

The Effects of High Concentration Copper Pulses on Mortality and Settlement of the Acorn Barnacle, *Balanus glandula*

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ABSTRACT

Historically, most research on the effects of pollutants on acorn barnacles has centered around eliminating them from an area. They are among the most severe of marine foulants, and among the most difficult to keep from ship hulls. Copper paint or plating is the most common method for preventing barnacle recruitment on deep water vessels, but this can cause waters around ships and ports to have periodic spikes in copper concentration with unknown effects. In addition, sewage outfalls and runoff along coastline can result in already elevated levels, exacerbating the effects. Acorn barnacles are generally able to cope with high copper concentrations. In order to copper tolerance in the cypris larvae of a common acorn barnacle, 81 *Balanus glandula* larvae were collected off the west side of San Juan Island, WA, and placed in copper solutions similar in concentration to levels during copper spikes, and allowed to sit for 36 hours. All experimental larvae died without settling.

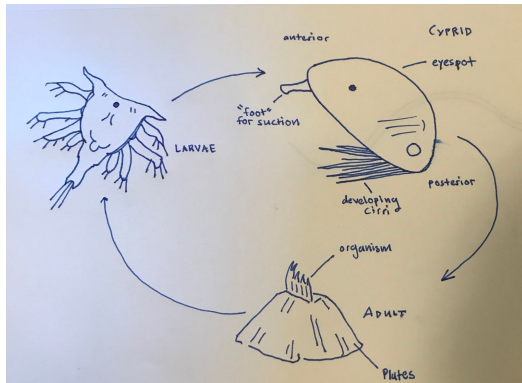
INTRODUCTION

Worldwide, the acorn barnacle is one of the most severe fouling organisms in the marine environment (Aldred & Clare 2008). Its calcareous plates can develop on ships, causing drag, reducing vessel speed, and scugging up turbines or rudders, slowing commercial freighters and affecting the maneuverability of warships (Aldred & Clare 2015, Tribou & Swain 2015). The gregarious settlement pattern of most species means that once a single cyprid has settled, more are quick to follow (Gaines et al. 1985). With such vested commercial and military interests, solutions to the problem of ship bottoms from becoming barnacle bastions have been well

researched for over a century. Historically, the most common remedy was to use copper (Tribou & Swain 2015).

Aside from mercury, copper is the most toxic heavy metal pollutant present in temperate seawater. On copper painted surfaces, barnacle wall plates form poorly, if at all, resulting in high fall off rates post-recruitment (Crisp & Austen 1960, Tribou & Swain 2015). As a result, it has become a key weapon in the war between humans and marine foulants. In terms of antifoulants, it is the cheap and dirty option, and thus the most popular. Over 90% of US Navy ships have their hulls painted with an ablative copper coating, which adds up to over 1.1 million meters squared of toxic heavy metal paint exposed to open seawater (Tribou & Swain 2015). The coatings have been designed so that, as the outermost layer becomes sludged with biofilm, it dissolves off into the surrounding water to create a biocidal “halo” in the area immediately around the hull. In the wake of a deepwater vessel, copper concentrations can reach over 1950 mg per liter (Tribou & Swain 2015). After an in-water hull cleaning, this rises threefold. In areas, such as the Salish Sea, that already experience elevated copper levels due to urban and industrial outfalls, the sharp spikes in concentration that accompany a deepwater vessel or hull cleaning can push ambient metal content from tolerable to toxic (Munier & Benndell 2018, Tribou & Swain 2015). With the completion of the Trans-Mountain pipeline through British Columbia

bringing in deepwater vessels, these spikes should increase in frequency from ever



y few years to every few months (Gaydos et al. 2015).

Within the next several decades, the importance of these periodic copper spikes is set to increase along the inland Washington and B.C. coasts. Changing weather patterns are predicted to increase rain and flood intensity, bringing in washes of contaminated water to marine environments from industrial and urban centers (Musselman et al. 2018, Yazzie & Chang 2017). For waters around major Canadian ports such as Victoria, B.C., and Vancouver, B.C., copper contaminated runoff arriving in bursts may be enough to create “danger zones” around outfalls and deepwater vessels where few species survive (Krogh et al.).

Traditionally, the dominant research focus with regards to acorn barnacles has been how to eliminate them from an area, rather than how to conserve them. With the exception of members of the genus *Amphibalanus*, recruitment of acorn barnacles is known to be substantially lower on copper coated surfaces, leading to their ubiquity (Crisp & Austen 1960). However, unlike other benthic organisms, such as algae or mussels, the effects of copper in the water are mixed (Amara et al. 2018). As a result, it serves as an excellent stand-in at high concentrations for how more sensitive organisms may react at lower concentrations. If it can kill a barnacle, it can probably kill anything else small (Amara et al. 2018). Barnacles are also a quotidian piece of the northeastern Pacific intertidal, serving as important prey for some species of sea star and

whelk (Connell 1969). Their reactions to sudden copper spikes could dramatically affect species composition in the rocky intertidal, either by allowing new species to colonize spaces once occupied by barnacles, or by colonizing new spaces once occupied by less copper-tolerant organisms (Berlow 1999). Little to no research has been published on the effects of a copper pulse on one of the Salish Sea's common acorn barnacles, *Balanus glandula*, despite its prominence along our shores.

The goal of this paper was to rectify that by examining *B. glandula* copper tolerance during the cypris larval stage, when the larvae are competent to settle. Overall, we predicted substantially lower settlement rates in cyprids exposed to a copper pulse for 36 hours compared to controls kept in typical seawater. We also predicted higher mortality rates in copper exposed larvae, both during the pulse and in the days following.

METHODS

Balanus glandula cyprids were collected on May 22, 2019, from the surface waters around Friday Harbor Laboratories' docks using an 80 micron mesh plankton net attached to a steel pole. Collections occurred thrice, at 9:30 am, 2:45 pm, and at 4:00 pm. The morning collection was done by dragging the net behind a rowboat just off the docks, while both afternoon tows were done from the dock, on the windward side. The batches were combined, and diluted using filtered seawater to make cyprids easier to spot and identify. Samples of roughly 60mL each were taken and examined under a dissecting microscope at low magnification (0.8x) to sort out the correct species from other plankton. 81 total were collected, placed into a jar of cold filtered seawater, and kept at sea temperature, at 10.1 degrees celsius. To ensure no cyprids

settled before the treatments began, all cypids were then transferred into millipore filtered seawater, with no available surfaces to settle on besides clean glass, which discourages settlement (Hills et al. 2000). They were then kept overnight in a sea table.

Eight 250 mL beakers were made embryo safe through an acid wash in 1.0M HCl for ten minutes, twenty rinsings with tap water, and ten rinsings with reverse osmosis water. After drying, CuSO_4 was measured using an Ohaus Navigator Portable balance, with a range of 200g to 0.01g (Ohaus Corporation, Parsippany, New Jersey, USA) and placed into solution using filtered seawater. CuSO_4 was selected because it would quickly break down into copper ions in solution, mimicking the paint on ship hulls, while any pH changes would be stabilized by natural buffers in seawater. This stock solution was then diluted further to create all experimental concentrations, 0.05M, 0.01M, and 0.001M. The lowest concentration is roughly equivalent to concentrations found just off copper paint on ship hulls. All beakers held a volume of 100 mL. Due to a miscount, there were only two 0.05M beakers, compared to three each for the other treatments and the control. Three control beakers were taken from stocks that had been previously made embryo safe, and were filled from the same source of filtered seawater used to dilute the copper solutions.

Cyprids were taken from their jar one by one and placed into beakers at random, without knowledge of which beaker belonged to which treatment. 7 cyprids were placed into each beaker, and the extra 4 cyprids released. Early reactions to the solution were recorded, and the beakers labelled with an assigned letter, the concentration, and the time cyprids were placed in solution. All beakers were sealed using Parafilm and placed in a sea table to maintain them at 10.1 degrees celsius, before being left to sit for 36 hours to simulate a copper shock. Beakers

were then examined in the same order, and counts taken of how many cyprids had settled, how many were still swimming, and how many had died. Death was distinguished from settlement based off orientation along the bottom. Settled barnacles sit on their heads, vertically in the water with their posterior end up. Dead cyprids lay horizontal, with dorsal or ventral side, or flank, facing up. While it was originally intended that the experiment continue for another day, with all larvae transferred into clean beakers of seawater to observe settlement behavior, this was rendered unfeasible. In all experimental treatments, all cyprids died without settling.

RESULTS

No statistical tests were performed, given that there was no difference whatsoever between treatments. In total, 58 cypris larvae died, 56 of which were in treatments, 2 of which were in controls. Of those in treatments, all larvae in 0.05M and 0.01M solutions sank instantly and presumably died shortly after, although no close observations were recorded. The remaining 19 from the controls were released without further observation.

DISCUSSION

Given our results, it seems evident that lower copper concentrations must be tested in the future. Since all larvae died regardless of concentration, it is impossible to determine if mortality and copper are linked linearly, or otherwise. It is possible that very low concentrations of copper could be beneficial to developing *B. glandula*, given its role in metabolism (Bernard & Lane 1960). The original query of this experiment, whether copper spikes reduce settlement rates after the metal has dispersed, remains to be answered, since no larvae were left to settle. Certainly, it

is indubitable that copper spikes reduce settlement in affected populations, but not by inhibiting settlement so much as inhibiting life.

Previous studies have shown that earlier stages, such as nauplii and cyprid larvae (Fig. 1), are more sensitive than adult barnacles to heavy metals, and will experience a sharp drop in oxygen intake and a quick death by suffocation caused by copper entering their system (Crisp & Austen 1960, Johnson et al. 2002, Rao et al 1986). In the case of 0.001M Cu^{2+} and above, it appears to result in a very quick death indeed.

While the results of this experiment seem somewhat obvious, they do have dramatic implications for *Balanus glandula* populations in this region. It is evident, based off these results, that spikes in copper levels similar to those found during vessel hull cleanings and sewage outflows are strong enough to kill barnacle larvae over the course of a single day. In a still harbor or similar area with low water flow, where most hull cleanings and outflows occur, contamination can sit for long periods of time without much mixing, killing all larvae near to the source. While rainfall and floods are impossible to control, there are ways to mitigate the strength of copper pollution within our area.

The negative effects of sewage outflows can be reduced through wastewater treatment, particularly in large coastal cities such as Victoria, B.C. Currently, Victoria releases untreated sewage regularly (Krogh et al. 2018). While constant flows such as this do not create the characteristic copper spike examined herein, they do elevate copper levels in the vicinity of the outflow, increasing the likelihood of copper concentration reaching critical levels (Krogh et al. 2018). In addition, heightened background copper reduces overall biodiversity, as well as genetic diversity within species, making communities more susceptible to disease (Ma et al. 2000,

Taylor et al. 1998). Wastewater management schemes, meanwhile, such as that of Vancouver, B.C., *would* cause copper spikes: during periods of heavy rain or flooding, the city diverts untreated sewage into the stormwater system, which is then ejected into the sea with runoff (Krogh et al. 2018). Installing or updating wastewater treatment systems around the Salish Sea would go far towards reducing the frequency and severity of copper spikes.

In terms of deepwater vessels and hull cleanings, the best solution is to look at temporal and spatial factors. Acorn barnacles abound on nearshore docks and pylons, where copper spikes from hulls are most likely to occur. For major hull cleanings, which release three times the level of copper per day compared to ordinary vessel service, avoiding barnacle die-offs in the surrounding waters can best be achieved by undertaking any sprucing up from October to December, before barnacle larvae are released (Tribou & Swain 2015). During the spring and summer, when waters are chock full of larvae, strategic positioning of dock lights could be used to discourage larvae from exploring copper-rich areas near boats. Since nauplii and cyprids are attracted towards light, and cyprids explore a wider range of surfaces and at higher speeds during the night, it is possible to direct the population away from large ships (Hills et al. 2000). This has the added benefit of helping to keep docks and hulls clear of barnacle larvae. A more efficacious, if more costly, solution is to replace copper paint with alternative antifouling solutions (Li & Ning 2019). Recent legislation has led to a boom in alternative antifoulants research, although most are not yet in regular use (Li & Ning 2019). Without dramatic overhauls of commercial and military fleets, it is inevitable that copper spikes will continue to occur in waters across the globe.

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