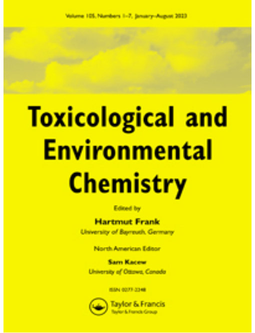
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Estuarine copper concentrations following boat-wash down and subsequent accumulation in blue mussels

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ABSTRACT

Antifouling paints may contain copper which prevents the attachment and growth of organisms on vessel hulls. In the study, estuarine copper concentrations and its dispersal are determined as well as whole-body copper burden of *Mytilus edulis* deployed at 8 sites in the estuary, following boat wash-down in Lymington estuary, United Kingdom. The copper concentrations half a meter away from a boat wash-down was quickly diluted within 4 h. But 24 h following a wash down, copper levels remained 10- to 20-fold elevated above the levels 30 m away from the source. Copper concentrations in the sediment half a meter from the point source were about 100-fold greater than within the marina but the marina sediment concentrations were still above the effect range low. Mortality of *M. edulis* was not observed, but copper body burden 24 h following a wash down event was up to tenfold elevated for 5 d before returning to normal within 21 d.

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salinity;
body-burden;
sediment copper

Introduction

Biofouling is the undesirable accumulation of organism on submerged surfaces causing an increase in drag and fuel consumption of ships and recreational boats (Amara et al. 2018), as well as the spread of invasive species (Wan et al. 2021). Antifouling paints are biocidal products applied to boats and ships, as well as other submerged structures, to prevent the adhesion and accumulation of organisms on the surface. Tributyl-tin (TBT) based antifouling paints proved to be extremely effective but have been shown to severely affect reproduction in marine

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organisms (Bryan et al. 1987). Consequently, the International Maritime Organisation implemented a world-wide ban on TBT in 2003, and currently most antifouling paints contain copper and zinc and a variety of organic biocides (Amara et al. 2018; Paz-Villarraga, Castro, and Fillmann 2022). The Biocidal Products Regulation (European Parliament and Council 2012) regulate application of these products and state that they should be applied at the minimum to induce an effect (e.g. removal of biofouling organism), and for Cu the effect release rate is between 3 and 22 μg $cm^{-2} d^{-1}$ (Lagerström et al. 2020).

In the UK the 76/464/EEC directive (EEC 1976) sets Environmental Quality Standard (EQS) for dissolved Cu at 5 μg L^{-1} (expressed as an annual average). However, site specific aquatic Cu concentrations are affected by dilution and sedimentation that are also dependent on the specific physicochemical parameters such as pH, salinity, dissolved oxygen and temperature (Beck and Sañudo-Wilhelmy 2007). Even though copper is an essential micronutrient and acts as a co-factor for several enzymatic processes, at high concentrations it is toxic (Bury, Walker, and Glover 2003), and there is growing environmental concern over the quantity of Cu entering the aquatic environment from boating activity (Karlsson, Ytreberg, and Eklund 2010; Bighiu, Eriksson-Wiklund, and Eklund 2017; Wrange et al. 2020). Copper toxicity is associated with Cu^{2+} or Cu^{+} ions (Campbell 1995) but in natural waters ionic Cu forms only a small fraction of the total Cu present due to complexation with carbonate, hydroxide, chloride, and dissolved organic matter (Stumm and Morgan 1996).

There are two fundamental paths by which copper from antifouling paints can enter the aquatic environment; via direct liberation from the paint surface through normal use (Ytreberg, Karlsson, and Eklund 2010; Lagerström et al. 2020), or by means of pressure washing of pleasure craft hulls and other maintenance activities directly on the foreshore (Thomas, McHugh, and Waldock 2002). Pressure washing is carried out to remove impurities such as attached marine organisms, salts and slime, and to detach the old paint from the ships hull (Champ 2003). During this process, wastewater containing high levels of dissolved copper are directly discharged into the river, estuary or coastal waters (Champ 2003), and because the paints are polymer-based they also form part of the microplastic pollution entering our oceans and subsequent release of biocides as they degrade (Gaylarde, Neto and Monteiro da Fonseca 2021).

This study reports on the fate of total and dissolved (defined as the concentration filtered through a 0.45 μm filter) copper concentrations in a marina and Lymington estuary, United Kingdom, following high pressure hosing operations. The copper dispersion and dilution from this point source was assessed in the surface waters at 9 sites, 6 within a 30 m radius of the point source. Copper concentrations were also assessed over the tidal cycle. From these values an estimate of the contribution of boat-wash down to the total copper input to the estuary could be made. The impact of copper to biota is dependent on bioavailability and this was assessed by measuring copper soft body copper content in the blue mussel (*Mytilus edulis*) deployed at 8 sites for 9 d before a wash-down event and then the subsequent 21 d thereafter.

Materials and methods

Sampling was carried out along Lymington River, Hampshire in southern United Kingdom and within one of its marinas. Tides in this region are semi-diurnal and are unusual in that they exhibit a double peak or 'stand' over high water, with a well-defined low water of relatively short duration, and a fast, scouring ebb. Lymington River provides moorings for approximately 1600 leisure craft, the current study was from a marina with approximately 250 berths. In addition to the permanent moorings the river receives approximately 9000 visiting yachts per annum, most of which arrive during the peak summer season. Nine sites were chosen for the study (Figure 1 and Table 1). Among the sampling stations, sites

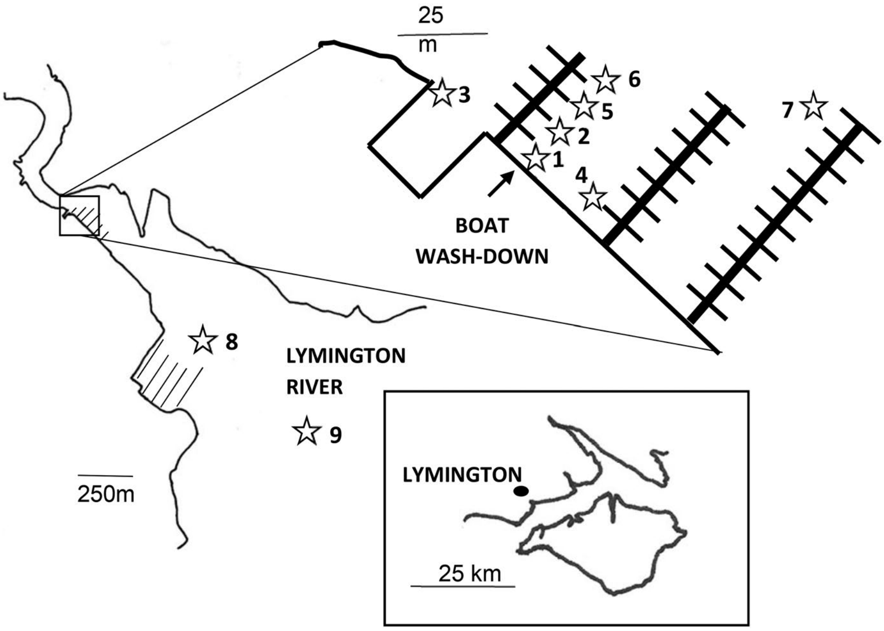

Figure 1. Map of study area at Lymington, Hampshire, United Kingdom, on the Lymington River feeding including sampling stations at the marina pontoon and at the mouth of the river as it enters the Solent.

Table 1. Location and position of sampling stations, distance from point source and typical surface water parameters recorded at HW in Lymington River.

Site	Location	Position Latitude	Position Longitude	Distance from point source (m)	*T* (°C)	*S* (ppt)	*C* (mS cm^{-1})	pH	DO (mg l^{-1})	DO (% sat)	TDS (g l^{-1})	Turbidity (NTU)	Background Copper Total (μg l^{-1})	Background Copper Dissolved (μg l^{-1})
1	Wash-down run-off *point source* (ps)	50°45′26.57″N	01°32′00.58″W	0	19.1 ± 0.04	0.5 ± 0.04	0.9 ± 0.01	8.3 ± 0.06	9.5 ± 0.04	101.8 ± 1.1	0.6 ± 0.04	572.4 ± 3.3		
2	PS river entry point	50°45′26.69″N	01°32′00.48″W	0.5	16.8 ± 0.03	16.6 ± 0.2	24.2 ± 1.1	6.5 ± 0.04	7.3 ± 0.01	82.4 ± 0.5	17.6 ± 0.2	3.6 ± 0.8		
3	Upstream of PS	50°45′27.10″N	01°32′01.36″W	30	16.8 ± 0.2	17.1 ± 0.8	27.7 ± 1.2	7.6 ± 0.01	6.9 ± 0.3	79.2 ± 3.3	18.0 ± 0.8	2.9 ± 0.6		
4	Downstream of PS	50°45′26.43″N	01°31′59.85″W	30	16.7 ± 0.2	15.3 ± 2.1	25.0 ± 3.1	7.5 ± 0.04	7.0 ± 0.2	79.2 ± 1.1	16.3 ± 2.0	3.2 ± 1.4		
5	Offshore from PS	50°45′27.04″N	01°32′00.69″W	20	16.8 ± 0.3	16.1 ± 0.1	26.3 ± 1.4	7.5 ± 0.2	7.1 ± 0.1	80.6 ± 1.6	17.1 ± 0.1	2.7 ± 0.6	6.7 ± 1.6	3.8 ± 1.3
6	Offshore from PS	50°45′27.36″N	01°32′00.20″W	30	16.8 ± 0.2	14.8 ± 0.3	24.2 ± 0.5	7.5 ± 0.1	7.2 ± 0.1	81.6 ± 1.7	15.8 ± 0.4	3.4 ± 0.7		
7	Berthon Marina reference site	50°45′27.70″N	01°31′54.07″W	150	17.5 ± 0.1	18.5 ± 0.8	29.8 ± 1.1	6.9 ± 0.01	7.1 ± 0.02	82.8 ± 0.0	19.4 ± 0.8	4.5 ± 0.1	6.3 ± 1.4	4.6 ± 1.9
8	Yacht Haven entrance	50°45′06.04″N	01°31′30.71″W	850	17.0 ± 0.3	28.8 ± 1.4	44.5 ± 2.0	8.0 ± 0.01	7.3 ± 0.7	85.7 ± 4.7	28.9 ± 1.3	3.1 ± 0.5	6.8 ± 0.2	6.6 ± 0.1
9	River mouth	50°44′45.43″N	01°31′09.71″W	1500	16.8 ± 0.1	33.8 ± 0.1	51.3 ± 0.2	8.1 ± 0.05	8.0 ± 0.8	91.3 ± 3.3	33.4 ± 0.1	3.6 ± 0.6	6.1 ± 1.0	3.9 ± 0.1

Mean values ± standard deviation of temperature (*T*), salinity (*S*), conductivity (*C*), pH, *dissolved oxygen* (DO), *total dissolved solids* (TDS) and turbid

7–9 were considered reference sites because they are located outside of the marina.

Sample collection

Clean sampling techniques were used through out the study. Prior to use, all glass and plasticware was thoroughly cleaned in a 5% (v/v) Decon-90 solution for 24 h, rinsed in high purity (18.2 MΩ cm) Milli-Q water, and then transferred to a 10% (v/v) nitric acid (Merck Life Scientific, Gillingham, Dorset, United Kingdom) bath for a further 24 h before being rinsed again in Milli-Q water and air dried prior to use.

Estuarine and marina water samples were collected 0.1–0.3 m from the surface in 1 L LDPE sample bottles. Discrete water samples (5 L) were collected at different depths using Niskin bottle (General Oceanics Inc., Miami, Florida, USA) attached to a dynema rope and triggered at the appropriate depth by a lead messenger attached to the rope. For every water sample taken, *in-situ* physical parameters (temperature. salinity, conductivity, pH, dissolved oxygen, total dissolved solids, and turbidity) were also measured by deploying a YSI 6600 Sonde multi-parameter probe (YSI Inc., Yellow Springs, Ohio, USA) from either the pontoon or boat and recording the data on a YSI 650 MDS data-logger (YSI Inc., Yellow Springs, Ohio, USA).

Estuarine and marina surface sediment samples (approximately top 25 cm) were collected using a 0.25 m^2 stainless steel mini-Van Veen grab from either the pontoon or boat. Three separate sediment samples were taken from sites 2–9.

To assess the effects of tidal flushing on copper concentrations, a tidal cycle survey was performed at the marina reference site (site 7) (Figure 1). Water samples from different depths (surface, 1 m, mid water column and bottom) were taken at high water (HW), medium water (MW) and low water (LW) at site 7 on two consecutive days.

Total and dissolved copper was determined from each water sample. Four sub-samples were prepared onsite, comprising of two 10 mL non-filtered (total) samples and two 10 mL (dissolved) samples filtered through sterile 0.45 μm cellulose acetate (Merck Life Scientific, Gillingham, Dorset United Kingdom) disposable filters into 15 mL polypropylene conical centrifuge tubes (BD Falcon™). Samples were preserved with high purity concentrated nitric acid (Merck Life Scientific, Gillingham, Dorset United Kingdom), at a concentration of 0.1% (v/v).

As a first step in assessing the input of copper biocides associated with hosing activities, the wash-down from two pleasure crafts (1 yacht and 1 motorboat) was collected and analyzed for total and dissolved copper (site 1). *In situ* surface waters samples at time of wash-down

(T0) were also taken at sites 2–6. To gain a perspective of dilution/dispersion of the wash-down run-off within the marina, this process was repeated at $T = 1$, 4 and 24 h following the wash-down event.

Mussel deployment

Cultured mussels were sourced locally from Oakford Oysters (Blandford Forum, United Kingdom). At sites 2–8, 15 mussels of 54.1 ± 8.0 mm were transplanted at each site. The mussels were placed in oval plastic meshed cages and suspended at a depth of 1 m. Deployment times were 1, 5, 10, 15 and 30 d respectively ($N = 3$ per exposure). A boat was washed down on day 9 of the deployment.

Copper analysis

Sediment samples were oven dried at 60 °C to constant weight in ceramic crucibles and ground using a pestle and mortar and sieved through a 63 µm sieve. Samples (1 g) were then digested under reflux in 10 mL high purity concentrated nitric acid (Merck Life Scientific, Gillingham, Dorset, United Kingdom) for 5 h, filtered through 1.2 µm Fisherbrand® glass microfibre paper and made up to 50 mL using Milli-Q water.

Whole Cu body burden quantification was performed on the whole-body tissues of *M. edulis*. The entire soft parts of the mussels were removed the bulked soft parts were weighed, as well as measuring the individuals shell length and weight. Samples were oven dried at 80 °C to constant weight, cold digested in 5 mL of high purity concentrated nitric acid (Merck Life Scientific, Gillingham, Dorset, United Kingdom) for 72 h and finally made up to 50 mL using Milli-Q water.

For each sample (aqueous, sediment and mussel) measurements of copper were made using graphite furnace atomic absorption spectrometry (4100ZL, Perkin-Elmer, Llantrisant, Mid Glamorgan, United Kingdom). In all cases triplicate analyses of samples were performed with the acceptable relative standard deviation between repeat analyses being < 10%. No internal standards for sediment or tissue copper concentrations were measured, thus values are based purely on the standard curve made using commercial standards (1000 mg L^{-1} Cu standard, VWR, Lutterworth, Leicestershire, United Kingdom). All sediment and mussel samples were frozen (−20 °C) within 2 h of collection.

Data analysis

Contour plots, generated in Sigma Plot version 10.0, were used to illustrate dispersion/dilution of total and dissolved copper in surface waters emanating from the point source at time of wash-down (T0), through to T24. The X axis is distance offshore, Y axis is distance up/downstream, and the Z axis is Cu concentration values in 2D space. The exponential equations derived between linear sites (eg, [2, 3], [2, 4], [2, 5, 6], [3, 6] and [4.6]) of known Cu concentration, were used to predict the concentrations between them.

Significant differences in copper sediment concentrations between sites and mussel body burden (of all exposure times) for all sites against the control mussel burden were assessed via a one-way univariate analysis of variance (ANOVA). Differences in mussel body burdens between sites and exposure times and differences in aqueous total and dissolved copper concentrations between depth and tidal state were assessed using a two-way ANOVA. A *post-hoc* Tukey's multiple comparison test was applied to identify where significant differences.

Results

Tidal influence on copper concentrations

A summary of the mean values of temperature, salinity, conductivity, pH, dissolved oxygen (DO), total dissolved solids (TDS) and turbidity, recorded at HW along Lymington River, at each sample site is provided in Table 1.

Background Cu levels; defined as copper concentration in surface water samples taken at sites outside of the marina at HW and where no boat wash-down activity had taken place 48 h previously varied between 6.1–6.8 μg L^{-1} and 3.8–6.6 μg L^{-1} for total and dissolved, respectively (Table 1).

There was no significant affect of depth on total Cu concentrations (Figure 2) at site 7. Conversely, depth displayed a significant affect on dissolved Cu concentration ($F_{3,60}$ = 4.46, $p = .007$), which varied between 6.74 and 2.83 μg L^{-1} between surface and bottom samples, respectively. There was also a significant interaction between depth and tidal state which influenced dissolved Cu concentrations ($F_{6,60}$ = 3.43, $p = .006$) (Figure 2). There was a significant difference between surface and bottom dissolved Cu concentrations at HW and LW ($p < .05$).

Boat wash-down – copper input

The wash-down water from two pleasure craft (1 yacht and 1 power boat) was analysed for total and dissolved copper. During these events the total Cu concentrations at discharge were measured at between 1140 and 4420 μg Cu L^{-1} (average 2780 ± 2320). The estimated volume of

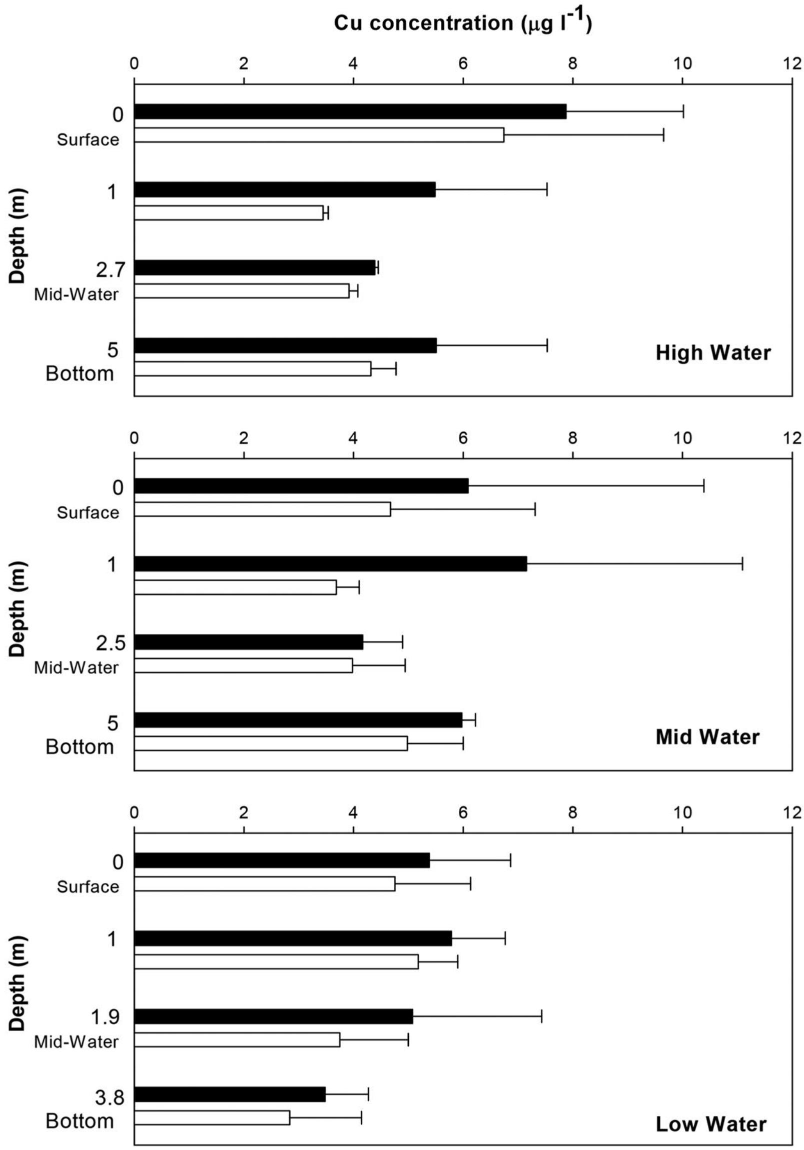

Figure 2. Background total (black bars) and dissolved (open bars) Cu concentrations (µg L^{-1}) taken from reference site 7 over a 6 h tidal cycle [High Water (A), Mid Water (B) and Low Water (C)]. Values represent an average of 3 separate measurements ± SD.

water used in a wash down was 375 L which would give an estimate based on average total copper of 1.04×10^4 mg Cu released per boat.

Upon discharge into the marina at site 2, Cu concentrations in wash-down run-off were immediately diluted approximately 10-fold (Figure 3(a)). Concentrations 30 m up-, down-stream and offshore from site 1 at T0 were 12, 15, and 6 µg Cu L^{-1}, respectively. At T1, discharge from site 1 was still entering the marina, though at reduced concentration (total copper 11.3 mg Cu L^{-1} Figure 3(b)). Due to the scaling of

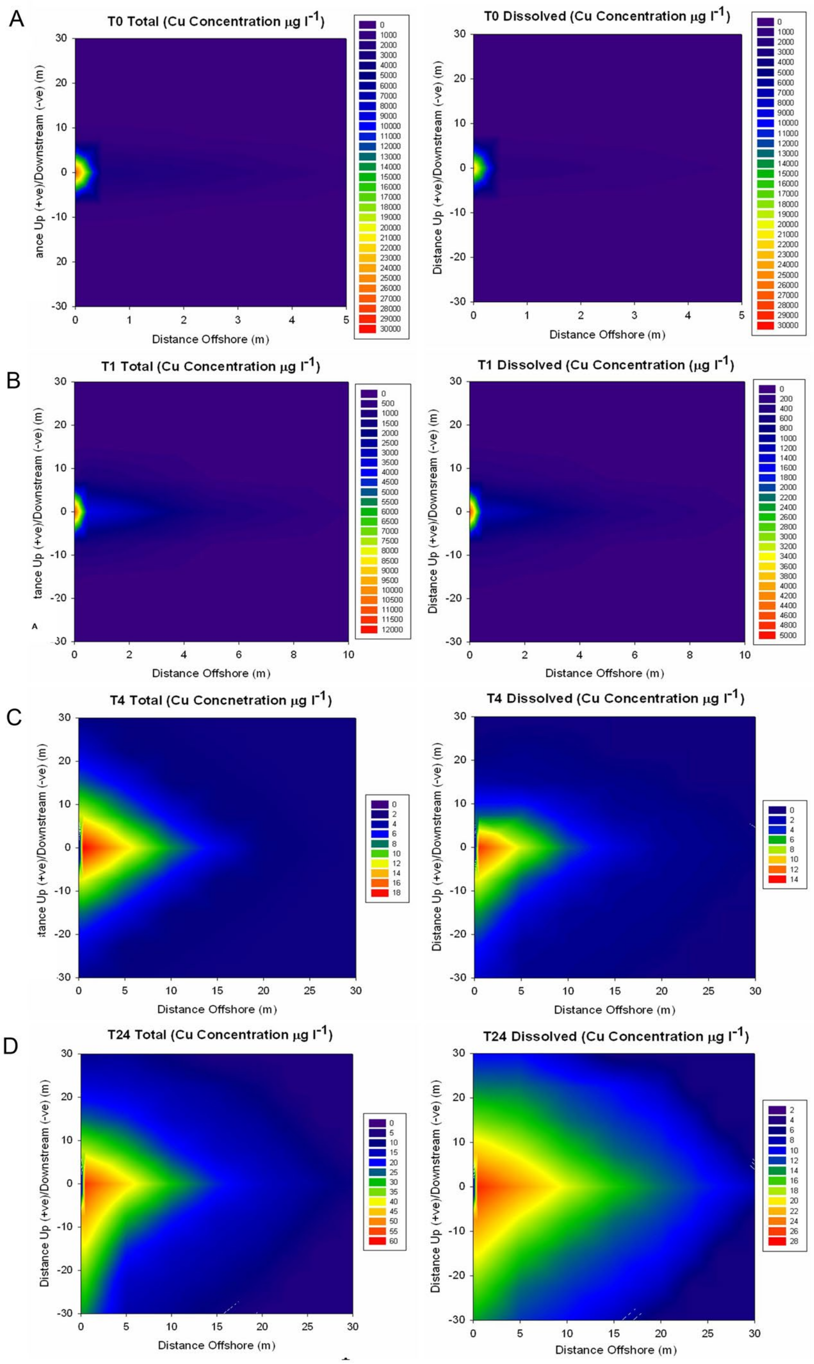

Figure 3. Contour plots of Cu dispersion (µg L^{-1}) from point source at (A) T0, (B) 1 h, (C) 4 h and (D) 24 h following a boat wash-down event. Figures on the left are for total Cu and right dissolved Cu concentrations. Note differences in x-axis and legend scales.

the Cu concentration on the contour maps it is difficult to visualise the Cu concentrations 30 m downstream and offshore at T1, however, these had increased significantly to 20 (site 4) and 10 μg L^{-1} (site 6) (Figure 3(b)).

By T4 (4 h), discharge from site 1 had ceased and water Cu greatly diminished (see the vastly different scales on the contour maps, Figures 3(a,c)), though Cu levels at site 2 were still elevated at 18 and 13 μg L^{-1} for total and dissolved, respectively and showed background concentrations beyond a 5 m radius of site1 (sites 3–6; Figure 3). However, at T24 Cu at site 3 and 4 (30 m from discharge point) were elevated and where higher at site 2 compared to at T4; 56 and 27 μg Cu L^{-1} for total and dissolved, respectively.

Sediment metal concentrations

Cu concentrations within sediment were variable among sites and ranged between 7810 and 15 mg kg^{-1} dry wt at sites 2 and 9, respectively (decreasing with distance from point source) (Figure 4). The difference in mean concentration of Cu in sediment at the 8 sampling sites were statistically different ($F_{7,14}$ = 101.11, $p < .001$) with site 2 Cu concentrations being significantly greater than all other sites and Cu concentrations at sites 8 and 9 being significantly less (Figure 4).

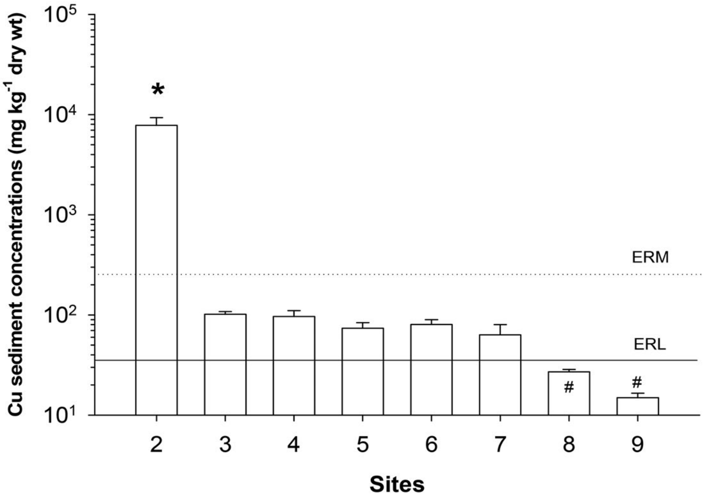

Figure 4. Cu levels (mg kg^{-1} dry weight) in sediment at all sampled sites. Values represent average + SEM, $N = 3$. * indicates significant greater than all other values and # significantly less (One-way ANOVA post-hoc Tukey's multiple comparisons test $p < .05$). The solid line represents the Effects Range Low (ERL) and the dashed line the Effect Range Medium (ERM) (Long et al 1995).

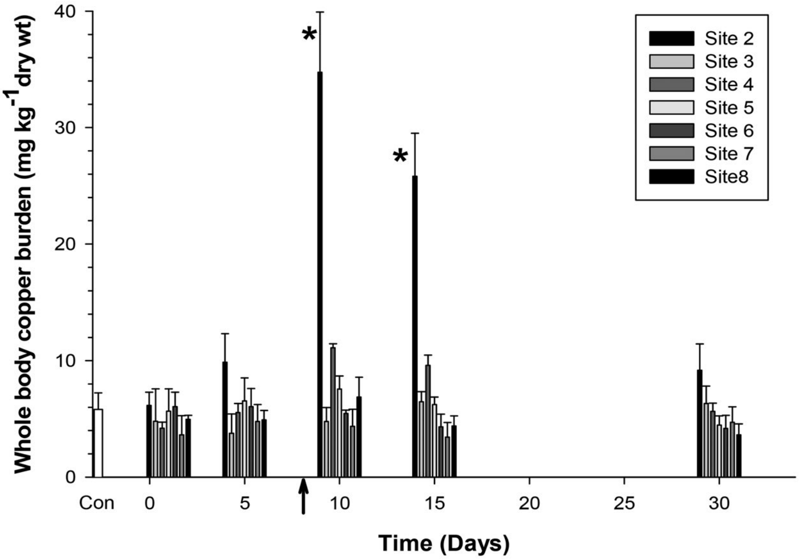

Figure 5. Cu body-burdens (µg g^{-1}dry weight) in *Mytilus edulis* at varying exposure times for sites 2–8. Open bar indicates the values in mussels before deployment (Con), the legend on the figure shows the bar shades corresponding to each site. The arrow indicates the time of a boat wash down. Values represent average + SEM, $N = 3$, * indicates significantly greater body burdens at those time points (Two way ANOVA *post-hoc* Tukey's multiple comparisons test $p < .05$.

Blue mussel (Mytilus edulis) whole body copper burdens

Mussel body burdens were variable between sites and time of exposure and ranged from 3.4 (site 7, day 15) to 34.7 µg Cu g^{-1}dry wt (site 2, day 10) (Figure 5). Results showed that levels in the vicinity of the point source (sites 2) were significantly higher than those elsewhere ($F_{2, 105} = 4.00$, $p < .02$). Individual sampling site also had a significant effect on mussel body burden ($F_{6\ 70} = 33.66$, $p < .001$). The *post hoc* Tukey's test demonstrated a significant difference between site 2 mussel body burden and all other sites, including the pre-deployed controls ($p < .05$). Time of exposure had a significant effect on mussel body burden ($F_{4,70} = 13.00$, $p < .001$) demonstrating a significant difference between day 10 and days 1, 5 and 30, and day 15 and days 1 and 30 for mussels collected at site 2 ($p < .05$) (Figure 5).

Discussion

The current study shows that approximately 1.04×10^4 mg (10.4 g) of Cu is released from a single boat wash down. This causes an exceptional high concentration of Cu immediately close to the point of discharge (i.e. 11.4–44.2 mg total Cu L^{-1} at site 2, 0.5 m from the discharge point) which is rapidly dispersed within 4 h, but still remains elevated above background water concentrations 24 h following the wash down. The high copper levels measured in this study exceeded the predicted solubility of inorganic Cu (~500 µg Cu L^{-1} at pH 8 and 25 °C). The reason for this pseudo-saturation is unclear; no copper carbonate precipitates were observed, but may have been present, alternately the Cu measured may be part of a soluble organic pool of Cu (Singh and Turner 2009). Despite these high Cu concentrations, no *M. edulis* mortalities were observed. The Cu from the wash-down was bioavailable with *M. edulis* transplanted at site 2 (e.g. the mostly heavily impacted by the wash-down) showing a significant accumulation of Cu, maximum recorded 34.7 µg Cu g^{-1} dry wt. This level of accumulation was surprisingly low considering the exceptionally high concentrations of copper in the surface water, suggesting that the copper may not be readily available potentially incorporated in to microplastic particles derived from the paint (Gaylarde, Neto and Monteiro da Fonseca 2021). However, a similar level of accumulation have been observed in *M. edulis* exposed to particles of antifouling paint (~4 mg Cu L^{-1} and a viscera concentration of ~60 µg Cu g^{-1}) in the laboratory for 16 h followed by a 24 h depuration period (Turner, Barrett, and Brown 2009) and in the gastropod *Littorina littorea* the head and foot accumulated ~80 µg Cu g^{-1} on exposure to 10 mg/L of antifouling paint over 5 d (Gammon, Turner, and Brown 2009).

A previous study by Jones and Bolam (2007) found that Cu concentrations in 4 sites around the UK representative of marinas, harbours and estuaries did not exceed the EQS value of 5 µg L^{-1}, with a range of dissolved Cu between 0.30 and 6.68 µg L^{-1}. In contrast, our background dissolved Cu concentrations were either close to this standard or exceeded it, 3.8–6.6 dissolved µg L^{-1}. These values were also higher than those measured in the adjacent estuary with similar pleasure craft activity River Hamble (1.1–5.9 µg Cu L^{-1}, Environment Agency personnel communication). Despite the current EQS being based on dissolved Cu this may not reflect the bioactive fraction of Cu, which is often referred to the labile Cu fraction and is measured via differential pulse stripping voltammetry. In the survey by Jones and Bolam (2007) they estimated that the ratio of labile Cu was between 10 and 30% of the measured total fraction and would suggest that the background labile Cu concentrations in Lymington estuary are likely not to pose an ecological risk, and this may be reflected in the lower-than-expected accumulation of copper in the tissues of the deployed mussels (Figure 5). However, research conducted by Bowman, Readman and Zhou (2003), who investigated seasonal variability in antifouling booster biocide Ingarol 1051 in a UK marina, demonstrated highest mean concentrations in surface waters during winter months (November, December and January). This probably results from the high intensity of wash-down and re-application

Table 2. Estimated yearly copper input from a marina of 250 boats.

% of boats treated with Copper(I) oxide[a]	99–100
Cu leachate rate[b] (µg cm^{-2} d^{-1})	8.2
Surface area for 45m boat below the water line[a](m^2)	30.7
Daily leaching input (mg/d)	2517.4
Estimated marina leaching load (kg/year)	229
Estimated hosing input[c] (kg/year)	10.4
Estimated marina hosing load from 250 boats (kg/year)	2.6
Total Cu input from a 250-boat marina (kg/year)	**231.6**
% input from leachate	99
% input from hosing	1

[a]Obtained from Boxall et al. (2000)
[b]Obtained from Valkirs et al. (2003)
[c]Values from current study.

of antifouling paints to boats removed from the water during the autumn (Thomas, McHugh, and Waldock 2002). Bowman, Readman and Zhou (2003) went on to demonstrate concentrations also increased in the period April to July when newly treated vessels are usually returned to the marina for the boating season, and the highest leaching rates occur just after application (Hall et al. 1999). Thus, our measurement of Cu concentration during the summer may be low, and further surveys would be necessary to ascertain the seasonality of dissolved and labile Cu spikes.

The contribution from boat wash-down or leaching to the overall estuarine loading is uncertain due to the potential for inputs from other non-point sources, such as urban run-off. However, an estimate of the relative contribution of each of these boating activities can be made (Table 2). The estimated Cu release rate from a boat hull varies between 3.7 and 20 µg Cu cm^{-2} d^{-1} (Schiff, Diehl, and Valkirs 2004), and for the purpose for this calculation a value of 8.2 µg Cu cm^{-2} d^{-1} (Valkirs et al. 2003) is used. A typical sized vessel in the marina is 45 m^2 boat whose surface below the water line is 30.7 m^2 (Boxall et al. 2000), which equated to 2517.4 mg Cu being released per day. In a marina of 250 boats the total discharge would be 629 g of copper per day. If it is assumed that the number of boats in the marina is constant throughout the year (this is unlikely; there is a large influx of transitory visitors during the summer months) then a total of 229 kg Cu yr^{-1} would be leached from these boats. If we also assume that each boat is cleaned once a year, and thus an additional 2.6 kg Cu yr^{-1} will enter the estuary from the wash-down activities for the 250 boats. Based on these calculations, the majority of copper input from boating activity is via leaching (98.8%) and only 1.12% is derived from hosing activities (Table 2). The biogeochemical fate of copper from each source will differ, the comparatively low amounts of copper liberated from the paints over a long duration through standard boat use will be readily diluted and dispersed thus reducing the immediate environmental risk. Whereas the copper input from wash-down takes place over a very short time window with concomitant sharp increase in copper concentration in the immediate area of the point source (Figure 3).

Our results suggest that most of the copper within the estuary (Figure 2) was in the dissolved phase, despite this the sediment samples near to the point discharge are exceptionally high in copper (Figure 3). In other studies, it has been estimated that roughly 75% of the wash-down input is associated with particulate matter that will become integrated with bottom sediments (Thomas, McHugh, and Waldock 2002). In comparison to water concentrations, sediment metal concentrations are indicators of the long-term accumulation of metals in water bodies and are a legacy of metal pollution (Luoma and Rainbow 2008). The National Oceanic & Atmospheric Administration (NOAA) sediment quality guidelines for sediment give Effects Range-Low (ERL) and Effects Range Median (ERM) values of 34 mg Cu kg^{-1}, and 270 mg Cu kg^{-1} (Long et al. 1995). Only sites 8 and 9 fell within the ERL concentration, though all sites, bar site 2, fell within the ERM. Site 2 exceeded the ERM concentration by almost a factor of 30. The fate of antifouling paint particles (Gaylarde, Neto, Monteiro da Fonseca 2021) and copper entrained within sediments is not understood, but it has been estimated that remobilisation of other metals (not necessarily from antifouling paint) such as Ag (Rivera-Duart and Flegal 1997) and Ni (Topping and Kuwabara 2003) from the benthic sediment during tidal fluxes may contribute significantly to the dissolved metal concentrations in San Franscisco Bay.

Conclusion

The study shows that there is a significant input of copper from boat wash-down activities, which is rapidly dispersed. This copper loading, despite being exceptionally high, was not acutely toxic to adult *M. edulis*. However, the mussels close to the source did show high body burdens shortly after the wash-down. These body burdens dropped off and returned to control levels within 15 days of exposure indicating that these organisms can regulate copper. The rise in copper input from diffuse urban sources poses a serious threat to the coastal environment, thus a reduction in the input of copper from any source will be beneficial. A number of advancements in technology, such as closed loop systems, cyclonic filtration and ozone treatment have resulted in a reduction in copper at point-source from boat wash down activities. But our estimates indicate that the majority (98%) of copper entering estuaries from boating activity is a result of leaching from the hulls.

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