2014

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Effects of dissolved and dietary Microcystin on clearance rates of Wedge Clams (*Rangia cuneata)* in the tidal fresh James River

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Introduction

Microcystin (MC) is a hepatotoxin found in eutrophic lakes and estuaries around the world (Paerl et al. 2001). MC is a secondary metabolite and is a highly stable compound which is produced by cyanobacteria (blue-green algae) including *Microcystis* (Pearson et al. 2010). MC is known to have adverse effects on diverse consumers, though its effects on benthic filter-feeders are not well-studied.

Wedge clams *(Rangia cuneata)* are the dominant benthic filter-feeders in the tidal fresh James based on annual surveys by the Chesapeake Bay Program (CBP). Our recent work has shown that *Rangia* grazing rates are lower during periods when MC concentrations are elevated (Wood et al. in review).

The objective of this study was to determine if low clearance rates by *Rangia* in the presence of MC could be replicated in the laboratory through controlled exposure to dissolved and dietary MC. We used commercially available Microcystin (Abraxis) to measure clearance rates of James River clams fed natural seston in the presence of varying concentrations of dissolved MC. We also compared clearance rates of James River clams fed seston from two sites: James (high MC) and Pamunkey (low MC) before and after the onset of cyanobacteria blooms. We hypothesized that clearance rates of James River clams feeding on James seston would be lower during periods when MC was present.

Methods

Two experiments were performed to test the effects of dissolved MC on *Rangia* clearance rates (May and June 2013). Four experiments were performed to assess the effects of MC in diet: three were conducted prior to the onset of MC production in the James (April, May, and June) and one experiment was performed during the period when MC was elevated in the James (September). All *Rangia* used in these experiments were collected from the tidal fresh portion of the James River near the VCU Rice Rivers Center.

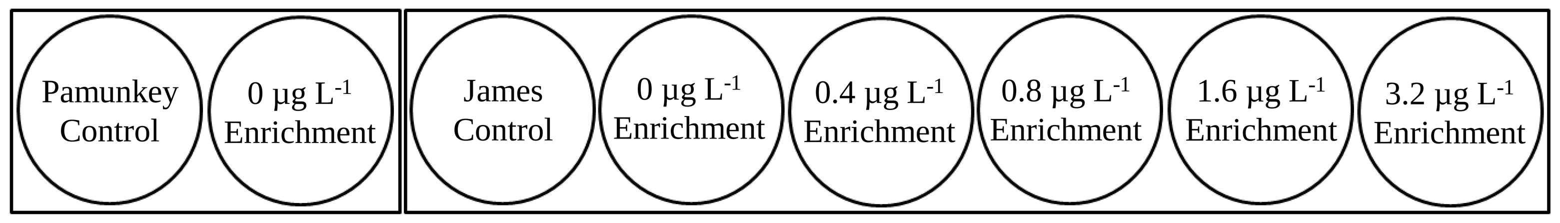
For the dissolved MC exposure, clams were placed in 3-L mesocosms containing a 50:50 mixture of filtered and unfiltered water from the James. A small water pump was used to maintain particulate matter in suspension and mesocosms were kept in the dark to limit phytoplankton growth. Pervious experiments showed that MC did not have a significant effect on *Rangia* clearance. The mesocosms were spiked with dissolved aqueous MC to create enrichment levels of 0, 0.4, 0.8, 1.6 and 3.2 µg L^{-1}. Each treatment level was performed in triplicate.

For the dietary study, we incubated clams in water from the James and Pamunkey Rivers. The water from the Pamunkey was not diluted so that CHL*a* and POC concentrations in the two source waters would be more similar. Experimental conditions were otherwise the same as for the dissolved MC experiments.

We derived clearance rates based on differences in the rate of change in CHL*a* or POC concentrations between control (no clams) and experimental (with clams) mesocosms. CHL*a* and POC concentrations in the mesocosms were determined at 0, 1 and 2 h. CHL*a* was measured following extraction in buffered acetone for 18 hours and analysis on a Turner Design TD-700 Fluorometer. POC was measured on a Perkin-Elmer CHN analyzer. Slopes of these regression lines were used to determine the biomass-specific clearance rate based on mass removed, concentration in water, and dry weight (tissues only) of clams (Coughlan 1969).

Grazing rate (L g DW^{-1} h^{-1}) = [(slope((mg L^{-1}) / h^{-1}))* mesocosm volume (L)) / clam dry mass](g DW^{-1}) / (average concentration (mg L^{-1}))

Experimental Setup



Figures

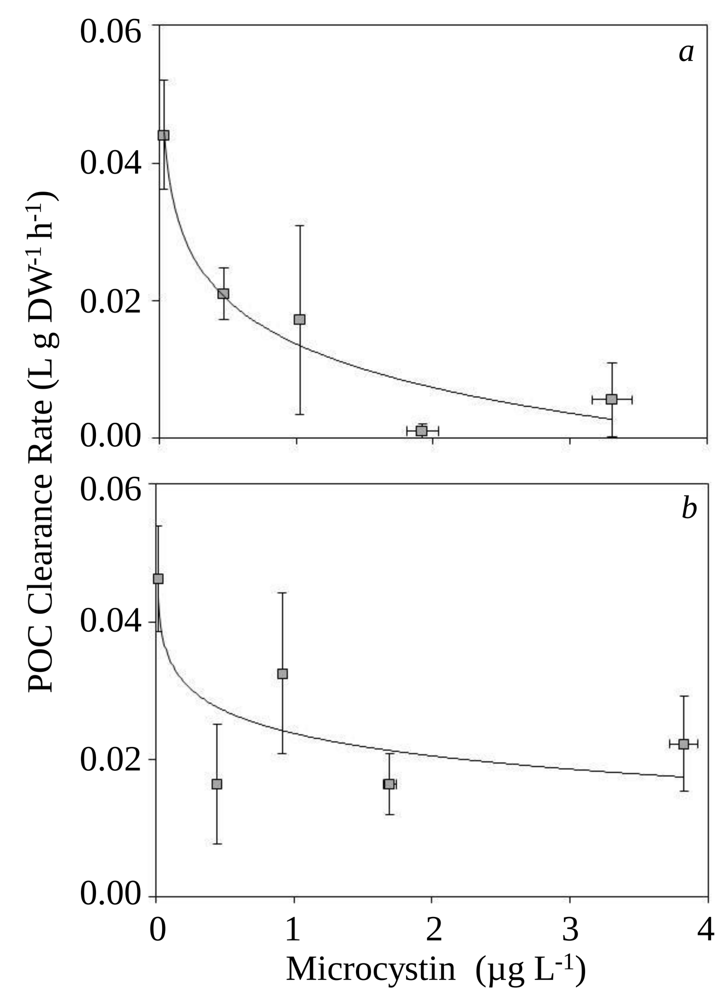


Figure 1. Effects of dissolved microcystin on clearance rates of the wedge clam based on experiments performed in May (*a*) and June (*b*). Error bars denote SE.

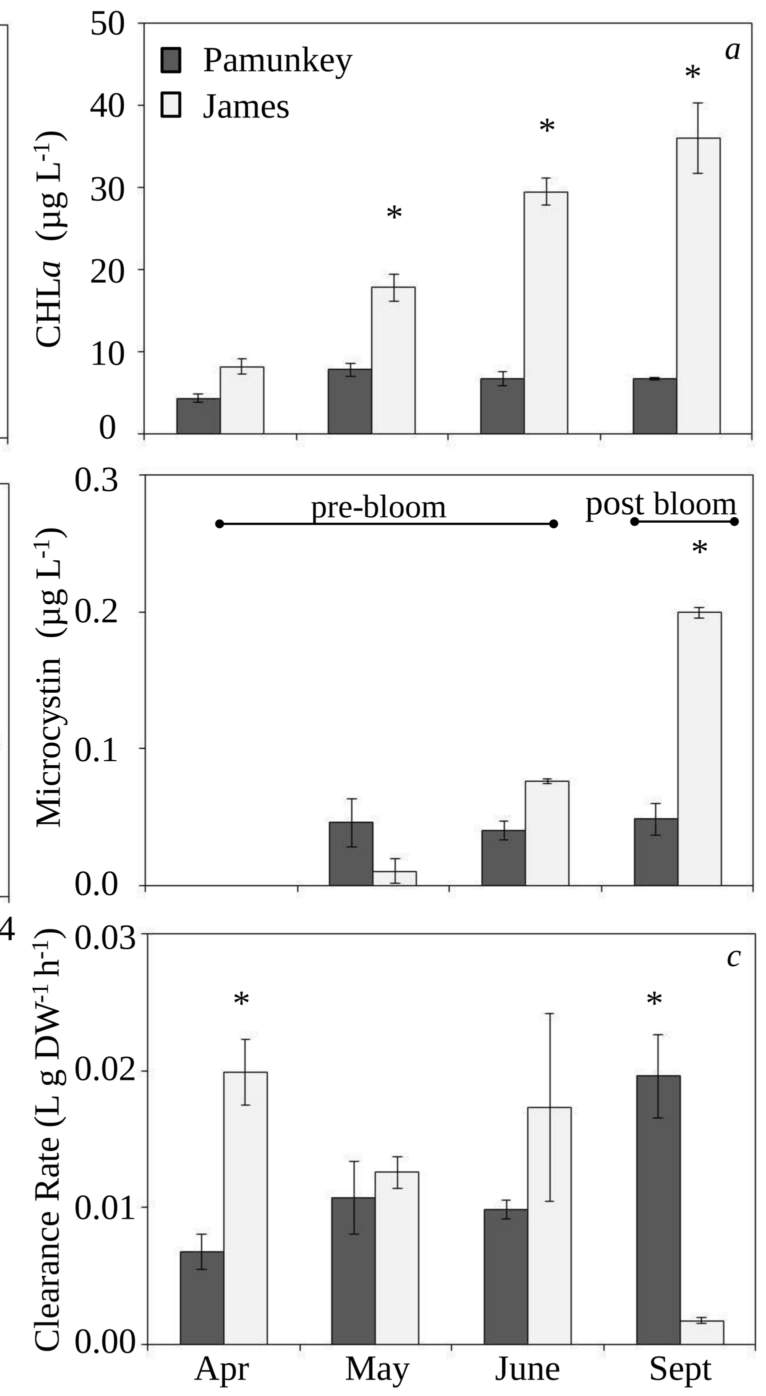


Figure 2. Results from dietary exposure study *a)* CHL*a* concentrations in water from the two sources, *b)* microcystin concentrations in water from the two sources, and *c)* clearance rates of clams

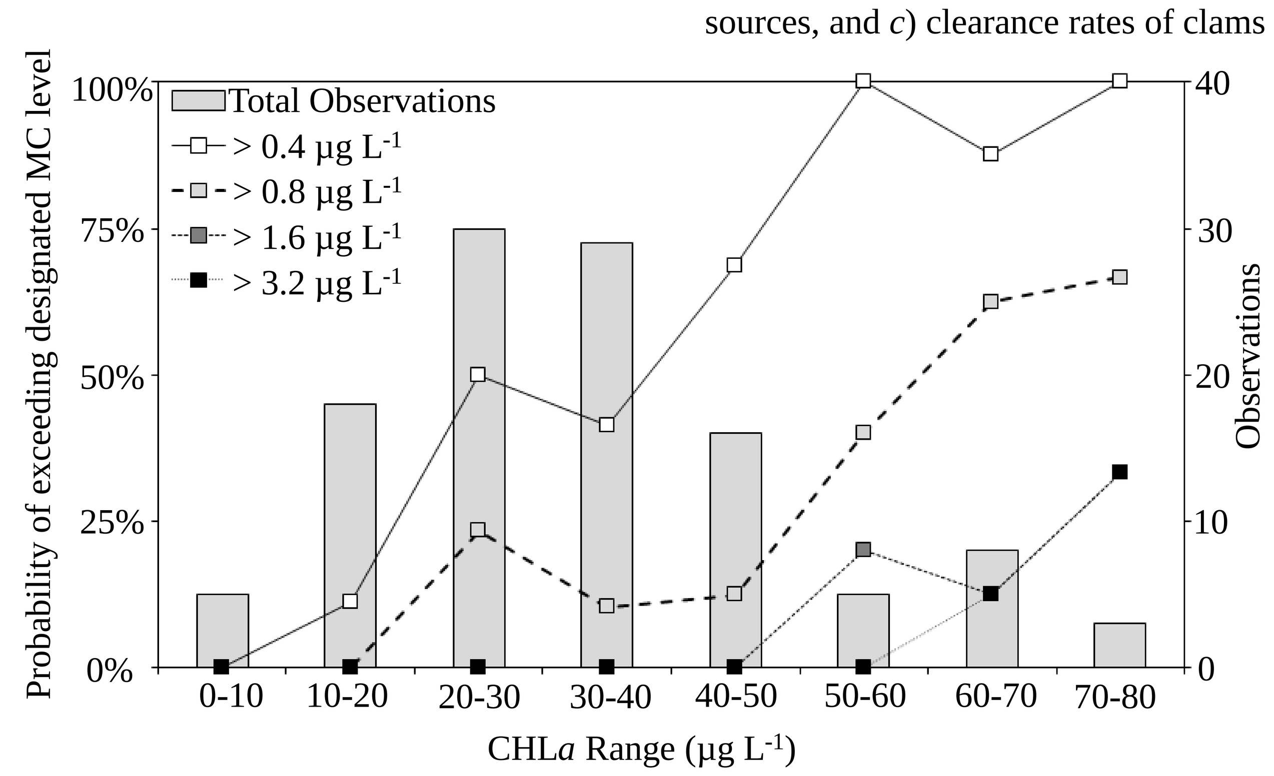
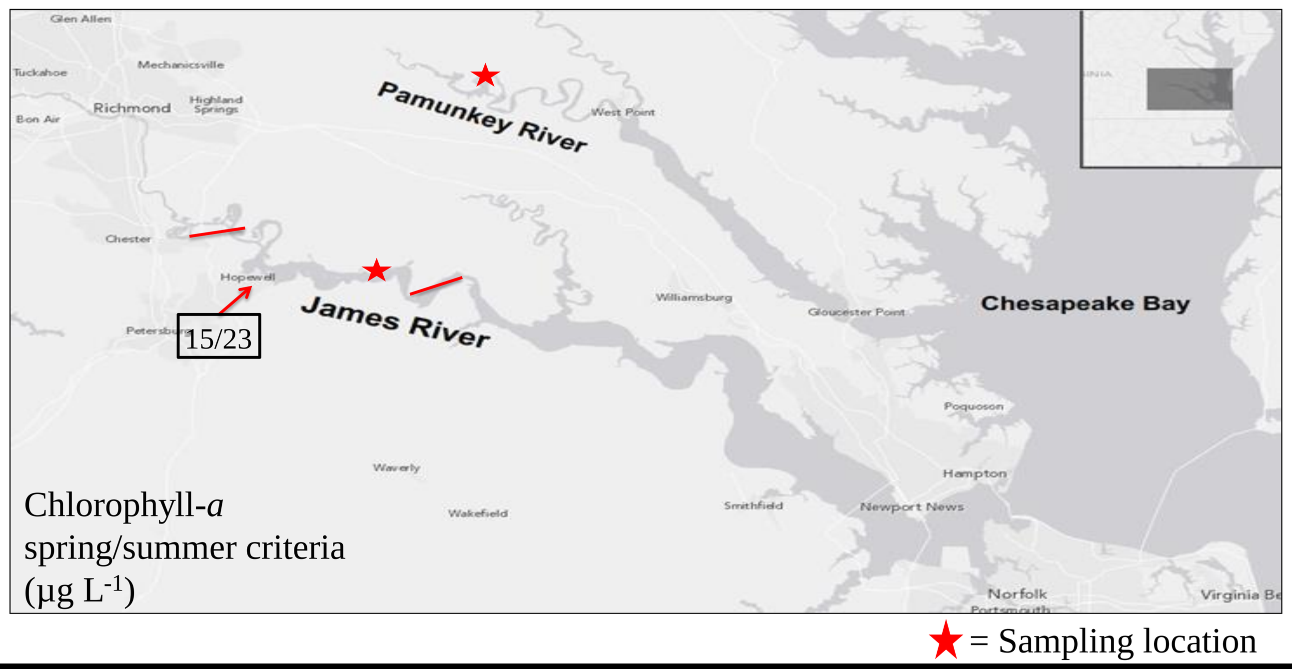


Figure 3. The probability of exceeding a given MC concentration as a function of CHL*a*. Data used for this analysis are weekly measurements of CHL*a* and MC obtained at multiple stations in the tidal fresh James during July-September of 2011-13 (bars denote number of observations at each CHL*a* range).

Study Sites



★ = Sampling location

Results

Exposing Wedge Clams to dissolved MC resulted in a reduction in their clearance rates (Figure 1). The two replicate experiments performed in May and June yielded similar results and showed a curvilinear reduction in clearance rates with increasing MC concentrations. To compare results from the two experiments, we derived a parameter 'CR_{50}' - the MC concentration at which clearance rates were reduced by 50%. For the May experiment, the curvilinear model provided strong fit to the data (R^2 = 0.94) and yielded a CR_{50} value of 0.40 µg MC L^{-1}. In June, the dose-response curve was somewhat less consistent (R^2 = 0.61) and yielded a higher CR_{50} value (1.15 µg MC L^{-1}).

Dietary experiments showed that in the absence of MC, clearance rates were higher among clams fed James River seston (April) or not significantly different between James and Pamunkey seston (May and June; Figure 2). During the September experiment, MC concentrations were higher in the James (0.20 µg L^{-1}) relative to the Pamunkey (0.05 µg MC L^{-1}). When elevated MC was present in the James (September), clearance rates were lower for clams fed James River seston. During the September experiment. CHL*a* concentrations were also higher in the James (36 µg L^{-1}) relative to the Pamunkey (6.7 µg L^{-1}) suggesting that the difference in clearance rates could be due to higher food availability in the James. However, higher CHL*a* concentrations in the James were also observed in the preceding two experiments (May and June) when no significant difference in clearance rates was detected.

Results from the exposure studies were used to assess the likelihood that *Rangia* populations in the James would experience MC concentrations sufficient to cause a reduction in their clearance rates (Figure 3). The probability that MC would exceed the lowest threshold for observable effects (0.4 µg L^{-1}) was low (<10%) when CHL*a* <20 µg L^{-1}, moderate (~50%) when CHL*a* was 20-40 µg L^{-1} and high (>75%) when CHL*a* >40 µg L^{-1}.

Discussion

Our results suggest that exposure to Microcystin in both dissolved and dietary forms diminishes clearance rates of *Rangia*. Other studies have shown that bivalves have the ability to control filtration intake when exposed to contaminants (Sabatini et al. 2011 Wildridge et al. 1998 Prepas et al. 1997 Hartwell et al. 1991). Furthermore, Kryger and Riisgård (1988) suggest that the presence of undesirable nutritional elements may interfere with filtration. Our results suggest that Microcystin may have similar effects on benthic filter-feeders and support our earlier observations of reduced clearance rates during periods when MC is present. These findings further suggest that blooms of toxic cyanobacteria may have deleterious effects on ecosystem services provided by benthic filter feeders.

Benthic suspension feeders provide a valuable ecosystem service by filtering suspended particulate matter from the water column thereby enhancing water clarity and suppressing algal abundance (Cerco and Noel 2010, zu Ermgassen et al. 2013). The presence of the toxin may cause *Rangia* and other consumers to avoid exposure through decreased feeding and in turn lead to long-term declines in consumer abundance and diminished top-down control on algal blooms.

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