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Combined effects of water flow and copper concentration on the feeding behavior, growth rate, and accumulation of copper in tissue of the infaunal polychaete *Polydora cornuta*

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Abstract We performed an experiment in a laboratory flume to test the effects of water flow speed and the concentration of aqueaous copper on the feeding behavior, growth rate, and accumulation of copper in the tissues of juvenile polychaetes *Polydora cornuta*. The experiment included two flow speeds (6 or 15 cm/s) and two concentrations of added copper (0 or 85 μg/L). Worms grew significantly faster in the faster flow and in the lower copper concentration. In the slower flow, the total time worms spent feeding decreased significantly as copper concentration increased, but copper did not significantly affect the time worms spent feeding in the faster flow. Across all treatments, there was a significant, positive relationship between the time individuals spent feeding and their relative growth rate. Worms were observed suspension feeding significantly more often in the faster flow and deposit feeding significantly more often in the slower flow, but copper concentration did not affect the proportion of time spent in either feeding mode. The addition of 85 μg/L copper significantly increased copper accumulation in *P. cornuta* tissue, but the accumulation did not differ significantly due to flow speed. There was a significant interaction between copper and flow; the magnitude of the difference in copper accumulation between the 0 and 85 μg/L treatments was greater in the faster flow than in the slower flow. In slow flows that favor deposit feeding, worms grow slowly and accumulate less copper in their tissue than in faster flows that favor suspension feeding and faster growth.

Keywords Copper · *Polydora cornuta* · Benthos · Suspension feeding · Deposit feeding · Flume experiment

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Introduction

Considering that laboratory-based toxicity assays are usually conducted under controlled, static and potentially over-simplified conditions, they often do not adequately mimic ecologically relevant variables or reflect in situ conditions (Moreira et al. 2005). This is particularly true for whole-sediment toxicity testing, which is usually completed under static conditions (U.S. Environmental Protection Agency 1994) and does not integrate key environmental variables, especially water flow. Furthermore, many sediment assays use mortality as the end-point of toxicity, despite the fact that sublethal endpoints such as growth rate, reproductive capacity and various behaviors are increasingly regarded as more sensitive metrics that can be achieved in relatively shorter experiments (Moreira et al. 2005; Rosen and Miller 2011). In additional, all animals accumulate trace metals into their tissues (Rainbow 2002) and, when preyed on, these accumulated metals transfer to higher trophic levels (Mendez and Green-Ruiz 2005; Rainbow et al. 2004). Measuring the accumulation rates of metals in animal tissues is, therefore, another sublethal endpoint that is key to understanding the broader cycling of metals in food webs and ecosystems (Rainbow 2002; Rainbow et al. 2004).

This study examined sub-lethal endpoints of feeding behavior, growth rate, and accumulation of copper in the tissues of juvenile spionid polychaetes, *Polydora cornuta,* in a laboratory flume experiment to explicitly incorporate effects of water flow on copper accumulation and sublethal toxicity. Like most spionids, *P. cornuta* is an interface feeder (Dauer et al. 1981) that switches between deposit feeding and suspension feeding in response to near-bottom flow and the flux of suspended particles (Bock and Miller 1995; Taghon et al. 1980; Taghon and Greene 1992). Laboratory flume experiments have revealed that the growth rates of juvenile *P. cornuta* are greatly enhanced when flow favors suspension feeding (Hentschel and Herrick 2005; Hentschel and Larson 2005; Taghon and Greene 1992). More recently, transplantation experiments have shown that *P. cornuta* growth rates in situ vary over small spatial scales in relation to tidal elevation (Matthews and Hentschel 2011) and over short time scales in relation to major rain events (Colvin and Hentschel 2011).

Spionids are common members of many soft-sediment communities, often having densities over 10,000 individuals m^{-2} (Hentschel and Larson 2006; Taghon 1992; Zajac 1991). Their tube-building, feeding, and bioturbation activities alter the sediment and affect other members of the community (Bolam and Fernandes 2003; Thrush et al. 1996). These worms also are important prey to higher trophic levels (Stehlik and Meise 2000; Tomiyama et al. 2007; West et al. 2003) and are among the first colonists of disturbed sediments (Levin et al. 1996; Pearson and Rosenberg 1978). Along with other opportunistic spionid species, the abundance of *P. cornuta* in estuaries has been used as an indicator of habitat quality (Dix et al. 2005). *P. cornuta* is distributed along the east coast of Northern America and has been introduced to the Pacific Coast as well (EPS 2001). Several species of spionids, including *P. cornuta,* are often used as indicators of pollution based on their abundances in situ or their performance in laboratory assays (Dix et al. 2005; Callier et al. 2009; EPS 2001). The abundances of spionids and the broader structure of infaunal communities also have been widely used to assess the recovery and restoration of human-impacted estuaries (e.g., Levin et al. 1996; Zajac and Whitlatch 2001).

Although spionids in general and *P. cornuta* in particular have been well studied in the ecological and toxicological literature (EPS 2001), interactions between near-bottom flow and the sublethal effects of copper (or any other metals) have not been tested explicitly for potential deleterious effects. Here, we performed a two-factor experiment in which flow speed and the concentration of aqueous copper were manipulated in a fully factorial design to measure effects on *P. cornuta* feeding behavior, growth rate, and accumulation of copper.

Methods

Organism and sediment collection

Live *P. cornuta* were collected during low tide from the eastern bank of Oneonta Slough in the Tijuana River National Estuarine Research Reserve (TRNERR, +32.56617°, −117.13119°) by sieving the top 3–5 cm of surficial sediment in the field (0.5 mm mesh) and then transported to San Diego State University's Coastal and Marine Institute Laboratory (CMIL), where they were sorted under a stereomicroscope. Individual *P. cornuta* were removed from their sediment tubes, and undamaged juveniles ranging in length from 3 to 7 mm were selected for the flume experiment. These juveniles were maintained in petri dishes with defaunated field-collected sediment and 5-μm filtered seawater (FSW) for 1–3 weeks until being placed in the flume. All seawater used in out experiments was originally pumped from Scripps Pier (+32.86618, −117.25438) and transported to CMIL. Field-collected sediment was defaunated by freezing, thawing, sieving to remove particles larger than 0.3 mm, and freezing a second time. Aliquots of frozen defaunated sediment were thawed just prior to use with worms.

Experimental design

The experiment was conducted in a Plexiglas laboratory flume located at CMIL (Fig. 1). The flume is similar to one described in Shimeta et al. (2004). The channel of the flume is 140 × 25 cm (L × W). Flow is driven by a 0.5-hp pump, and speeds are adjusted by partially closing valves that either direct water to flow through the channel or divert it into a reservoir. Turbulence was dampened by channeling the water through two bundles of plastic soda straws

Fig. 1 Picture of laboratory flume used to conduct experiments

(0.5 × 9.5 cm, D × L) that were located 19 cm apart from one another at the head of the flume channel. The channel bottom includes a recessed box (15 × 20 × 8 cm, W × L × D) located 84 cm downstream from the soda straws. The recessed box is centered in the channel, providing 5 cm of space between the edges of the recessed box and the channel sidewalls. A water chiller (Aqua Logic, Inc) was connected inline to maintain water temperature at 20 °C.

The experiment included two flow speeds (6 and 15 cm/s, as measured 1.5 cm above bottom) with or without copper (Final concentration: 0 or 85 µg/L, nominal). Each combination of flow and copper was replicated 3× in a fully factorial design and consisted of separate 6-day flume runs (i.e., a total of 12 flume runs). The combination of flow speed and copper concentration was assigned randomly for each flume run. After each flume run, the flume was drained, sediment was removed from the recessed box, and the empty flume was rinsed with 2 % nitric acid and then rinsed again with FSW. Flow speeds were chosen based on prior evidence of significant effects on *P. cornuta* growth rates due to sediment resuspension (Hentschel 2004). In the present study, the critical erosion velocity for fine components of the sediment was ~12 cm/s (Colvin pers. obs.). Flow speeds were measured 1.5 cm above the bottom of the flume with a Marsh-McBirney Flow-Mate 2000.

To obtain a final aqueous concentration of 85 µg/L (nominal) in the flume, 12.5 mL of a 477 mg/L copper chloride stock solution (verified by Enviromatrix Analytical, Inc., San Diego, CA) was added to 70 L of FSW. The FSW was verified that it did not contain copper (non-detect, 0.0 µg/L) at the onset of the study by Enviromatrix Analytical, Inc. That particular concentration of copper (85 µg/L) was chosen after determining in a 96-h static range finding pilot study that 85 µg/L caused only 5 % mortality of *P. cornuta* (i.e., LC5). The addition of 85 µg/L copper is well below the maximum concentrations of aqueous copper in the TRNERR. As reported by Meyer and Gersberg (1997), previous studies have found mean copper concentrations of 116 µg/L and concentrations in the first flush of a rain event reaching up to 400 µg/L in the Tijuana River, which drains entirely downstream to the TRNERR. Sediment used in the experiments was analyzed prior to and after a representative flume run for each flow speed for copper concentrations by Enviromatrix Analytical, Inc. Copper concentrations at the beginning of an experiment were 0.55 µg/g and were 0.53 and 0.76 µg/g at the termination of the 6 and 15 cm/s flow speeds, respectively.

Each flume run included 60 *P. cornuta* juveniles that were randomly assigned to subsets for measuring growth rates, feeding behaviors, or copper accumulation in a given flow speed and copper concentration. To measure relative growth rates (RGR), we anesthetized 30 individuals in 5 % MgCl$_2$ and photographed each individual using a Sony

HDR-HC7 camcorder connected to a Leica MZ 12.5 stereomicroscope and a Macintosh computer with iMovie software prior to starting each 6-day flume run. Using ImageJ software, we measured the body length of each individual and its width at five locations to calculate its body volume as the sum of four conical frustums (Hentschel 2004). The volume of each frustum was calculated as

$$V = (\pi/3) \bullet L \bullet \left[(W_1/2)^2 + (W_1/2)(W_2/2) + (W_2/2)^2 \right],$$

(1)

where V is the volume of the frustum, L is the length of the frustum, and W_1 and W_2 are the two width measurements at each end of the frustum. After measuring each individual's initial body volume, worms were transferred to FSW for 15 min. Twenty four of these measured worms were then placed in individual wells of a Falcon 24-well plastic plate (12.8 × 8.5 cm) containing defaunated sediment. Worms built their sediment tubes and acclimated to their wells for 2 h in a temperature-controlled room (20 °C) prior to initiation of a flume run.

The remaining six pre-measured worms were placed in individually numbered vials that facilitated observations of feeding behaviors in addition to measurements of these individuals' growth. The vials consisted of two concentric cylinders (both 2 cm high) made from 2-mm thick polypropylene tubing and sealed at the bottom with Hot Melt Glue (Colvin and Hentschel 2011). After filling a vial with defaunated sediment, a pre-measured worm was placed into the central cylinder (5 mm inner diameter). The outer cylinder (16 mm outer diameter) ensured that the worm was located near the center of the entire vial and aided in the recovery of the worms. These worms acclimated to their vials for 2 h as indicated above.

To provide additional *P. cornuta* tissue for the measurement of copper accumulation, each flume run also included an additional 30 worms that were divided equally among the wells of a Falcon 6-well plate (12.8 × 8.5 cm). These 30 worms were not measured for body size and instead were directly acclimated to their sediment wells for 2 h, as described above.

At the beginning of each flume run, the two welled plates and the six individual worm vials were placed into the recessed box in the flume channel. The individually numbered vials were located in a row (1.8 cm between each vial) along the edge of the box (5 cm from the flume's sidewall) to facilitate direct observations of each individual's feeding behaviors. The two multi-well plates containing worms were located in the center of the box, with the 24-well plate upstream of the 6-well plate. Defaunated sediment was added to fill the recessed box and smoothed over the plates and vials so that the sediment and the tops of the well plates and vials were flush with the bottom of the flume channel.

The flume was then filled with FSW to a final volume of 70 L and a water depth of 7.5 cm in the channel.

To provide a controlled food source, a non-living algal slurry (Shellfish Diet 1800, Reed Mariculture, Campbell, CA) was added to the flume. The slurry concentration was 2×10^9 cells/mL and consists of *Isochrysis* sp, *Pavlova* sp, *Tetraselmis* sp, and *Thalassiosira weissflogii*. At the start of each experiment, 1.5 mL of the concentrated slurry was added to the well mixed reservoir of the flume and an additional 0.5 mL was added daily for the remainder of each 6-day flume run.

Growth rate measurements

Following each 6-day flume run, individual wells and vials were rinsed with a stream of FSW to expose worms' U-shaped sediment tubes. Worm tubes were then gently removed from the wells and vials with forceps, and worms were teased out of their tubes and re-measured to calculate each individual's final body volume. RGR were calculated according to Fisher (1921) as:

$$RGR = [\ln(V_f) - -\ln(V_i)]/time, \tag{2}$$

where V_f and V_i are measurements of each individual's final and initial body volume, respectively (Hentschel 2004). Relative growth rates were analyzed using a 2-factor ANOVA with flow speed and copper concentration as the main factors ($n = 3$ replicate flume runs for each treatment).

Observations of feeding behaviors

Near the end of each 6-day flume run, just prior to the removal of the worms from the flume, individuals in the 6 vials were monitored for their feeding behaviors using a scan-sampling procedure (Martin and Bateson 2007). Every 2 min over the course of 30 min we observed the instantaneous behavior of each of the six individuals. A light was placed on the opposite side of the flume channel from the observer and backlit the worms' feeding palps to facilitate observations. Behavioral data were treated as counts of observations in each behavioral category seen during the observation period. Behaviors were categorized as: no activity, deposit feeding, or suspension feeding. Occasionally, worms were observed with one feeding palp on the sediment surface and the other palp raised into the near-bottom water. This behavior was quantified as half a count towards deposit feeding and half a count towards suspension feeding. The total number of observations for each of the three behavioral categories was averaged for each flume run, and the proportion of time engaged in each behavioral category was calculated for each combination of flow speed and copper concentration.

Data were arcsine square-root transformed prior to analyses. We decided a priori to perform two analyses of the behavior data. First, we performed a 2-factor ANOVA that tested whether there was a difference in the time spent feeding (as opposed to not feeding) among the flow and copper treatments ($n = 3$ replicate flume runs for each treatment). Second, we analyzed the observations of feeding activity more closely by performing a 2-factor ANOVA that tested the proportion of time that feeding worms were suspension feeding (as opposed to deposit feeding) among the flow and copper treatments ($n = 3$ replicate flume runs for each treatment).

Copper accumulation

We determined the concentrations of copper in the tissues of worms before and after each 6-day flume run. Prior to each flume run, 60 worms were randomly assigned to initial tissue analyses rather than being included in the flume run and were randomly divided into two samples to determine the initial concentration of copper in worm tissue. At the end of each flume run, the 30 worms placed in the six-well plate were pooled to form one sample for tissue analysis, and the 30 individuals measured for growth were pooled to form a second sample. The pair of initial samples and the pair of final samples for each flume run were each averaged to provide a single estimate of the initial tissue concentration and a single estimate of the final tissue concentration for each flume run at a given flow speed and aqueous copper concentration. Before measuring the copper concentration of worm tissues, the worms were placed in a Petri dish containing FSW for 1 h to allow evacuation of gut contents and limit the influence of ingested sediment on the analysis of worm tissues. Gut clearance was confirmed by visual inspection using a microscope. On a few occasions, some individuals were given an additional 1 h to clear their guts. Samples were then frozen until all could be processed concurrently. Worms subjected to tissue analysis were then placed into pre-weighed, acid-washed vials, dried in an oven at 60 °C, weighed, and then subjected to microwave digestion and routine metal analysis (Deheyn and Latz 2006). Each sample received 500 μL of 45 % ultra pure nitric acid (Optima, Fisher) and underwent microwave digestion (Ethos One, Milestone Inc.) at 80 °C for approximately 4 h. The resulting samples were diluted with 5 mL Milli-Q water, shaken and allowed to settle for 24 h. The supernatant was then transferred to polystyrene tubes and measured with an ICP-OES Perkin Elmer Optima 3000 DV analyzer, which was calibrated before every run for quality control by successive dilution of a 100 ppm certified multi-element instrument calibration standard solution (Fisher Scientific). For quality assurance/quality control, a 1 ppm multi-element standard was run approximately every

20 samples over the course of a run and the intra-run variation was 102.9 ± 6.8 % (mean $\pm$ standard deviation), with a range of 96.1–112.8 %. Method blanks (analytical tubes subject to the same treatment but without containing any sample) were analyzed concurrently with samples to ensure consistency of the equipment and minimal contamination during processing of the samples. Measurements from the blanks were subtracted from the sample readings to determine the copper concentration of each sample. To ensure accuracy of the digestion process, processing of a standard reference marine organism tissue (DOLT-1) was conducted by Deheyn and Latz (2006) with recovery from the expected concentration for Cu at 114.6 %. Similar digestion procedures for marine tissues that were employed by Deheyn and Latz (2006) were utilized in this study.

The accumulation of copper in worm tissue during each 6-day flume run was calculated by subtracting the initial copper concentration from the final copper concentration measured for each flume run. The effects of flow speed and aqueous copper concentration were tested with a 2-factor ANOVA ($n = 3$ replicate flume runs for each treatment, except that the samples from one of the three flumes run at 15 cm/s and 85 µg/L copper were lost during processing).

Results

Relative growth rate

On average, we recovered 77 % of pre-measured individuals from each flume run (i.e., 23 of the 30 individuals initially placed in the flume). In a few additional cases, worms were
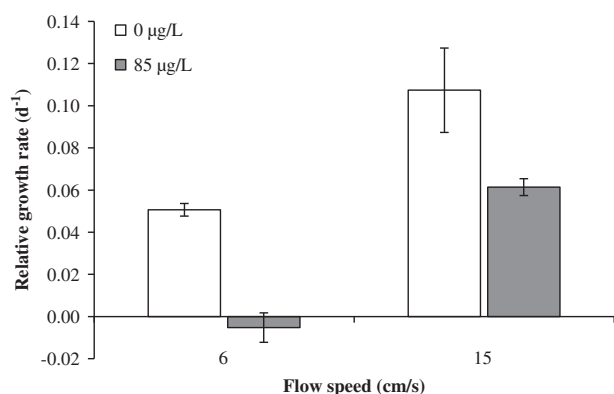
damaged during removal from their well or vial; these damaged worms were not re-measured and not included in the growth rate analyses, but their tissue was included in the analysis of copper accumulation. All worms collected at the end of each flume run were alive and active. The proportion of worms recovered was not affected significantly by either flow speed ($F_{1,8} = 0.376$, $p = 0.557$) or copper concentration ($F_{1,8} = 0.094$, $p = 0.767$). The relative growth rate (RGR) of *Polydora cornuta* differed significantly between the two flow speeds ($F_{1,8} = 32.324$, $p < 0.001$) and between the two copper concentrations ($F_{1,8} = 22.012$, $p = 0.002$) (Fig. 2). There was not a significant flow–copper interaction ($F_{1,8} = 0.209$, $p = 0.660$, Fig. 2). The mean RGR within flumes at the 6 cm/s and 85 µg/L treatment resulted in a slight loss of body volume and a slightly negative RGR, while the other three treatment combinations all resulted in mean increases in body volume (Fig. 2).

Feeding behaviors

Every behavioral category was observed at least once in each flume run. The proportion of observations of active feeding (as opposed to worms not feeding) did not differ significantly between the two flow speeds ($F_{1,8} = 0.311$, $p = 0.592$) or between the two copper concentrations ($F_{1,8} = 1.797$, $p = 0.217$), and there was no significant flow–copper interaction ($F_{1,8} = 3.099$, $p = 0.116$). Due to the nature of this organism to change feeding behaviors at different flow speeds, closer analysis of only flumes running at the slower flow speed was conducted and they revealed that active feeding was observed less frequently in the higher copper concentration (*t* test, $p = 0.004$, Fig. 3). When
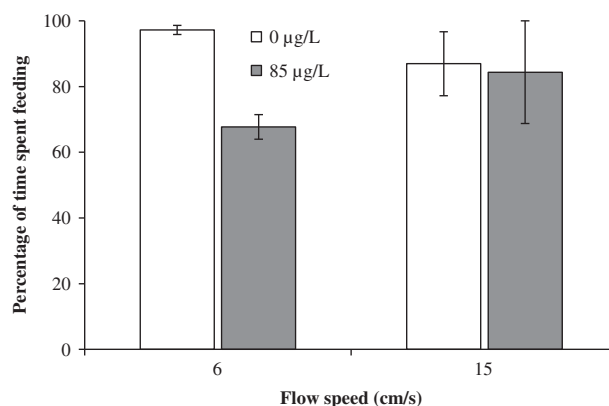

Fig. 2 Relative growth rates (RGR) of juvenile *P. cornuta* under different combinations of flow speed and copper concentration. Bars are mean $\pm$ SE ($n = 3$) when no copper was added (*white*) or when 85 µg/L was added (*gray*). Effects of flow and copper were statistically significant without significant interaction (flow: $F_{1,8} = 32.324$, $p < 0.001$; copper: $F_{1,8} = 22.012$, $p = 0.002$, interaction: $F_{1,8} = 0.209$, $p = 0.660$)

Fig. 3 Percentage of time *P. cornuta* spent feeding (as opposed to not feeding) under different combinations of flow speed and copper concentration. *Bars* are mean $\pm$ SE ($n = 3$ replicate flume runs) when no copper was added (*white*) or when 85 µg/L was added (*gray*). At the 6 cm/s flow speed, there was a statistically significant difference in the percentage of time worms spent feeding in the 0 vs. 85 µg/L copper treatments (*t* test, $p = 0.004$)
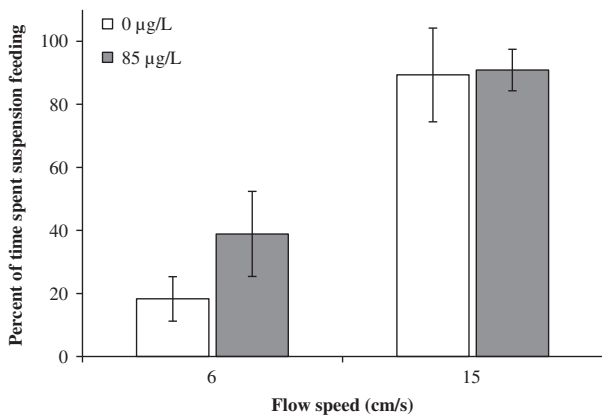
Fig. 4 Percentage of time that actively feeding *P. cornuta* spent suspension feeding (as opposed to deposit feeding) under different combinations of flow speed and copper concentration. *Bars* are mean $\pm$ SE ($n = 3$ replicate flume runs) when no copper was added (*white*) or when 85 µg/L was added (*gray*). There was a statistically significant effect of flow speed on the percentage of time spent suspension feeding ($F_{1,8} = 36.331$, $p < 0.001$), but not a significant effect of copper ($F_{1,8} = 0.098$, $p = 0.762$) or a flow-copper interaction ($F_{1,8} = 2.521$, $p = 0.151$)
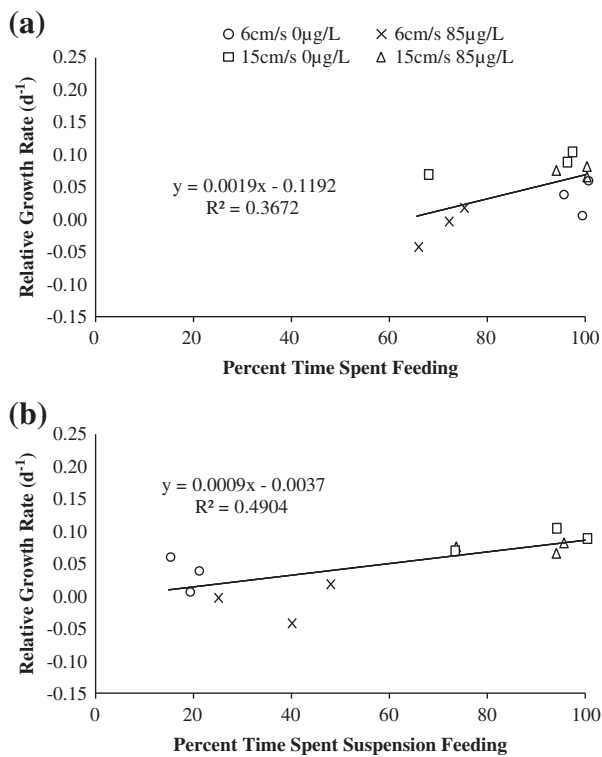

Fig. 5 Mean relative growth rates (RGR) of *P. cornuta* individuals vs. the percentage of time each individual spent **a** actively feeding (as opposed to not feeding) or **b** suspension feeding (as opposed to deposit feeding). *Different symbols* distinguish data from each of the flow-copper treatments. A significant correlation was observed between RGR and percentage of time spent feeding ($p = 0.037$) and between RGR and the percentage of time spent suspension feeding ($p = 0.009$)

observations of feeding worms were categorized more closely as either suspension feeding or deposit feeding, suspension feeding was observed significantly more often in the faster flow ($F_{1,8} = 36.331$, $p < 0.001$), but copper concentration had no effect on the proportion of time spent suspension feeding ($F_{1,8} = 0.098$, $p = 0.762$) and there was no significant flow–copper interaction ($F_{1,8} = 2.521$ $p = 0.151$) (Fig. 4).

The six individuals that were observed for feeding behaviors also were measured for RGR. Across all 12 flume runs, there was a significant linear regression between an individual's RGR and the proportion of time it spent feeding ($F_{1,12} = 5.764$, $p = 0.037$) (Fig. 5a). There was also a significant relationship between an individual's RGR and the proportion of time it spent suspension feeding relative to deposit feeding ($F_{1,12} = 10.279$, $p = 0.009$) (Fig. 5b).

Copper accumulation

All flume runs showed accumulation of copper in the worms' tissue. Initial copper concentrations in the worms ranged from 65 to 86 µg/g, and did not differ significantly between the two flow speeds ($F_{1,7} = 1.333$, $p = 0.286$) or between the two copper concentrations ($F_{1,7} = 0.392$, $p = 0.551$). The average copper accumulation in the tissues of the worms as calculated by subtracting the worms' initial copper concentration from their final copper concentration measured for each flume run ranged from 12.6 to 47.1 µg/g. The accumulation of copper during 6 days was significantly greater in the 85 µg/L treatment than in the 0 µg/L treatment
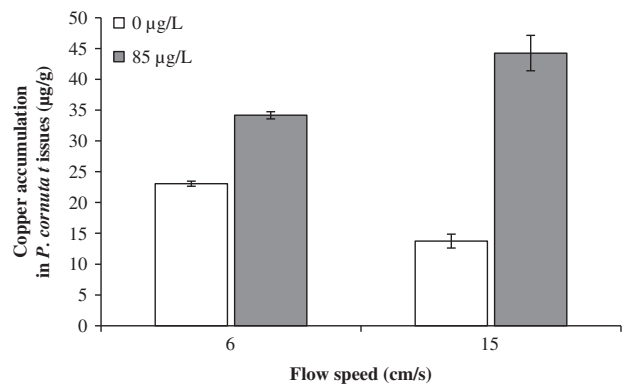

Fig. 6 Mean copper accumulation in the tissues of *P. cornuta* under different flow speeds and copper concentrations. Copper accumulation during each 6-day flume run was calculated by subtracting the worms' initial copper concentration from their final copper concentration measured for each flume run. Bars are mean $\pm$ SE ($n = 3$ replicate flume runs, except for the treatment at 15 cm/s and 85 µg/L where $n = 2$) when no copper was added (*white*) or when 85 µg/L was added (*gray*). There was a statistically significant effect of copper concentration and a significant flow-copper interaction on the accumulation of copper in *P. cornuta* (flow: $F_{1,7} = 0.106$, $p = 0.754$; copper: $F_{1,7} = 297.56$, $p < 0.001$, interaction: $F_{1,7} = 64.561$, $p < 0.001$)

($F_{1,7} = 297.56$, $p < 0.001$), but did not differ significantly between the two flow speeds ($F_{1,7} = 0.106$, $p = 0.754$) (Fig. 6). There was, however, a significant interaction between copper and flow speed ($F_{1,7} = 64.561$, $p < 0.001$); the magnitude of the difference in copper accumulation between the 0 and 85 µg/L treatments was greater in the faster flow than in the slower flow (Fig. 6). Post-hoc Tukey multiple comparisons revealed that the accumulation of copper differed significantly among all flow–copper combinations ($p < 0.003$).

Discussion

The relative growth rates (RGR) of juvenile *P. cornuta* measured in this experiment are consistent with results of previous laboratory flume experiments that found faster growth when flow and the flux of suspended food favor suspension feeding (Hentschel 2004; Hentschel and Herrick 2005; Hentschel and Larson 2005, 2006). As the flow rate increases above the critical erosion velocity for the sediment used (~12 cm/s, Colvin pers. obs.), organic content can become more available to both deposit and suspension feeding worms and lead to increased feeding activities and resulting increased growth rates. Our experiment is, however, unique in also testing the effects of sublethal concentrations of copper and in simultaneously quantifying feeding behaviors and growth rates of individual spionids subjected to metal exposure in flowing near-bottom water. Our data provide clear evidence that RGR correlates positively with the time a worm spends suspension feeding. The addition of copper suppressed feeding activity at slower flows in which deposit feeding is common, but did not alter suspension-feeding activity that occurs during faster flows. A possibility is that suspension feeding is a more energetically favorable mode of feeding where the worms coil their feeding palps in the overlying water and may be an advantageous behavior that reduces drag (Shimeta et al. 2004). In slow flows, the worms may have to expend more energy locating food particles on the surrounding benthos and this the difference that was observed in the presence of copper was just made more apparent in the scenario in which the worms have an increased metabolic requirement. Our initial experiment on this subject tested only two flow speeds and two copper concentrations and testing a greater range of flow speeds or copper concentrations might reveal an interaction between flow speed and copper concentration. Under slow flows that favor deposit feeding, worms will grow less and accumulate metals into their tissues less than they will if conditions favor suspension feeding. In the treatments where no copper was added, it was observed that the slow flow treatment accumulated more copper than the fast-flow treatment (Fig. 6). It is feasible that due to the deposit feeding behavior that is more prevalent in the slower flow, the worms were accumulating and concentrating copper that was found in the sediment. Under the suspension feeding behavior, the worms would not come ingest the sediment particles as frequently and therefore wouldn't accumulate copper at as high of a rate as the deposit feeding worms.

Seasonal rain events have the potential to be a significant source of both dissolved and particulate pollutants such as metals into downstream coastal environments into habitats in which these animals are common (Joshi and Balasubramanian 2010; Schiff and Tiefenthaler 2011; Cánovas et al. 2012). The accumulation and subsequent toxicity of these materials in coastal ecosystems can harm organisms and the functioning of ecosystems (McCahon and Pascoe 1990) and potentially increase the rates at which metals are accumulated into worm tissues. Bioavailability of metals will likely change as a result of the changing water and sediment chemistries as a result of stormwater runoff (Strom et al. 2011; Simpson and Bately 2007; Zhang et al. 2014). Rain events do, however, also reduce salinity, which has long been considered a central variable affecting the ecology of estuarine benthos (Sanders et al. 1965). Colvin and Hentschel (2011) measured RGRs of *P. cornuta* in situ during the rainy season and found that worms grew very little during and shortly after major rain events (mean RGR within 1 week of rain = 0.02 d^{-1}). That study then suggested that a cessation of feeding activity during periods of low salinity was the most likely mechanism explaining these reduced RGRs during the rainy season. Additional studies evaluating feeding activities as a function of salinity would be supportive to line of thought. Toxicity associated with storm run-off is an alternative explanation for the reduced growth rates observed, and data from our laboratory flume experiment show that sublethal concentrations of copper can reduce feeding activity at slower flow speeds and RGR. Mendez and Green-Ruiz (2005) also found that body size was reduced in juveniles of the deposit-feeding polychaete *Capitella* when exposed to aqueous copper.

In our flume experiment, there was not a significant interaction between the effects of flow speed and the concentration of aqueous copper on either *P. cornuta* RGR or feeding activity. Again, we caution that our initial experiment on this subject tested only two flow speeds and two copper concentrations. Testing a greater range of flow speeds or copper concentrations might reveal an interaction between these factors. For example, Sabater et al. (2002) observed an effect of current speed on diatom growth at low and high speeds, but not at intermediate velocities when copper was included in their experiment. Additionally, the feeding behaviors observed for this study was near the termination of each flume run. It would be of interest to see if observations of feeding behaviors would be different if observed for longer periods or possibly at different period

throughout a given flume run to tease out possible latent effects that might be occurring.

As expected, we found that adding aqueous copper increased the accumulation of copper into worms' tissue. The greatest concentration of copper in worms' tissues occurred in the faster of the two flow speeds and the greater of the two concentrations of aqueous copper we tested, while the lowest accumulation of copper was measured in worms feeding in the faster flow without added copper. These results suggest that the increased biomass of rapidly growing worms in flows that favor suspension feeding will reflect the concentration of chemicals present in the water. If the concentration of aqueous copper is relatively high, copper is likely to bind to suspended food particles (i.e., primarily non-living microalgae in our laboratory experiment), be consumed by the suspension-feeding worms, and incorporated into worm tissues as worms grow. If the concentration of aqueous copper is relatively low, suspended food particles will be relatively free of copper and the concentration of copper in worm tissues will be diluted by the relatively clean food. The seawater used in this study was filtered to 0.45 μm therefore reducing the likelihood of complexation of metals to organic matter. However, with the addition of the algal-slurry as a food source, metals may adhere to the organic matter and form strong metal-organic complexes (Simpson et al. 2014) and render some metals non-bioavailable to this particular organism. In this way, suspension-feeding spionids can potentially transfer particle-bound toxicants from the water column to the benthos. Alternatively, metal-bound organic matter may be incorporated into the tissues dependant on the digestibility and residence time of the food in the gut of the organism matter as well as the binding strength with the metals (Selck et al. 1999; Lee et al. 2000). When or where flow speeds are relatively slow and worms tend to deposit feed more often, the reduced growth of worms ingesting nutritionally dilute deposited particles can lead to more moderate rates of copper accumulation in worm tissues. For example, a greater proportion of inorganic mineral grains compared to organic-rich particles that have lower specific gravities and can be suspended in the benthic boundary layer and become non-available as a food source. Similarly to metals in water, metal bioavailability in sediments is complex and is influenced by numerous factors such as speciation, sediment properties (grain size, total organic carbon, oxygen depletion zones within the sediments), sediment-water partitioning and exposure pathways (Strom et al. 2011; Simpson and Bately 2007) . In particular, the sensitivity of an organism to metals in sediment is also driven by the bioavailability of metals in various dissolved phases (i.e. pore water, burrow water, or overlying water) (Strom et al. 2011). In nature, a complete quantification of the rates at which metals accumulate into the tissues of interface-feeding benthos will require knowing the concentrations in the water and sediment, water and sediment characteristics, as well as the feeding rates on suspended and deposited particles.

Bioassays conducted in still water are grossly oversimplified with respect to the feeding biology of interface-feeding benthic organisms such as *P. cornuta*. In systems where *P. cornuta* and other spionids are common, the central role of water flow in the benthic boundary layer must be considered when assessing the toxicity and cycling of metals or other pollutants. Stampfli et al. (2013) found that hydrological and other abiotic stressors increased the sensitivities of a freshwater community to an insecticide toxicant and are of importance for risk assessments. In particular, answering questions related to the cycling of metals through estuarine food webs requires knowledge about the many interactions between feeding, growth, and water flow in addition to the concentrations of toxicants in the water or sediment.

Metal toxicity either from water or sediments is extremely complex. The laboratory flume study helps to distinguish the relative effects of flow and metal spiking on the feeding behavior, growth rates and rates of accumulation within a controlled setting. It was shown that RGR correlated positively with the time a worm spends suspension feeding. The addition of copper suppressed feeding activity at slower flows in which deposit feeding is common, but did not alter suspension-feeding activity that occurs during faster flows. However, when the worms were suspension feeding in the higher flow speeds with the increased copper concentrations, new tissue is being made with food particles that are laden with copper, and thus are going to accumulate copper at a faster rate. Being able to understand the effects of flow and metals on *Polydora* and understanding its rates of accumulation under different situations allows us to gauge the availability of metals to organisms of higher trophic levels that happen to feed upon them.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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