Estimates of environmental loading from copper alloy materials

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ABSTRACT
Metal release rates were measured from four different copper alloy-based materials used by the aquaculture industry: copper sheet machined into a diamond mesh, copper alloy mesh (CAM), silicon bronze welded wire mesh, and copper sheeting, and compared with conventional nylon aquaculture net treated with a cuprous oxide antifouling (AF) coating. Release rates were measured in situ in San Diego Bay using a Navy-developed Dome enclosure system at nine different time points over one year. As expected, copper was the predominant metal released, followed by zinc and nickel, which were fractional components of the materials tested. Release rates followed a temporal trend similar to those observed with copper AF coatings applied to vessel hulls: an initial spike in copper release was followed by a decline to an asymptotic low. Leachate toxicity was consistent with prior studies and was directly related to the metal concentrations, indicating the alloys tested had no additional toxicity above pure metals.

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Introduction
Aquaculture accounts for more than half of the total seafood supply worldwide. In 2012, aquaculture had a production of 66.5 million tons, or about 43% of the total seafood supply (Food and Agriculture Organization of the United Nations (FAO) 2014), and surpassed 50% of the total seafood supply by 2014 (FAO 2016). The growth of sessile marine organisms on the fish enclosure materials (biofouling) significantly impacts aquaculture production. Fouled nets reduce water flow and can negatively impact fish health, can act as reservoirs for disease, and can cause structural deformation or even failure of the net materials, possibly allowing fish to escape (Braithwaite et al. 2007; Fitridge et al. 2012).

Industry standards for finfish farming operations are comprised of steel frame pens with nylon mesh netting, often coated in antifoulants (e.g. Braithwaite et al. 2007). Fouling still occurs despite these coatings, requiring expensive replacement or in situ cleaning of nets (Willemsen 2005; Ayer et al. 2016). To reduce biofouling as well as associated environmental impacts, and to improve aquaculture output and safety, fish pens made out of copper alloys are now being developed and tested. Copper is a material desirable for aquaculture pens as it is naturally resistant to biofouling, being generally toxic to microorganisms (Borkow and Gabbay 2005); copper can be toxic to other organisms depending on concentration and other parameters such as water hardness and dissolved organic carbon (Eisler 1998; Di Toro et al. 2001; Arnold et al. 2005). Yigit et al. (2018a) found that compared to a conventional net fish pen, a tested copper alloy mesh (CAM) pen had qualitatively less fouling; this was interpreted by the authors as the explanation for apparent higher dissolved oxygen in waters inside the CAM pen, and the associated faster growth rates for Gilthead Seabream (Sparus aurata) in the CAM pen. The CAM pen was also more structurally sound and required less cleaning than the conventional net pen (Yigit et al. 2018a). Gonzalez et al. (2013) found that CAM pens had higher net economic outcomes compared with traditional net pens. However, while Yigit et al. (2016) found that tissues from fish grown in CAM pens did not have metal concentrations exceeding the safety limits for consumption, Yigit et al. (2018a) noted that the...
environmental effects of copper leaching from CAM pens had not been thoroughly studied.

In this study, metal leaching rates for four copper alloy materials and one traditional coated-nylon net material were evaluated in a 365 day field test in San Diego Bay, California. In situ metal leach rates were measured using a Dome-system originally designed to assess leaching from ships' hulls (Seligman and Neumeister 1983). These leach rates were extrapolated to estimate total environmental loading that would occur from aquaculture applications of each material, given likely maintenance and replacement schedules. Chronic toxicity of the leachate from each material type, using embryos of the purple sea urchin (*Strongylocentrotus purpuratus*), was assessed periodically throughout the year to determine whether leachate from the copper alloy materials was potentially more toxic than, for example, copper-based antifouling (AF) paint or pure copper. Finally, theoretical deployments of 50 CAM aquaculture pens were modeled in both San Diego Bay CA (an arid, Mediterranean bay with a low flushing regime) and Sinclair Inlet, WA (a cold water, high flushing regime environment) to assess overall environmental loading in these representative scenarios.

### Material and methods

#### Test materials

Five different materials designed to resist biofouling that are used in aquaculture and/or other underwater applications were tested for in situ metal leaching rates and embryo-larval toxicity. The common characteristic, and the base for their ability to prevent fouling, is that they are either made primarily of metallic copper, or they are covered with an AF coating that has copper as its main component. The test materials (Table 1) included copper alloy mesh stock formed from a 65% copper - 35% zinc alloy (“CAM”), 97% bronze - 3% silicon weld wire mesh (“Bronze”), a stock sheet of 90% copper - 10% nickel (“Sheet”), an expanded mesh (“Mesh”) that was made from the same production run as the 90:10 Cu:Ni sheet, and conventional nylon netting coated with cuprous oxide-based AF paint (“Net”). The nylon Net obtained for this study was directly from an end user, not the manufacturer, and lot specific concentrations of copper were unavailable. However, Edwards (2012) reported 13-17% Cu$_2$O in AF-coated nets. Each of the 5 materials had 3 replicates, which were randomly positioned in exposure racks and assessed for leach rates at each time point over the course of the 365 day study.

| Sample ID | Material Composition | Surface area (cm$^2$) |
|-----------|----------------------|----------------------|
| CAM       | Copper alloy mesh Cu 65%, Zn 35% | 613                  |
| Bronze    | Silicon bronze wire Silicon 3%, Bronze 97% | 1,839               |
| Sheet     | Flat copper-nickel sheet Cu 90%, Ni 10% | 707                  |
| Mesh      | Expanded copper-nickel sheet mesh Cu 90%, Ni 10% | 613                  |
| Net       | Nylon netting (with AF coating) Cu, Zn (coating) | 1,532.5              |

Surface area refers to the two-dimensional area that the material covers as a single layer, and not the overall surface area of the exposed material.

#### Field sampling

#### Material preparation

Test materials (3 replicates of each material) were secured to a 25.4 cm (10 in) diameter polyvinyl chloride (PVC) ring with plastic tie-wraps (Figure 1) and suspended within a fiberglass frame (Figure 2). Each given test material replicate was located at least 20.3 cm (8 in) from the other replicates, and all frames were arranged such that the test materials were equally exposed to open water and did not face towards one another. A total of 10 frames were deployed to accommodate all of the replicate samples. The Bronze and Net materials used 3 and 2.5 layers of material, respectively, to ensure sufficient measurable metal concentrations in leachate and to reduce analytical measurement error, based on pilot testing. The Sheet material was directly connected to the fiberglass frame. Leachate concentrations were normalized to the surface area of a single layer of material to allow for direct comparison across all sample types. The mesh sizes of the tested materials were CAM: 4 × 4 cm (1.6 × 1.6 in), Bronze and Net: 2.5 × 2.5 cm (1 × 1 in), and Mesh: 1 × 2 cm (0.4 × 0.4 in).

The materials were exposed to natural seawater from 23 August 2011 to 22 August 2012 (one year) at the Naval Information Warfare Center (NIWC) Pacific’s Harbor Research Platform System (HaRPS) deployed in San Diego Bay, CA (32°42′19.28″N, 117°14′10.08″W). The fiberglass frame was hinged in the middle, allowing the lower portion, where the replicates were attached, to be positioned vertically in the water column during long-term seawater exposure.
During leachate testing, the racks were raised slightly and swung on their hinges so that the testing materials were oriented horizontally but were maintained below the water’s surface. This allowed access to materials for testing, while minimizing exposure to air. Individual frames were deployed vertically below 1 m depth facing NNW and configured with a single replicate of each material in variable positions in the water column across all frames. The materials were used as received from the manufacturers, with no further treatment to their surfaces.

**In situ leach rate testing**

The Dome-system is considered the most accurate method for estimating passive leach rates *in situ* from AF coatings on ships’ hulls and similar surfaces (Finnie 2006), and was expected to be the most accurate method for making similar release rate
measurements from copper alloy mesh and other aquaculture materials for this study. Laboratory standard methods for release rate determination of paint systems (ASTM International (ASTM) 2006, ASTM 2007; International Standards Organization (ISO) 2007a, ISO 2007b) are useful for understanding relative leach rates; however, it is widely recognized that these methods do not produce data representative of release rates in a natural environment for AF products, and are not suitable for deriving environmental loading estimates (Schiff et al. 2004; ASTM International (ASTM) 2006, ASTM 2007; Finnie 2006). The Dome-system and method, in contrast, allow for testing to occur in a natural seawater environment and produce realistic environmental loading values compared to laboratory methods. This system isolates a volume of ambient water over a surface, and provides water circulation in a closed loop, thus allowing for a confined volume of ambient seawater to be exposed to leaching effects from a given surface. The system allows small aliquots of the confined seawater to be withdrawn at regular intervals, from which leachate release rates are then determined.

The “Dome-system” and method developed by the US Navy (Seligman and Neumeister 1983) and described in detail by Seligman et al. (2001) and Valkirs et al. (2003) were used for evaluating passive leaching was measured ten times throughout the course of the 365-day exposure for each material. The first leach rate measurements utilizing the Dome-system were initiated 24 h after the materials were deployed (day 1), with subsequent measurement events on days 8, 15, 29, 43, 64, 91, 183, 273, and 365. The experiments required a modified application of the Dome-system technique (designed to adhere to a ship’s hull). Each sample ring was removed from the exposure frame, maintained underwater, and placed under the seawater-filled Dome apparatus, which was then adhered to a clean plexiglass surface in order to isolate the material and conduct leach rate sampling. One representative frame from each material type was photographed above water before Dome testing on each measurement day. Careful attention was paid during testing to leave biofilms and fouling intact and undisturbed, however, during the later stages of testing, from day 90 onward, fouling that was adhered to the PVC rings required removal or trimming to reduce the sample profile and allow for the Dome-system to properly seal (the rings did not contain antifouling characteristics to avoid sample interference). In all cases, a concerted effort was undertaken to leave the sample surface intact, as it was before testing. However, even minor disturbances to the fouled surface may impact the leach rate.

During each individual test, five 50 ml aliquots of water were withdrawn from the Dome at 15-min intervals (0, 15, 30, 45, and 60 min). Approximately half of each sample was filtered through a 0.45 μm disc filter, while the remaining half was unfiltered, allowing measurements of dissolved and total fractions of each metal, respectively. Samples were acidified to pH ≤2 with quartz-still-grade nitric acid (Q-HNO₃) in a High Efficiency Particle Air (HEPA) class-100 all polypropylene working area. The normalized planar surface area of the material under the Dome-system was as 613 cm² for the CAM, Bronze, Mesh and Net, and 707 cm² for the Sheet (Table 1).
Ancillary field data
Dissolved organic carbon (DOC) samples from ambient water were collected concurrently with leach rate testing. DOC samples were immediately acidified with concentrated quartz-still-grade sulfuric acid. Samples were sent to a commercial laboratory for analysis. In addition, pH, salinity, dissolved oxygen and temperature were measured at each sampling date in the vicinity of the frames with an In-Situ Troll 9500, to help inform and understand any potential data anomalies encountered during testing. The Troll 9500 sensor was calibrated with appropriate standards either the day before or on the day that the Dome-system measurements were conducted. These ancillary data were used as inputs to the Biotic Ligand Model (following Di Toro et al. 2001) to predict how these ancillary parameters may have influenced the bioavailability of copper and associated toxicity. Although no formal evaluation of biofouling was performed, photographs were taken of each test material during all sampling events to document biofouling development.

Laboratory methods
Metals
Quantification of metal concentrations followed trace metal clean sampling techniques throughout the collection, handling and analysis (USEPA 1996). Metal concentrations were measured with a Perkin-Elmer SCIEX ELAN DRC II inductively-coupled plasma mass spectrometer (ICP-MS; U.S. Environmental Protection Agency (USEPA) 1994). As necessary, to minimize matrix-related interference, samples were diluted with 0.1 N HNO₃ made up in 18 MΩ cm⁻¹ water. The diluted samples were injected directly into the ICP-MS via a Perkin-Elmer Autosampler 100, while the undiluted samples were preconcentrated with a Perkin-Elmer FIAS 400 flow injection system, following the methodology presented by Ndung’u et al. (2003), using a Toyopearl AF-Chelate-650M resin from Tosoh Co. Analytical standards were made with Perkin-Elmer multi-element standard solution (PEMES-3) diluted in matrix-matched 1 N HNO₃, and were analyzed at the beginning and end of each run. The analysis also included measurement of the Standard Reference Material® (SRM) 1643e, Trace Elements in Water, from the National Institute of Standards and Technology. This SRM is certified with a mass concentration of 22.76 ± 0.31 μg l⁻¹ copper, 62.41 ± 0.69 μg l⁻¹ nickel, and 78.5 ± 2.2 μg l⁻¹ zinc. Analytical recoveries for these elements in SRM 1643e throughout the series of ICP-MS runs were in average (±1 standard deviation [SD]) 95 ± 7% copper, 95 ± 7% nickel and 99 ± 6% zinc. Duplicate samples (10% of total samples) were analyzed during each ICP-MS run, with average agreement in the measured concentrations of 3.7 ± 3.8% copper, 4.0 ± 3.5% nickel, and 4.5 ± 3.8% zinc. Blanks made up of 1 N HNO₃ were analyzed every 5 samples in each ICP-MS run, with average concentrations of 0.0093 ± 0.044 μg l⁻¹ copper, 0.056 ± 0.062 μg l⁻¹ nickel, and 0.15 ± 0.070 μg l⁻¹ zinc. The method limit of detections, defined as three times the SD of the procedural blanks, were 0.13 ± 0.13 μg l⁻¹ copper, 0.19 ± 0.11 μg l⁻¹ nickel, and 0.21 ± 0.18 μg l⁻¹ zinc.

Dissolved organic carbon
DOC concentrations were measured using the SM 5310 C persulfate oxidation analysis method at Columbia Analytical Services, Inc. (Kelso, WA). The method detection limit was 0.2 mg l⁻¹ DOC.

Chronic toxicity testing
Gravid purple sea urchins (S. purpuratus) were field-collected locally in San Diego, CA and delivered to the NIWC Pacific Bioassay Laboratory. Testing was conducted in accordance with standard methods (USEPA 1995) to assess percent normal embryo-larval development. Tests were conducted on ≤4 h old S. purpuratus embryos, using a composite of the leachate samples extracted at each of the five time points during Dome-system testing, and diluted in an appropriate concentration series with 0.45-μm filtered San Diego Bay seawater. This test was completed for each replicate of the five material types. Laboratory controls were tested concurrently with each toxicity test and statistical comparisons were performed against the controls using Comprehensive Environmental Toxicity Information System (CETIS) Software, Version 1.9.2.8. Point estimate analyses were conducted to determine median effective concentrations (EC50), the percentage of the leachate sample required to adversely affect 50% of the test population. A linear regression of log transformed (to meet assumptions of normality and homoscedasticity) EC50 values and leachate copper concentrations was performed to determine if a linear relationship exists. The composited leachate samples were hand delivered to the NIWC Pacific Bioassay Laboratory and stored at 4 °C until ready for use.

Leach rate calculation
Leach rate estimates were derived from the time series of samples associated with each Dome deployment. The initial Dome volume was approximately 2.8 l; after each
aliquot was removed, the measured metal concentration was corrected for the volume of remaining water during the test. The measured metal concentration, multiplied by the system volume, provides the total mass of a given metal within the Dome-system. These mass values were regressed against sampling time (0, 15, 30, 45, and 60 min) to calculate the metal release rate from the slope of the fitted line. If regression coefficients were <0.75, those data were not included in the overall leach rate calculations. Values were normalized to the surface area of the test panel or mesh material covered by the Dome (Table 1), resulting in a final release rate in units of \( \mu g \text{cm}^{-2} \text{d}^{-1} \) for each metal analyzed.

**Life cycle loading calculation**

The surface area of an example aquaculture farming pen (30 x 30 x 12 m) was combined with leach rate data and a generic lifecycle model (Earley et al. 2014) to estimate environmental life cycle loadings (total amount of copper released during the usable deployment life of the material) from aquaculture farming pens made from the CAM or Net materials. Only the CAM pens made from the CAM or Net materials. Only the CAM and the Net aquaculture materials were considered for this calculation, as these are the only materials that would likely be used for building aquaculture pens. The other tested materials might be used for other in-water applications.

The cumulative loading \( (CL) \) over a given time interval \( (x_0, x_n) \) was approximated from leach rate measurements \( (R) \) using the following equation (Earley et al. 2014):

\[
CL_{x_0, x_n} \approx \sum_{x_0}^{x_n} (x_1 - x_0) \frac{R(x_0) + R(x_1)}{2} + (x_2 - x_1) \frac{R(x_1) + R(x_2)}{2} + \ldots + (x_n - x_{n-1}) \frac{R(x_{n-1}) + R(x_n)}{2}
\]

Equation 1:

where: \( CL_{x_0, x_n} \) = the cumulative copper loading (\( \mu g \text{cm}^{-2} \)) from day \( x_0 \) through \( x_n \); \( x_n = a \) series of consecutive time points (days) during which release rate measurements were made beginning with day \( x_0 \) and ending with day \( x_n \); and \( R(x_n) \) = the measured release rate (\( \mu g \text{cm}^{-2} \text{d}^{-1} \)) for time point \( x_n \).

Typical copper release rate patterns have an initial spike in concentration, followed by a decline to an asymptotic low or a pseudo-steady state (PSS) (Valkirs et al. 2003; Earley et al. 2014). Copper loading estimates over four years, equivalent to the expected life cycle for a CAM pen, were made for both materials. For this comparison, it was assumed that the Net material would require cleaning at 6 months intervals and replacement every 24 months; note however that cleaning frequency could be adjusted using the equations herein to inform site-specific conditions. Following a cleaning event or replacement, it was assumed that the measured initial copper release rates would occur, followed again by reduction to PSS. This would allow for the most conservative, or worst case, copper loading rates over the product life cycle. These episodic variations in release rates based on cleaning or replacement were used in the loading calculation for the four-year period. The CAM pens would require no prescribed cleaning or replacement intervention, and would continue to leach metal at PSS after a single initial release.

A schematic diagram of the release rate patterns for each material is shown in Figure 4.

The PSS is the average release rate (\( \mu g \text{cm}^{-2} \text{d}^{-1} \)) over the period of time when rates have stabilized to an asymptotic low. Specifically, this is at least 24 days after starting exposure and contains four or more data points where the arithmetic mean between two consecutive points differs from the final calculated weighted mean release by not more than 15% (ASTM International (ASTM)) 2006). Building from Equation (1), the PSS was calculated by the following equation:

\[
PSS_{x_a, x_n} = \frac{CL_{x_0, x_n}}{(x_n - x_a)}
\]

Equation 2:

where: \( PSS_{x_a, x_n} \) = the pseudo steady state loading rate (\( \mu g \text{cm}^{-2} \text{d}^{-1} \)), which occurs after day \( x_a \) and \( x_a = \) the time after which the copper release rates asymptote to PSS.

To capture the cumulative copper loading during the initial release period, the following equation was used:

\[
IL_{x_0, x_n} = CL_{x_0, x_n}
\]

Equation 3:

where: \( IL_{x_0, x_n} \) = the initial release loading (\( \mu g \text{cm}^{-2} \)), which occurs before day \( x_n \).

The total copper loading based on a materials life cycle was thus estimated using the above variables with the following equation:

\[
\text{Life Cycle Loading}_{b,n} = ([IL_{x_0, x_n}] \times (\Sigma \text{E}_\text{cleaning} + \Sigma \text{E}_\text{replacement})) + (PSS \times \Sigma D_{PSS})
\]

Equation 4:

where: \( \text{Life Cycle Loading}_{b,n} \) = Cumulative copper release (\( \mu g \text{cm}^{-2} \)) between time points \( x_s \) and \( x_f \) the time over which the material is exposed to water;
$\Sigma E_{\text{cleaning}} = \text{the total number of regularly scheduled material cleaning events over a given life cycle period; }$

$\Sigma E_{\text{replacement}} = \text{the total number of regularly scheduled material replacement events over a given life cycle period (which includes the initial placement of material); }$ and $\Sigma D_{\text{PSS}} = \text{the total number of days at which PSS releases are anticipated to occur.}$

**Numerical modeling**
Metals leached into the water column from fish pens will be transported by currents and/or may partition into other reservoirs such as sediments. The numerical hydrodynamic fate and transport model Curvilinear Hydrodynamics in 3D (CH3D), was applied to examine the transport and fate of metals from hypothetical fish pens using the leaching rates measured above for both San Diego Bay, CA and Sinclair Inlet, WA. CH3D is a boundary-fitted finite difference, Z-coordinate model developed by the U.S. Army Corps of Engineers Waterways Experiment Station (Johnson et al. 1991) to simulate hydrodynamic currents in four dimensions (x, y, z and time), and the fate and transport of metals in estuaries and coastal environments under these current forcings (Sheng 1990; Wang and Richter 1999). Hydrodynamic models used in this study include hydrodynamic mixing due to tides only. This is sufficient since tidal forcing is the largest factor governing hydrodynamic mixing in these two waterbodies.

The grids of the CH3D models were previously defined for San Diego Bay and Sinclair Inlet. These CH3D models have been previously implemented to simulate copper and other AF biocide concentrations from hull paint in San Diego Bay (Wang et al. 2004), concentrations of copper and its species from other sources in San Diego Bay (Chadwick et al. 2008), sewage outfall discharge and dilution in Sinclair Inlet (Wang and Richter 1999; Wang et al. 2004), and to assess total maximum daily loading for fecal coliform in Sinclair and Dyes Inlets (Johnston et al. 2009). For both model simulations completed here, simulated copper concentrations are assumed to derive completely from the fish pen loads, with no other background concentrations or inputs from the ocean boundary, as these background and ocean boundary contributions have been considered in previous studies. Supplementary Table 1 describes the model conditions used for both CH3D runs in this study.

Hypothetical CAM fish pen loads were located in two grid cell locations each in the San Diego Bay entrance channel bend and the Clambay entrance to Sinclair Inlet, respectively. At each location, inputs from a total of 50 fish pens were simulated, each with a copper leach rate of 193.3 g pen$^{-1}$ day$^{-1}$ (the calculated four-year lifecycle loading rate of a 30 $\times$ 30 $\times$ 12 m pen divided by 1,460 days), resulting in an estimated total copper load from 50 pens of 9.665 kg day$^{-1}$. Half of this copper load was evenly
introduced to the entire water column at each of two model grid cells for each water body. During the simulations, the hydrodynamic model was activated from a cold start, running for 48 h before the copper loads entered into the simulation. A steady state of the simulated copper concentrations was reached after 10 days in San Diego Bay and 30 days in Sinclair Inlet.

Results and discussion
Ancillary environmental parameters and DOC
The ancillary environmental parameters followed the annual seasonal pattern, and remained within a relatively small range. The range in temperature was 15.39 to 20.65 °C with an average of 17.97 °C. Salinity ranged between 32.00 and 34.22, with a mean of 32.98. pH ranged from 7.87 to 8.57, with a mean pH of 8.13, and DOC ranged from 0.72 to 2.13 mg L⁻¹, with an average concentration of 1.38 mg L⁻¹.

In general, the ancillary parameters indicated that the seawater conditions remained relatively moderate throughout the exposure. The exposure and resulting leach rates measured here are therefore representative of the temperate conditions in San Diego Bay, which may differ of those from more extreme latitudes, or tropical sites.

Copper leach rates and biofouling of aquaculture materials
Release rates of copper from the materials followed a temporal pattern with high copper release rate in the initial stages of the deployment, followed by an asymptotic decrease to a small constant release rate, or pseudo steady state (PSS) in about 30 days (Figure 5, Supplementary Table 2). However, the magnitude of the initial release was different between the types of material. The copper alloys had initial releases of copper on the order of 100 to 200 µg cm⁻² d⁻¹, while the treated nylon net initially released about 10 µg cm⁻² d⁻¹. In comparison, typical AF coatings on ships’ hulls initially release copper at a rate of about 50 µg cm⁻² d⁻¹ (Earley et al. 2014). Note that for leach rate quantitation, as undertaken for this study, careful handling of all test materials to reduce potential disturbance to the test surfaces that could influence leach rates, is required. Small increases in leach rates during later stages of the experiment (Figure 5) are suspected to be related to minor disturbance to the test material surfaces during late stages of testing.

The PSS asymptotic low release rate was achieved in ~ 30 days and ranged between 1.4 and 6.9 µg cm⁻² d⁻¹ for copper alloys, and < 0.5 µg cm⁻² d⁻¹ for the treated net (Figure 5). The pattern of a rapid decrease in metal leach rate to a low PSS leach rate observed here (Figure 5) is similar to those previously reported.
for panels covered with epoxy and ablative coatings (Seligman et al. 2001; Valkirs et al. 2003; Earley et al. 2014). The PSS of copper alloys measured here (1.4, 3.9, 2.9, and 6.9 μg cm⁻² d⁻¹ for Sheet, Mesh, Bronze, and CAM, respectively), is similar to that measured from typical AF coatings as well (3.0-5.7 μg cm⁻² d⁻¹; Earley et al. 2014).

When exposed to seawater, a protective corrosion-product film forms on copper-based materials (Francis 1986; Tuthill 1987). Informal visual assessments of the test materials throughout the experiment, particularly apparent on the Sheet material (Figure 6), indicated development of such a protective film, and is consistent with the observed decrease in copper leach rates to an asymptotic low, at which point the film would be fully developed (Tuthill 1987).

The difference in pseudo steady-state release rate influences biofouling control. The traditional net had the lowest PSS release rates of <0.5 μg cm⁻² d⁻¹, but it also had the most biofouling growth, based on visual assessment, during the deployment timeframe (Figure 6). In contrast, the PSS release rate for the four copper alloy materials, 1.4 to 6.9 μg cm⁻² d⁻¹, was able to prevent biofouling growth on those materials for longer periods (Figure 6). Among the copper alloy materials, the Bronze and Net had multiple layers of material. These samples also appeared to have the highest degree of biofouling, which may be an artifact of the layering that created a three-dimensional matrix, and may have increased the capability for fouling organisms to gain a foothold, despite the AF chemical properties of the materials also being increased with the multiple-layer approach.

**Table 2. Relevant results from toxicity testing using S. purpuratus embryo-larval development for each material type at each testing time point.**

| Material type | Leachate data metric | 1 | 8 | 15 | 29 | 43 | 64 | 91 | 183 | 272 |
|---------------|----------------------|---|---|----|----|----|----|----|-----|-----|
| CAM | Initial (μg l⁻¹) | 629 | 526 | 621 | 82 | 84 | 43 | 58 | 42 | 52 |
| | Max test (%) | 25 | 24 | 18 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Max test (μg l⁻¹) | 157 | 126 | 112 | 82 | 84 | 43 | 58 | 42 | 33 |
| | EC50 (%) | 6 | 3 | 30 | 29 | 44 | 96 | 59 | >63 | 33 |
| | EC50 (μg l⁻¹) | 38 | 16 | 21 | 24 | 25 | 19 | 56 | 25 | >33 |
| Bronze | Initial (μg l⁻¹) | 2,714 | 2,562 | 2,472 | 129 | 120 | 70 | 56 | 27 | 69 |
| | Max test (%) | 7 | 6 | 4 | 90 | 100 | 100 | 100 | 100 | 100 |
| | Max test (μg l⁻¹) | 190 | 154 | 99 | 116 | 120 | 70 | 56 | 27 | 69 |
| | EC50 (%) | 1 | 1 | 29 | 16 | 30 | 80 | 78 | 62 | 62 |
| | EC50 (μg l⁻¹) | 30 | 20 | 27 | 37 | 19 | 21 | 45 | 21 | 43 |
| Sheet | Initial (μg l⁻¹) | 1,076 | 371 | 103 | 56 | 38 | 35 | 28 | 17 | 8 |
| | Max test (%) | 13 | 34 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Max test (μg l⁻¹) | 149 | 126 | 103 | 56 | 38 | 35 | 28 | 17 | 8 |
| | EC50 (%) | 3 | 4 | 25 | 59 | >100 | >100 | >100 | >100 | >100 |
| | EC50 (μg l⁻¹) | 36 | 16 | 26 | 33 | >38 | >35 | >28 | >17 | >8 |
| Mesh | Initial (μg l⁻¹) | 1,153 | 1,153 | 854 | 77 | 31 | 17 | 14 | 10 | 29 |
| | Max test (%) | 8 | 9 | 12 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Max test (μg l⁻¹) | 92 | 104 | 102 | 77 | 31 | 17 | 14 | 10 | 29 |
| | EC50 (%) | 2 | 1 | 3 | 33 | 64 | >100 | >100 | >100 | >100 |
| | EC50 (μg l⁻¹) | 28 | 15 | 21 | 25 | 20 | >17 | >14 | >10 | >29 |
| Net | Initial (μg l⁻¹) | 249 | 77 | 47 | 27 | 26 | 12 | 9 | 17 | 18 |
| | Max test (%) | 74 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Max test (μg l⁻¹) | 184 | 77 | 47 | 27 | 26 | 12 | 9 | 17 | 18 |
| | EC50 (%) | 20 | 24 | 69 | >100 | >100 | >100 | >100 | >100 | >100 |
| | EC50 (μg l⁻¹) | 49 | 19 | 33 | >27 | >26 | >12 | >9 | >17 | >18 |

For each material, the following data are shown: copper concentrations in the initial leachate delivered to the bioassay lab (μg l⁻¹), the highest leachate concentration (%) tested for toxicity, the copper concentrations in the highest concentration sample (μg l⁻¹), the associated estimated median effective concentration (EC50) as a percentage leachate (%) and the associated EC50 copper concentration (μg l⁻¹).

a100% concentration not tested due to technician error.

**Purple sea urchin embryo-larval development toxicity tests**

This study paired chemical measurements from leachate obtained from Dome-system measurements with bioassays using test organisms and endpoints that are among the most sensitive to copper (USEPA 2003). Toxicity of the leachates became lower over time, evidenced by the increasing EC50 values of the leachate concentrations (Figure 7). This observed reduction in toxicity was directly related to the reduction in overall copper concentration in the leachates with time. Indeed, the measured (ICP-MS) copper concentration and EC50 values across all samples were strongly negatively correlated (r = −0.97). The results of the linear regression analysis show a significant linear relationship (p < 0.05, r² = 0.94), suggesting that
Leachate toxicity was driven by copper concentration. Although zinc was measured at concentrations (up to $67 \mu g \ l^{-1}$, data not shown) that approach effects concentrations for *S. purpuratus* larval development ($EC_{50} = 102 \mu g \ l^{-1}$; Phillips et al. 1998), the concentration in test samples was never elevated enough to be of concern due to dilution required to bracket the expected effects associated with copper.
These results suggest that the copper alloy materials are not more or less toxic to marine organisms, and do not pose an elevated environmental threat, compared with elemental copper.

The differences in the temporal trend and magnitude of the release rates between the four copper alloy materials and the traditional Net (Figure 5), as well as the rates of fouling development (Figure 6) were also reflected in similar patterns in toxic effects of the leachate. The Net leachate lost toxicity fastest, followed by the Sheet and Mesh. Leachate from the CAM and Bronze samples continued to exhibit some toxicity over the entire period tested. (Figure 7). Although Bronze samples continued to show toxicity, there also was increased biofouling relative to other materials that could be an artifact of layering the Bronze.

For the bioassays conducted, the sensitivity of S. purpuratus to copper was consistent with that historically observed for these organisms at NIWC Pacific in dilution water collected from North San Diego Bay (EC50 = 14.3 µg l⁻¹; Rosen et al. 2008). Normal responses in reference toxicant tests and controls met test acceptability criteria of at least 80% normal larval development (USEPA 1995) across all testing events.

**Biotic ligand model**

The bioavailability of copper was likely similar across sampling events based on the relatively narrow range in environmental parameters and DOC measured throughout the deployment. Ancillary field data were input into the Biotic Ligand Model to compare toxicity results and assess how environmental conditions may have affected the relative toxicity of leached copper to the test organisms. Water temperature had no effect. Low salinity, low DOC and high pH all increased copper toxicity (decreased the copper concentration at which toxicity occurred to the test organisms).

Low DOC and low salinity conditions can lead to higher copper toxicity, as there are fewer organic compounds available to complex copper, and fewer sodium and chloride ions that can compete with copper at organism binding sites, respectively (Di Toro et al. 2001). Both of these conditions, therefore, increase the bioavailability of copper. In this study, higher pH was counterintuitively related to increased toxicity. Higher pH can indicate increased water hardness and, therefore, more competition with copper to binding sites, similar to increased salinity; however, seawater pH can fluctuate rapidly, particularly in nearshore environments (e.g. Hofmann et al. 2011), and this result may not be robust, but rather a consequence of sampling rate.

The results from this study, and mechanism behind the Biotic Ligand Model (e.g. Di Toro et al. 2001; Bosse et al. 2014), suggests that the bioavailability, and therefore, the toxicity of metals leached from aquaculture pens will be affected by oceanographic conditions around and inside the pens. Thus, pens should ideally be located in areas where these conditions would not inadvertently increase the toxicity of leached metals in the surrounding environment.

**Life cycle loading**

Over a four-year life cycle, using the assumptions outlined above, the life cycle loading of copper from the CAM and Net materials was estimated as 12,060 µg cm⁻² and 1,480 µg cm⁻², respectively. Thus, for 30 × 30 × 12 m aquaculture pens, over four years, one pen would release ~282 kg or 35 kg of copper, if made from the CAM or Net material, respectively.

Earley et al. (2014) applied the same leaching rate and life cycle loading calculations to estimate copper loading entering San Diego Bay from passive and active leaching (after cleaning) from AF coatings on ships’ hulls. They found that over a three-year life cycle, between 4,157 and 13,017 µg cm⁻² of copper would enter the bay from a given ship’s hull, with the lower bound occurring without cleaning or vessel movement and the upper bound occurring with regular cleaning that did not follow best management practices. Using a standard recreational boat size of 40 feet (12.2 m) in length and a 13 foot (4 m) beam, one boat has an approximate average wetted surface area of 41 m² (Bakker and van Vlaardingen 2017). Using a conservative estimate of 8,000 µg cm⁻² of copper entering the water over a three-year life cycle, one standard-sized boat would therefore introduce ~4.3 kg of copper to the bay over four years. To put these values into context, San Diego Bay has ~9,000 slips (San Diego Harbor Police 2002). Assuming an average 40 foot boat length coated with copper based AF coating, the environmental loading of copper from these boats is equivalent to that from ~135 CAM pens.

**Numerical modeling**

CH3D modeling indicated that the copper concentrations in both San Diego Bay and Sinclair Inlet from fish pens constructed of CAM would generally attain
maximal values near the pens (loading source), and the values would decrease with distance from the pens, as expected (Figures 8 and 9). This source/dilution pattern is consistent with the overall hydrodynamics in these water bodies. On average, copper concentrations in a given grid cell fluctuated temporally along with tidal variability.

Simulated tidally-averaged mean copper concentrations in San Diego Bay ranged from 1.17-1.27 \( \mu g \ l^{-1} \), 1.19-1.61 \( \mu g \ l^{-1} \), and 0.92-1.12 \( \mu g \ l^{-1} \) from each of four cells located north of, in the middle of, and south of the simulated fish pens (Figure 8), respectively. Simulated tidally-averaged mean copper concentrations for Sinclair Inlet ranged from about 0.03-0.11 \( \mu g \ l^{-1} \) at two locations near the simulated fish pens (Figure 9).

Tidal current is the only driving force for the simulated transport and mixing of copper leached from the fish pens, and its effects are reflected in the fluctuations of simulated copper concentrations. Supplementary Figures 1–3 show time series of simulated copper concentrations. Compared to tidally-averaged values, fluctuation magnitudes of the simulated copper concentrations are high. During the neap tides, when tidal flushing is poor, temporal maxima were 3.4 \( \mu g \ l^{-1} \), 2.8 \( \mu g \ l^{-1} \), and 2.4 \( \mu g \ l^{-1} \), for the area within, north and south of the fish pen locations, respectively. The corresponding temporal maxima during the spring tides, when tidal flushing is active, are low, approximately equal to or lower than the tidally-averaged values (Supplementary Figures 1–3).

Compared to simulated tidally-driven fluctuations in copper concentrations associated with simulated fish pens in San Diego Bay, at Sinclair Inlet the differences of simulated copper concentration fluctuations during spring and neap tides are less pronounced, caused by relatively strong tidal action in Sinclair Inlet (Supplementary Figure 4). The stronger flushing and dilution effects for Sinclair Inlet are also reflected in the relatively larger difference of the simulated copper concentrations between the loading locations (near the simulated fish pens) and neighboring locations (Supplementary Figure 4). Stronger tidal currents induce stronger flushing and dilution, and leached copper is flushed or diluted more quickly with distance from the simulated pens in Sinclair Inlet compared to San Diego Bay.

Simulated copper concentrations were relatively uniformly distributed in the water column in San Diego Bay, which is consistent with the fact that San Diego Bay is shallow and circulation is driven by tides, resulting in vertically uniform hydrodynamics through the water column (Figure 10). In Sinclair Inlet, hydrodynamic flows are more stratified due to flow shear and density stratification. Poor vertical mixing in the relatively deeper water column in Sinclair Inlet lead to low simulated copper concentrations, in the range of 0.01-0.03 \( \mu g \ l^{-1} \) in the surface layer, and to higher concentrations near bottom with copper values up to 0.1-0.13 \( \mu g \ l^{-1} \). These higher concentrations of copper in the lower portion of water...
column in Sinclair Inlet are caused primarily by settling of particulate copper through the stratified water column. Contrary to that for San Diego Bay, settling-induced redistribution of copper concentrations in the water column dominated over the poor vertical mixing for Sinclair Inlet. Settling rates for San Diego Bay were obtained empirically from Chadwick et al. (2008) and for Sinclair Inlet obtained from a parallel copper fate and transport study (Johnston et al. 2009); these are listed in Supplementary Table 1.

Steady state copper concentrations attained a maximal value near the loading locations in both simulations. These maximal concentrations were 1.2 µg L⁻¹ for San Diego Bay and 0.12 µg L⁻¹ for Sinclair Inlet.

Both of these numerical copper-loading models used steady loads over the entire study time period, which in actuality represents a more conservative scenario, maintaining the steady state loads throughout the simulations. Alternatively, in dynamic loading scenarios, higher initial peak loads tend to be flushed out of the systems, and the loads that follow the initial peak, loads which are lower in magnitude, would tend to under-estimate the copper concentrations predicted from the steady state loading conditions.

Summary

This study focused on assessing the overall loading and potential toxicity to the surrounding environment of metals leached from copper alloy materials in contrast to traditional antifoulant-treated nylon aquaculture netting. Yigit et al. (2016, 2018b) previously investigated metals in fish and mussel tissues grown in and around CAM pens, respectively, and found elevated metals, but that all metals were below thresholds that would present a risk to human health through consumption. Similarly, Kalantzi et al. (2016) found elevated metal concentrations in nearby waters following deployment of a copper alloy net and an antifoulant-treated net, which decreased and stabilized with time. Those authors also found that experimental fish and mussels did not accumulate metals above recommended safety thresholds. However, it was not well known how much copper or other metals overall would leach into the ocean water from CAM pens and whether these levels would cause such high environmental loading as to be detrimental to the...
surrounding ecosystem. Further, it was not known whether the combination of various metals represented in these alloys might be more toxic and thus potentially more environmentally detrimental than each metal alone.

The study conducted here found that the copper, impregnated as cuprous oxide in the nylon Net, was more rapidly depleted compared to the metal alloys. This was apparent in both the Dome-system leach rate data and the visual assessment of biofouling. After <15 days, leaching rates were low and marine growth started occluding the net openings (Figures 5 and 6). In contrast, the CAM retained its AF properties with a low steady state copper release rate throughout the year of exposure (Figures 5 and 6).

Metal leaching rates from each material were determined using the Navy Dome-system, and used to calculate environmental loading estimates over the expected lifecycle of both traditional netting and copper alloy net materials. Overall, a CAM fish pen would contribute more than 15 times the amount of copper to the environment over a four-year grow-out cycle compared to a fish pen made of antifoulant-treated Net material. However, CAM fish pens retain their antifoulant properties and can be recycled at the end of their lifecycles.

Toxicity of the leachate was directly related to copper concentration, and decreased with time based on increasing concentrations of leachate that the sea urchin larvae could tolerate before showing significant abnormal development (Figure 7). No intrinsic difference in toxicity was observed among the materials, other than the relation to copper concentration.

Using the 3D hydrodynamic models previously developed and calibrated, fate and transport of copper leached from fish pens in San Diego Bay and Sinclair Inlet were simulated. The physical processes simulated include transport by tidal currents, vertical mixing and settling of particulate copper in the water column. These processes compete and interact with each other and resulted in simulated copper concentrations, which are consistent with the general transport and mixing behaviors in these two waterbodies. This study shows that, with the same copper loads in two different waterbodies, different magnitudes of simulated copper concentrations were obtained, relating to different capacities for transport and mixing differences.

The results from the current study indicate that environmental loading of metals from copper alloy materials is higher than from antifoulant-treated nets. However, using the weight of evidence approach presented here, the overall effect of this metal input is not interpreted to be environmentally detrimental. This difference (in metal release rates) is important for farming pen maintenance purposes, including factors such as control of water flow obstruction, steady oxygen availability, decrease in the probability for establishment of pathogen vectors and control on the total weight of the pen structure (Gonzalez et al. 2013).

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