due to a limited number of bowls in which we prioritized a higher number of replicates of copper solution treatments. The rims of all bowls were lined with a narrow strip of copper tape to ensure that snails did not climb out of the bowls. The bowl water level was approximately 2.5 cm below the top edge of the bowl (7.5 cm), ensuring the tape never came into contact with the water. We confirmed copper rims did not leach into the water by measuring the concentration of copper in the controls at the conclusion of the experiment (controls: 0.02 ± 0.05 ppm).

Snail behaviors and movements can be impacted by shadows and the movement of observers. Thus, bowls were haphazardly placed into large black plastic tubs (length × width × height: 99.1 × 37.5 × 43.8 cm) to prevent snails from seeing movement and shadows. Four periwinkle snails (15–20 mm total length) were placed into each bowl. Periwinkles generally spend most of their time on container walls and have very little behavioral interactions, which limits the likelihood of conspecific interactions affecting copper impacts (J. M. Hill pers. obs.). Further, periwinkles exhibit no response to conspecific cues (Duval et al. 1994). Exposing multiple individuals to pollutants in small containers is common in ecotoxicology, including studies in gastropods (Gomot-de Vaufleury & Bispo 2000, Relyea 2004, Bernot & Brandenburg 2013, Salice & Kimberly 2013, Besser et al. 2016). This design is also preferable to individual exposures, as copper tape is commonly used to manipulate small populations of snails within mesocosms or field environments. Consequently, exposing small groups of snails to copper tape solutions better recreates the use of copper tape in periwinkle experiments (Kimbro 2012, Soomdat et al. 2014) than individual exposures. One pre-selected snail per bowl was marked with a permanent marker for use in an emerge-and-right behavioral assay (see below). We examined the impact of copper treatments on periwinkle climbing, emergence, and righting behaviors each day for 2 d.

2.2. Climbing assays

In the marsh, submerged periwinkles will generally climb out of the water (Warren 1985) up the stems of marsh cordgrass. This innate predator avoidance behavior reduces their predation risk from predators that enter marshes on a flood tide (Warren 1985). Consequently, any impairments to climbing behaviors by copper are likely to increase snail mortality. In the lab, periwinkle climbing behavior typically occurs on any wall, necessitating the use of copper barriers (Kimbro 2012, Soomdat et al. 2014). These climbing behaviors also make it difficult to consistently expose periwinkle snails to copper-laced water. There are very few options to restrict snails to the water while also maintaining ecological realism. In our experiment, snails readily climbed the bowl walls of bowls but were able to reduce their exposure to copper by climbing out of the water just below the copper-lined rim. To compromise between allowing ecologically realistic behaviors and the need for consistent copper exposure, all snails (submerged and emergent) were knocked off the sides of the bowl once per day, ensuring equal disturbance.

We measured the climbing behavior of all snails in response to copper treatments over 2 d. After snails were initially placed into the bowls, we counted the number of snails (1–4) that had crawled up the sides of the bowls, out of the water after approximately 3 h (1 measurement per bowl). Snails were then knocked off the bowl sides back into the water. We repeated this measurement after 24 h for 2 total measurements. The number of snails out of water each day (Day 1, Day 2) was non-normal and heteroskedastic and resistant to transformation. We analyzed the number of snails out of water each day in each bowl to test for the effect of copper treatment, using a general linear model (GLM) in SPSS v.26, as GLMs are often robust to violations of statistical assumptions. However, we confirmed that a more conservative analysis using a non-parametric Kruskal-Wallis would not alter statistical conclusions ($p < 0.05$). Pairwise comparisons between treatments were made using a Games-Howell post hoc test for unequal variances, as this test is robust to non-normality (Ruxton & Beauchamp 2008). Statistical conclusions were also similar when using more conservative Dunnett's T3 or Tamhane post hoc procedures.

2.3. Emerge-and-right assays

We also examined the impacts of copper on snail coordination by measuring the amount of time it took for preselected snails (1 snail per bowl) to emerge from their shell and right themselves after being placed upside down. Snails can be dislodged from cordgrass stems in the marsh by waves, predators, or other animal movements. Failure to right themselves and quickly move may leave them vulnerable to predators. Snails used were pre-selected using preliminary righting assays in which snails were submerged in bowls upside down with their operculum closed. Snails that righted themselves in under 5 min were chosen for use in experimental assays. Previous assays in our lab have shown that there is considerable between-snail variation in righting times (<1–10 min) but less within-snail variation over time (J. M. Hill unpubl. data). Thus, using preliminary assays to select snails minimized between-snail experimental variation as well as reduced experimental timing and logistical constraints. As we excluded snails with longer righting times, results from this methodology cannot represent the effects of copper on the entire population of periwinkles.

Emerge-and-right behavioral assays were performed each day of the experiment (2 d total). Marked snails were removed from the bowls and gently agitated with forceps until snails retracted into their shells and closed their operculum. Snails were then placed back into respective treatment bowls upside down and their behaviors were video recorded (1 snail per bowl per day). Although most of the other snails were located on the walls, any snails on the floor of the bowls were moved to the edge to prevent their interference in the assay. In videos, snail emergence was defined by when both of the snail's antennae emerged outside of the shell. Snail righting was defined by when snails righted themselves completely from their overturned position. To assess if copper affected the ability of snails to emerge and right themselves, we examined the effect of copper treatment on the total number of snails that emerged and righted themselves via Fisher's exact test. We also examined if copper affected the speed at which snails emerged and righted themselves. Snails that did not emerge or right themselves within 10 min were not included in any time analyses. Snail emergence time (the elapsed time from when a snail is placed to when it emerges) was analyzed by a 1-way repeated-measures GLM (SPSS) for the effects of copper treatment and with time as a within-subject factor (Day 1, Day 2). Only 3 treatment groups (0, 0.1, and 0.2 ppm) were included in this analysis, as less than 3 snails in higher copper concentrations successfully emerged in under 10 min. Similarly, many snails did not right themselves within 10 min on Day 2. Consequently, we analyzed only the Day 1 righting time (righting time − emergence) and total time-to-right (righting time − placement time) for the effect of copper treatments, using a univariate GLM.

2.4. Recovery from copper exposure

To examine if snails recovered from 48 h of copper exposure, snails were removed from each treatment bowl and placed into 200 ml glass beakers (4 snails per beaker) filled with 100 ml of 10 ppt seawater. For another 48 h, we counted the number of snails (1–4; 1 measurement per beaker) that climbed out of the water at 3 h intervals (09:00, 12:00, 15:00, and 18:00 h). Snails were knocked back in the water after each measurement until a final documentation was recorded at 33 h of post-exposure recovery. Knocking snails back into the water resulted in multiple climbing attempts to ascertain recovery and/or sustained impacts of copper. As snail behavior was similar in all treatments after 24 h, we only analyzed the first 24 h of data for this time series. The number of snails out of water was analyzed by using a repeated-measures GLM to evaluate copper treatments as a between-subjects factor and time (3, 6, 9, 24 h post-exposure) as a within-subject factor.

3. RESULTS

3.1. Climbing assays

High concentrations of copper (≥1 ppm) and the copper tape treatment generally resulted in minimal movement of periwinkle snails, with few snails climbing out of the water or emerging and righting in behavioral assays. Lower concentrations of copper often impaired behaviors in comparison with controls. The number of snails crawling out of the water was significantly affected by copper on Day 1 ($F_{5,27}$ = 41.63, p < 0.001; Fig. 1) and Day 2 ($F_{5,27}$ = 46.30, p < 0.001). Periwinkle snails in controls generally occupied the bottom or sides of the bowls under the waterline, with only 1–2 snails emerging above the waterline. Both 0.1 and 0.2 ppm copper solutions induced more snails to climb out of the water, but this behavior was not always significantly different from controls. In contrast, high concentrations of copper (1 or 2 ppm) and copper tape halted snail movements and resulted in zero snails escaping the water on both Day 1 and Day 2. Snails in these treatments were generally sitting on the bottom of bowls with their opercula closed.

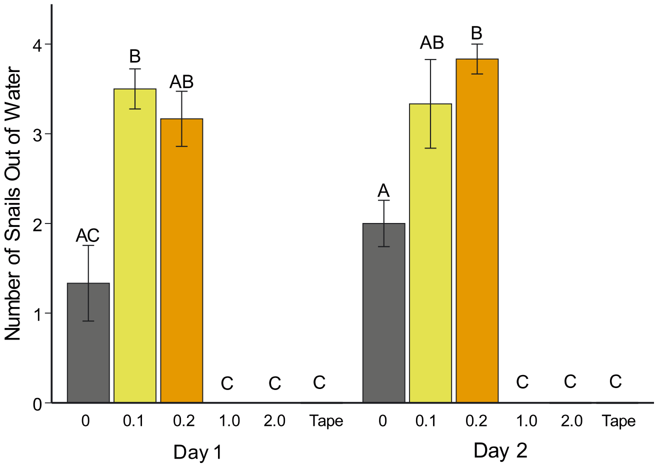

Fig. 1. Effects of copper (ppm) and copper tape on the number of snails escaping the water (mean ± SE). Snails in concentrations ≥1 ppm did not emerge from the water. Differing letters denote significant differences based on Games-Howell post hoc tests

3.2. Emerge-and-right assays

The reductions in snail mobility due to high copper concentrations were also apparent in emerge-and-right behavioral assays (Fig. 2). High concentrations of copper (1 or 2 ppm) and copper tape significantly reduced the number of snails that emerged from their shells within 10 min, with no snails emerging in the 2 ppm or tape treatments (Fisher's exact test, Day 1: p < 0.001; Day 2: p < 0.001; Fig. 2A). 100 % of snails emerged from their shells in control treatments on both days, whereas in low copper concentrations, generally only 66–83 % of snails emerged from their shells. Less than 33 % of snails in the 1.0 ppm copper concentrations successfully emerged in under 10 min on Day 1 and Day 2. In examinations of how long snails took to emerge from their shells (Fig. 2B), control snails were slower to emerge from Day 1 to Day 2, whereas snails exposed to low levels of copper exhibited similar emergence times each day (time ×

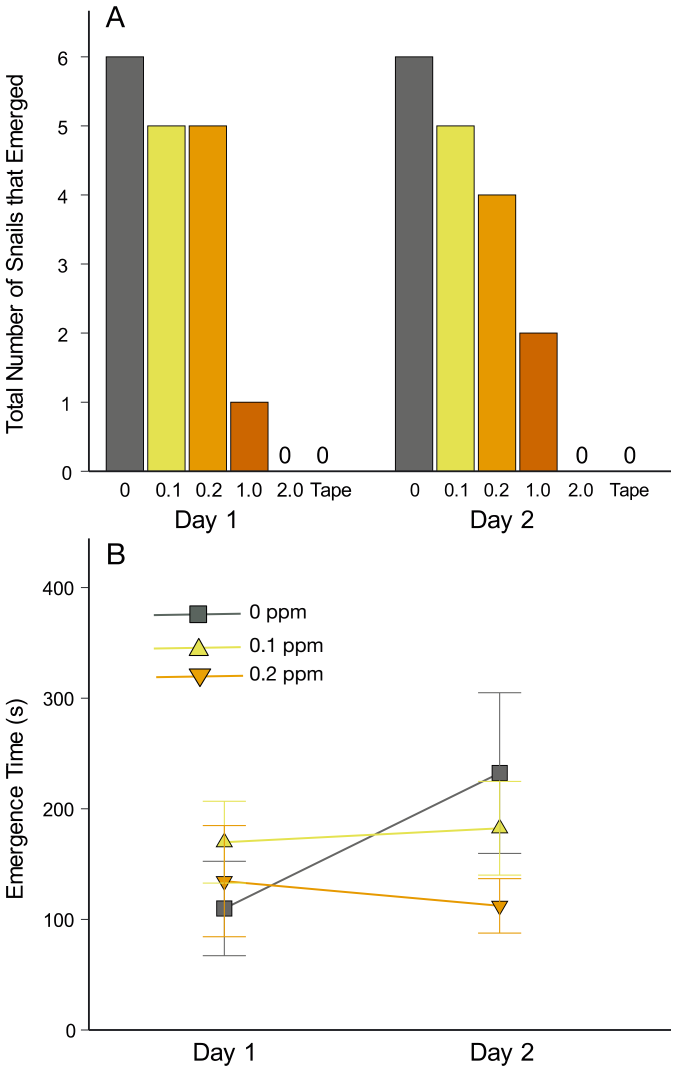

Fig. 2. (A) Total number of snails that emerged over the 2 d experiment and (B) mean (±SE) snail emergence time with varying copper treatments. No snails emerged in under 10 min in 2 ppm or copper tape treatments

treatment, $F_{2,12}$ = 5.2, p = 0.02; Fig. 2B). Consequently, snails in 1 and 2 ppm copper treatment emerged faster (mean ± SE: 1 ppm: 182.4 ± 42.34 s; 2 ppm: 112.25 ± 24.63 s) on Day 2 than controls (232.3 ± 72.64 s). Similar to effects on emergence, copper also reduced the number of snails that were able to successfully right themselves after being flipped over. Snails that did not emerge in higher concentrations of copper (1 or 2 ppm) and copper tape treatments also never righted themselves within 10 min (Fisher's exact test, Day 1: $p < 0.001$; Day 2: p = 0.006; Fig. 3A). Although snails were more likely to right themselves at lower concentrations of copper, copper did not significantly affect snail righting time (treatment, $F_{2,11}$ = 0.317, p = 0.735; Fig. 3B).

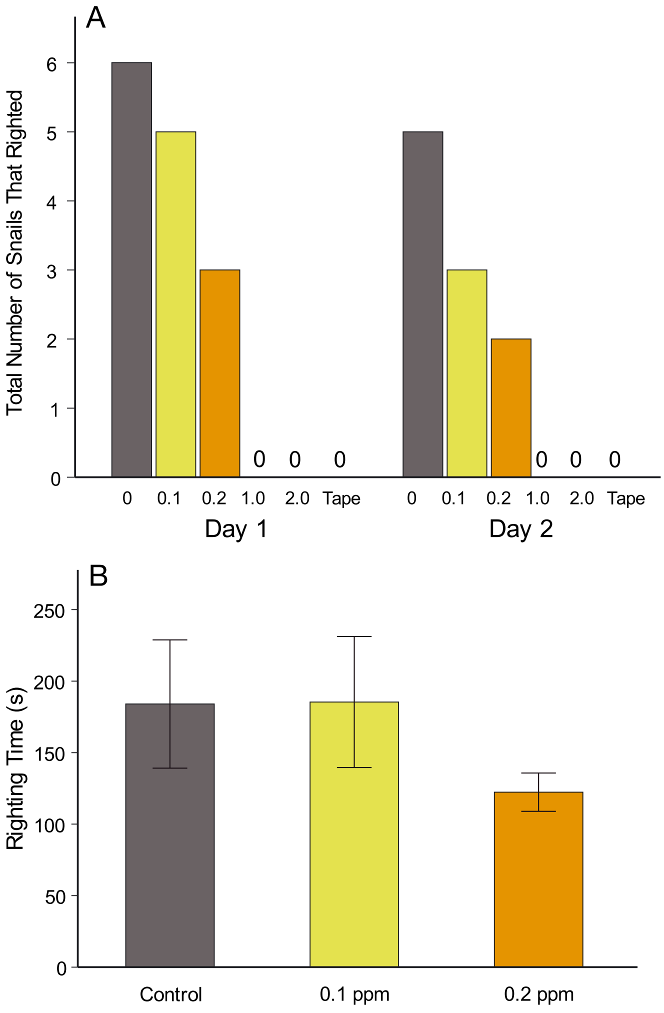

Fig. 3. (A) Total number of snails that successfully righted themselves and (B) mean (±SE) snail righting time in varying copper treatments. No snails righted in under 10 min in 1 ppm, 2 ppm, or copper tape treatments

3.3. Recovery from copper exposure

After being transferred into clean water, snails appeared to recover and climb out of the water (Fig. 4). Generally, snails that were exposed to higher concentrations of copper and copper tape treatments were quicker to crawl out of the water, resulting in a higher number of snails out of the water at early time intervals (time × treatment, $F_{15,81}$ = 2.848, $p \leq 0.001$). At 3 h, more snails had climbed out of the water after exposure to high concentrations of copper (2.0 ppm: 3.67 ± 0.33; 1.0 ppm: 3.83 ± 0.17) compared to control snails (2.33 ± 0.21) after 3 h. By 9 h into the post-exposure period, the number of snails out of the water in high concentrations of copper (2.0 ppm: 3.5 ± 0.34; 1.0 ppm: 3.83 ± 0.17) was similar to the control

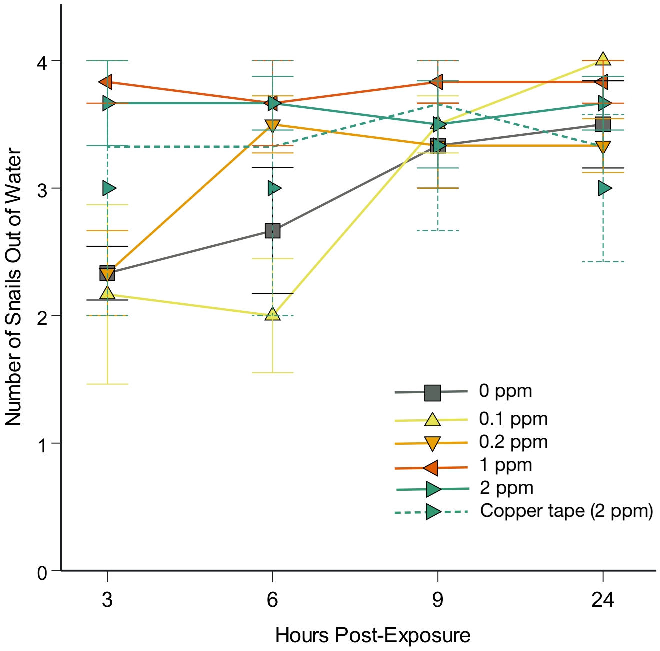

Fig. 4. Mean (±1 SE) number of snails escaping the water during post-exposure to copper treatments

treatment (3.33 ± 0.33). Although snails exposed to high concentrations of copper appeared lifeless in many parts of the experiment, all snails were alive and had resumed climbing behaviors at the conclusion of the experiment.

4. DISCUSSION

Our results indicate that copper tape can leach copper into water, resulting in alterations to snail behavior. Lower concentrations of copper (0.1 or 0.2 ppm) often resulted in behavioral avoidance of copper-impacted water, as snails in these treatments were more often found out of the water relative to controls. Low levels of copper also reduced the number of snails that successfully emerged and righted and increased the speed at which they emerged from their shells. In contrast, exposure to high copper concentrations and copper tape inhibited snail movements, drastically reduced the number of snails climbing out of the water, and stopped snails from emerging and righting. These results suggest that copper barriers can cause alterations to snail behavior which may produce experimental artifacts.

The sublethal effects of copper on snail behavior we observed are consistent with previous studies performed on differing snail species and are likely behavioral adaptations to avoid the toxic impacts of copper (Hughes et al. 1987, Holan et al. 2017, Osborne et al. 2020). For example, Osborne et al. (2020) documented that *Planorbella pilsbryi* snails were typically immobile in response to copper concentrations (>18 ppb), while snails in lower copper concentrations remained active. Additional studies have documented snails withdrawing into their shells with a closed operculum in response to copper (Hughes et al. 1987, Holan et al. 2017). The withdrawal of snails into their shells likely reduces their contact with the environment, effectively shutting the door on heavily polluted waters. This shutting response may be optimal when exposure to copper or other toxins may be brief and/or where brief exposure is likely to result in death. Copper-induced immobility likely carries a variety of fitness consequences, including delayed oviposition, reduced reproductive output, and behaviors that result in higher mortality rates (Holan et al. 2017, Osborne et al. 2020). When copper concentrations are lower, it may be more advantageous to emigrate from the habitat. Periwinkles in our experiment were more often out of the water and emerged faster when exposed to lower copper concentrations, suggesting a behavioral avoidance of copper-impacted waters.

After being placed in clean water, periwinkle snails that were previously exposed to high concentrations of copper and the copper tape were quickest to emerge from the water. This rapid response may be due to the release of toxic conditions that restricted all snail mobility resulting in an urgency to resume normal behaviors and/or emigrate from the area. This rapid recovery suggests periwinkles were behaviorally avoiding copper and that their immobility was not a result of copper toxicity. Rapid recovery from copper-induced immobility has also been documented in at least one prior study (Osborne et al. 2020). However, copper can accumulate in the tissues of snails, resulting in toxic effects including shell defects, growth inhibition, and death (Rogevich et al. 2008, 2009, Khangarot & Das 2010, Brix et al. 2011, Das & Khangarot 2011, Besser et al. 2016, Holan et al. 2017). We did not observe any mortality, demonstrating that 48 h exposure to copper concentrations up to 2 ppm is non-lethal. Lethal copper concentrations and sub-lethal behavioral impacts often vary with species and exposure period (Betzer & Yevich 1975, Nebeker et al. 1986, Rogevich et al. 2008). As our experiments were performed with snails housed under laboratory conditions for several months, it is possible periwinkles were more sensitive to copper than freshly collected snails. However, we also observed similar reductions in snail mobility in our preliminary investigations that used freshly collected snails. The similar alterations to snail behavior with both freshly collected and laboratory-maintained snails suggest snails in this experiment were not more sensitive to copper. Further experiments should be performed to fully assess the impacts of copper on periwinkle behavior and mortality for comparison with other species.

The sublethal impacts of copper tape on snail behavior could cause artifacts that alter the conclusions of predator–prey experiments. In response to predator cues and/or high tide, periwinkles migrate up cordgrass stems out of the water to avoid predators (Warren 1985). Snails in the rocky intertidal can also exhibit a variety of escape behaviors including climbing out of tide pools (Bullock 1953, Trussell et al. 2004, Morgan et al. 2016). In our experiment, low copper concentrations induced more snails to climb out of the water. Thus, low copper concentrations can result in increased avoidance behaviors, reducing predation, and resulting in an overestimation of non-consumptive effects. High copper concentrations caused reduced or complete immobility, which would likely impair the ability of periwinkles and other gastropods to avoid predators and/or recover from being dislodged (Kwan et al. 2015). Copper also reduces limpet gripping tenacity, potentially resulting in increased dislodgements and predation risk (Cartwright et al. 2006). Thus, high copper concentrations can cause an overestimation of predator consumptive effects and an underestimation of non-consumptive effects (Kwan et al. 2015). Copper-induced immobility may also alter grazing behaviors of periwinkles (Elfwing & Tedengren 2002, Das & Khangarot 2011). Copper has been shown to reduce grazing by as much as 51% in other species of marine snails (Elfwing & Tedengren 2002); however, copper has had little impact on limpet grazing rates in the field (Cartwright et al. 2006, Corte et al. 2017). At least one recent study suggests that copper can impair foraging of predatory snails (Pardal et al. 2021). Thus, many artifacts are likely to be species-dependent.

It is unknown whether copper tape used in previous mesocosm experiments affected periwinkle behaviors or caused experimental artifacts. As high copper concentrations immobilize periwinkles, it is unlikely that copper tape used in previous studies resulted in high copper concentrations since such extreme aberrant behavior is likely to be noticed. Other impacts on periwinkle orientation and/or climbing behaviors ultimately depend on the volume of water in the mesocosms and whether that water was flow-through or recirculated. Kimbro (2012) used 68 l tanks rimmed in tape, thus concentrations of copper in these tanks were likely to be low in comparison with our study. Soomdat et al. (2014) used 122 l tanks approximately half full, and water was refilled at regular intervals which would allow for copper to be flushed. The volume and/or turnover of water in these studies suggests that it was unlikely that tape caused major behavioral changes consistent with higher copper concentrations observed in our study. Both studies also manipulated the amount of water in their mesocosms to simulate tidal conditions, which would continually alter copper concentrations. As periwinkles can recover rapidly from copper exposure, any changing conditions that affect copper concentrations are likely to make artifacts on snail behavior difficult to detect.

Copper wire or copper sheeting used in previous studies (see Table 1) may also result in the mobilization of copper, altering the behavior of gastropods and causing experimental artifacts. However, the impact of these methods on gastropod behavior is dictated by both the susceptibility of the gastropod species to copper and the concentration of copper. The use of copper sheets, foil, and copper-based antifouling paints have traditionally been used in field environments where fluid conditions are more likely to disperse copper (Table 1). Thus, heavy metal contaminants may be less damaging under natural conditions and low concentrations (reviewed by Mayer-Pinto et al. 2010). However, there are only a limited number of studies that address sublethal behavioral effects in field conditions (Cartwright et al. 2006, Corte et al. 2017, Pardal et al. 2021). As our results suggest that copper can affect gastropod behavior but may also be difficult to detect due to rapid recovery, we would suggest that the use of copper enclosure methods, especially in laboratory settings, be avoided. If copper must be used, we suggest measuring copper levels within and around enclosures and using behavioral assays to examine any potential sub-lethal impacts to snail behavior which may affect experimental conclusions.

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