ABSTRACT

DUKE, ROBERT MICHAEL. Phytoplankton-Oyster Trophic Dynamics in a North Carolina Estuary. (Under the direction of Dr. Astrid Schnetzer).

Bogue Sound, part of the Albemarle-Pamlico system, which is the second largest estuary in the United States, represents coastal water bodies increasingly affected by urban development and population growth. Latter stressors link to excess nutrient loading (eutrophication), lowered water quality, and subsequent losses for the fisheries and tourism industry. Oysters can alleviate anthropogenic influences and provide important ecosystem services by improving water quality due to their filtration abilities and shoreline erosion control. While oyster feeding activities have been associated with declines in algal bloom formations in some coastal systems, little is still known about how top-down control may act as a primary factor in regulating prey abundance and assemblage composition. In this study, seasonal oyster grazing experiments with *Crassostrea virginica* showed that clearance rates varied with prey abundance, as well as prey type, during various seasons. Extrapolation of experimental results using *in situ* oyster abundances indicated that oysters can play an important role for water quality (i.e. chlorophyll removal) in Bogue Sound, where up to 82 % of the sound volume could be cleared per day.

Phytoplankton-Oyster Trophic Dynamics in a North Carolina Estuary

by
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DEDICATION

To all of the hard working scientists who strive to find links between phytoplankton and their grazers; may this be a look into the complex food web interactions within our world's oceans.

BIOGRAPHY

I grew up in Wilmington, N.C. where my interest for the ocean and the natural world arose. Since then, I have been dedicating my life to exploring and communicating the importance of the natural world and its relationship to all of its organisms.

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I want to thank my committee members for all their guidance throughout this process, as well as my lab and field members for your continuous support.

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INTRODUCTION

Oyster reefs provide important ecosystem services for coastal and estuarine systems, including the improvement of water quality via removal of particulate matter (which include phytoplankton), decreases in turbidity in shallow waters, erosion control of shorelines, and the creation of living habitat for an array of organisms (Haamer, 1996, Newell *et al.*, 2005, Officer *et al.*, 1982, Kellogg *et al.*, 2014). Over the last century, an estimated 85% of oyster reefs have been lost worldwide (Beck *et al.*, 2011) and decreases of 50% have been reported for the Albemarle-Pamlico Sound estuary in North Carolina (Oyster Reef Restoration Report by Nature Conservancy). Major efforts for reef restoration have been underway in NC, where the aquaculture of eastern oysters, *Crassostrea virginica*, accounts for $58 million in revenue annually (North Carolina Aquaculture Update, 2013). Nationwide, approximately 33 jobs are created per $1 million invested into a given restoration project (Bendor *et al.*, 2014). Also, oyster reefs provide an average of $860 per hectare a year of shoreline protection, with up to $85,998 per hectare annually in cases that oyster reefs are suitable substitutes for man-made structures (Grabowksi *et al.*, 2012). Incentives for the United States economy and anticipated benefits from ecosystem services that oysters provide are major drivers of continued oyster restoration efforts.

As links between increased oyster abundance and the removal of excess algal biomass have been ascertained for the Chesapeake Bay, along with other regions around the world (Cerco and Noel, 2007, Fulford *et al.*, 2011, Shumway, 1990), large gaps remain in our understanding on how much biomass may be removed in a given environment. Even less

data exists on how plankton composition is altered due to oyster feeding. Earlier estimates based on population abundances of *C. virginica* in the 1980's suggested a turnover of the entire Chesapeake Bay within ~325 days (Newell, 1988). In an effort to mitigate or prevent the development of harmful algal blooms, more recent modeling efforts indicate that significant increases in oyster numbers (~ 10-fold) could work as an effective complementary strategy to nutrient reduction in the Chesapeake Bay (Cerco and Noel, 2007). Currently, however, projected oyster restoration efforts are not expected to make a significant impact to water quality (Fulford *et al*., 2011).

To gain insight into the cause-effect relationship between oyster density and water quality, most field and laboratory studies have focused on calculating clearance rates (CRs) based on changes in chlorophyll *a* (chl *a*) concentration. Mean clearance rates (CRs) for different *Crassostrea* spp. on natural prey have been shown to range from <0.5 to 18 l h^{-1} g^{-1} (Gedan *et al.*, 2014, Li *et al.*, 2012, Newell *et al.*, 2005, Riisgård, 1988, Sroczyńska *et al.*, 2012), while maximum CRs of up to 24 l h^{-1} g^{-1} were reported for *C. madrasensis* using cultured phytoplankton (Rajesh et al., 2001). As CRs from these studies are applied to estimate clearance capacity within coastal environments, challenges arise due to the need to account for local physiochemical regimes, spatiotemporal variability in prey environments, and total oyster densities (e.g., Newell, 1988, Ramos *et al.*, 2008).

A limited number of studies have aimed to decipher trophic relations for oysters beyond chl *a* clearance by examining changes in prey groups or size fractions (e.g., Dupuy *et al.*, 2000, Wetz *et al.*, 2002, Stoecker and Adolf, 2008). Dupuy *et al.* (2000) determined that while

picoplankton (< 5 µm in size) dominated the Mediterranean Thau Lagoon in France, *C. gigas* showed a preference for microphytoplankton (> 5 µm size range). Stoecker and Adolf (2008) looked at oyster feeding from a different angle when they studied how feeding on bloom-forming dinoflagellates affected *C. virginica* survival within the Chesapeake Bay. The toxic dinoflagellate *Karolodinium veneficum* reduced the survival or maturation of the oyster larvae, therefore, greatly inhibiting the oysters' filtering ability. The multitude of problems that arise from harmful algal blooms and/or subsequent drawdown of dissolved oxygen, have shellfish growers concerned as bivalves become contaminated with algal toxins and cause shellfish poisoning, or as anoxic events lead to major shellfish die-offs (Shumway, 1990). A better understanding of how the quantity and quality of prey impact bivalve feeding will allow for a predictive understanding of how environmental perturbations will affect estuarine food webs.

This study aimed to examine prey-oyster dynamics in Bogue Sound which is located within the southwestern region of the Albemarle-Pamlico Sound estuary in North Carolina. Seasonal differences in grazing impacts of *C. virginica* on prey abundances were determined from field incubation experiments conducted in June and September 2014. Overall prey community structure was not affected by oyster feeding in either experiment; however, genus-specific CRs indicated that *C. virginica* selected for certain prey taxa. The latter trend was more apparent when overall prey abundances were higher, as observed in the September experiment compared to the June experiment. An inverse relationship between CR and oyster abundance was seen as prey abundances decreased during both months, but temperature

stress in September seemed to adversely affect feeding activity. Extrapolating CRs indicated that *C. virginica* can play an important role in improving water quality in systems such as the Bogue Sound via the removal of algal biomass.

MATERIALS AND METHODS

Study Site and Feeding Experiments

Oyster feeding experiments were conducted using natural protistan assemblages collected from Bogue Sound, NC (34° 43' 25.017N, 76° 45' 7.887W; Fig. 1). The impact of oyster feeding on prey assemblages (>20 μm in size) was studied by manipulating the number of filter feeders in exclusion/addition experiments and determining the effects on prey abundance and community structure (Table 1). Eastern oysters (*C. virginica*) were collected in June and September 2014 from a local hatchery on Cedar Island, approximately 60 kilometers northeast of the study site, within Pamlico Sound. For each experiment, ~130 oysters (30-50 mm in size) were acclimated for ~2 weeks to adjust to experimental conditions at the study site.

Feeding experiments were conducted in a seawater flow-through tank (4.3m x 1.2m x 0.5m) at the University of Chapel Hill's Institute of Marine Sciences outdoor facility that directly received water from the adjacent sound (pipe intake ~1 m above the bottom). The day before initial sampling, the oysters were transferred into 62 L bins that were kept submerged to allow for continued seawater exchange. The next morning, the flow-through system was temporarily turned off, any accumulated sediment or pseudo-feces was siphoned from each container, and all bins were refilled to a final volume of 60 L with freshly-supplied seawater from Bogue Sound (leaving oysters submerged at all times). After filling the bins, water flow was only re-established for the surrounding flow-through tank to maintain ambient water temperature, but no further seawater was supplied to the individual bins for the duration of

the experiments. Treatments included containers with 0, 2, 4, 8, and 16 oysters in triplicates for the June experiment, and 0, 2, 4 and 8 in triplicates in September (Table 1). Sampling was initiated within the hour of filling the containers, and several time points were taken to measure chl *a* and characterize prey assemblages throughout the ~ 3-4 hour incubations (experimental details in Table 1). Upon completion of each experiment, oysters were collected and stored in a freezer until analysis to determine ash-free dry weights (Lawrence and Scott, 1982), allowing for normalized estimates of feeding rates, per gram oyster.

Chlorophyll *a* Measurements

Water samples of 100 mL (200 mL for T0) were filtered through Whatman GF/F filters (0.45µm) immediately after collection, and filters were frozen (-80 ºC) until analysis, which entailed using a non-acidification protocol on a Trilogy fluorometer (Turner Design) as detailed in Welschmeyer (1994). Samples were analyzed within 2-3 weeks of field collection to minimize pigment loss due to extended freezing (Graff and Rynearson, 2011). To be able to put initial chl *a* concentrations from each experiment into context with overall phytoplankton dynamics within Bogue Sound, a 2-year time-series of weekly chl *a* samples collected nearby were also analyzed (Fig. 1).

Prey Abundances and Community Structure

Protistan assemblages where examined by settling seawater samples (60 -125 mLs) and preserving them with 2% Formalin or 10% acid Lugols following standard settling techniques (Stoecker *et al.,* 1994, Utermöhl 1958). Identification of general major taxa (i.e

diatoms, dinoflagellates, ciliates) was achieved to the genus level using light microscopy (Olympus DP73), and a taxonomic reference guide by Tomas (1997).

Calculation of Oyster Clearance Rates

CRs (in l h^{-1} oyster $^{-1}$ or l h^{-1} g^{-1}) were calculated based on changes in chl *a* concentration (μg l^{-1}) and cell abundance (cells mL^{-1}) between the start of each experiment and each proceeding time point, to determine how much water was cleared by an oyster in order to obtain its prey (Coughlan 1969, Dupuy *et al.*, 2000, Lonsdale *et al.*, 2009).

The formula used for CR is as follows:

$$\text{CR (per individual)} = V/n\ [(\ln C_0 - \ln C_t)/t_0 - a]$$

or

$$\text{CR (normalized per dry weight)} = V/DW\ [(\ln C_0 - \ln C_t)/t_0 - a]$$

Where V is the volume of water per container, n is the number of oysters per container, DW is the average dry weight of the oysters per container, C_0 is the initial prey density at t_0 (time in hours), C_t is the prey density at the time point in question, and a is the growth rate of the control containers in hours (Coughlan 1969). Information on abundance changes for major taxa or individual genera was used to estimate group and genus-specific CRs, which also allowed for insight into possible selective feeding by *C. virginica*. To assess whether other metazoan grazers (i.e., copepods) played a role in altering prey communities, we filtered 3 L of incubation water at the initial and final time points through a 100 μm mesh for abundance analyses.

Statistics

Multivariate analyses were employed using the PRIMER v6 statistics package (Clarke and Warwick, 2001) to compare CRs and to examine the impact of oyster feeding on overall prey community structure. CRs (derived from chl *a* and cell counts) were analyzed based on Euclidean distance measures, and compared among differing treatments, time points and experiments. This was done by employing one-way ANOSIM tests which resulted in R values and significance levels (Clarke and Warwick, 2001). A value of R = 0 implied no difference among groups of data being compared, and a value of R = 1 meant that group separation was so large that all dissimilarities among groups are larger than any dissimilarity within groups. Pearson correlation coefficients were computed to examine the linkage between CRs and chl *a* levels, and between genus-specific CRs and cell abundances (PRIMER v6).

PERMANOVA (Type 1, sequential at 9999 permutations) was used to test for significant differences ($p < 0.05$) among all CRs (from all time points) for differing genera and for differing cell concentrations (PRIMER v6). PERMANOVA tests resulted in a Pseudo-F value of 1 if large overlap among sample groups was observed (confirmation of the null hypothesis), whereas Pseudo-F values greater than 1 indicated little or no overlap between the compared groups (Anderson *et al.,* 2008). Differences among CRs for diatoms, dinoflagellates and ciliates and among CRs for the most common genera/major taxonomic groups were also examined using 1-tailed, paired t-tests using Microsoft EXCEL 2013.

Effects of oyster grazing on overall community structure were examined by using final relative abundances for all genera from replicate bottles (listed in Supplementary Table 1). The values were square-root transformed, and Bray-Curtis similarities were computed to test whether assemblage structure at the initial and latter time points were dissimilar (PRIMER v.6; Clarke and Warwick, 2001). The Bray–Curtis coefficient is not affected by joint absences of genera between samples (Clarke and Warwick, 2001).

RESULTS

Bogue Sound Environmental Characterization

Weekly chl *a* concentrations for Bogue Sound ranged from 1.2-13.0 µg L^{-1} from January 2013 to March 2015 (n = 78; average = 4.4 µg L^{-1}; Fig. 2). Initial chl *a* levels, at the onset of each experiment, were 2.3 µg L^{-1} in June and 3.8 µg L^{-1} in September. Overall chl *a* concentrations tended to be higher during summer and early fall at the nearby time-series site, but the two years differed considerably as biomass levels in 2013 generally exceeded those in 2014 (Fig. 2).

Oyster Clearance Rates

Individual CRs based on changes in chl *a* concentration declined with increasing oyster number in June (global $R = 0.66$; $p = 0.002$; ANOSIM), but the same observed trend was not statistically significant in September, when variability among triplicates was high (Fig. 3). Pairwise comparison for all treatments showed significant effects of oyster number on CR in June except for indistinguishable CRs in treatments that contained 4 versus 8 oysters (ANOSIM at $p < 0.05$). This inverse trend weakened if CRs were computed for latter time points (i.e., over longer incubation periods; not shown). Initial CRs (calculated over ~1 hour) derived for incubations that contained the lowest number of oysters (n=2), averaged 9.8 l h^{-1} g^{-1} (range = 6.5 to 14.6 l h^{-1} g^{-1}) and 6.9 l h^{-1} g^{-1} (range = 3.7 to 9.0 l h^{-1} g^{-1}) for June and September, respectively (Table 2). Overall, CRs in June were slightly higher than those in September (global $R = 0.18$; $p = 0.034$; ANOSIM), and in both experiments, CRs were inversely correlated with chl *a* concentration (Pearson correlations; r = -0.73 and -0.89 for

June and September, respectively; $p < 0.05$) independent of grazer densities (shown for treatments with 2 oysters over time in Fig. 4).

No difference was seen in oyster dry weights between June and September with an average ± standard deviation of 0.7 ± 0.3 g oyster^{-1} and 0.9 ± 0.3 g oyster^{-1}, respectively (Table 1). Water temperatures throughout the experiments ranged between 26 and 28°C in June, and 29 and 32°C in September, and no changes were detected in salinity (Table 1). Abundances for plankton metazoan grazers (mainly adult copepods, euphausiids and polychaetes) did not exceed 2 L^{-1} in June or September; thus their impact on protistan assemblages was considered negligible.

Taxa-specific oyster CRs were calculated based on cell counts for diatoms, dinoflagellates and ciliates (Fig. 5). Also, with diatoms dominating prey assemblages (Table 1), clearance rates were calculated for the most common diatom genera in the treatments that contained 2 oysters (Fig. 6). In instances where cell counts were zero (abundances below the lower threshold for microscopy counts with ~ 5 cells mL^{-1}; Fitzpatrick *et al*., 2010), a value of 0.5 was added to each sample to account for the logarithmic transformation when calculating the CR (McDonald, 2014). Negative CRs were excluded from further analysis. Individual counts for treatments with higher oyster abundances were not pursued since cell abundances dropped rapidly, limiting detectability of cells using our settling approach. In June, CRs for diatoms were significantly different from dinoflagellates and ciliates at 135 min, but no difference was found between the major taxonomic groups at 195 min (one-tailed, paired t-test, $p < 0.05$; Fig. 5A and Table 3). At the final time point, abundances had plummeted for

all prey groups (Fig. 5A). For September, no difference could be observed initially (at 90 min), and CRs for dinoflagellates were higher than that for ciliates at 120 min (Fig. 5B). At 180 min, CRs for diatoms had ramped up and were higher than those for either ciliates or dinoflagellates (one-tailed, paired t-test, $p < 0.05$; Fig. 5B).

Over the initial incubation period (~1 hour), diatom-specific CRs ranged between 14.9 and 23.7 l h^{-1} g^{-1} for the three most abundant genera in June; *Leptocylindrus*, *Asterionellopsis* and *Nitzschia*. In September, CRs were calculated between 5.4 and 49 l h^{-1} g^{-1} for *Leptocylindrus*, *Skeletonema* and *Pseudo-nitzschia* (Table 3). In September, *Melosira* contributed strongly to the initial prey assemblage and its rapid disappearance from any of the incubations below resulted in CRs of 110.3 l h^{-1} g^{-1}, with final abundances falling below the detection threshold.

Plotting all CRs against cell abundances from all time points, showed to be inversely correlated for genera *Leptocylindrus*, *Asterionellopsis* and *Nitzschia* in June (Pearson correlations; r = -0.86, -0.95 and -0.72; respectively; $p < 0.05$) and for *Leptocylindrus*, *Skeletonema* and *Pseudo-nitzschia* in September (r = -0.79, -0.95 and -0.84, respectively; $p < 0.05$; Fig. 6). The relationship between CRs and cell abundances could not be resolved for *Melosira* since the majority of counts fell below the lower detection limit of our settling approach, leaving a limited number of observations to statistically test (r = -1.12; $p < 0.05$). Multivariate statistics showed that cell abundance, but not prey type (genus), played a role in driving CRs in June (Pseudo-F = 2.6 at $P = 0.04$ for factor chl *a*). However, in September,

both factors had an effect on CRs, with prey type (Pseudo-F = 97.9 at $P = 0.001$) strongly outweighing the impact of cell abundance (Pseudo-F = 9.4 at $P = 0.001$).

Changes in Overall Prey Community Structure

Microscopy analysis allowed for the distinction of 43 genera/major groups (Supplementary Table 1). Multivariate statistics showed that community structure based on this microscopy data was not dissimilar among initial assemblages from June and September (SIMPROF, $p<0.05$; PRIMER 6). Furthermore, no consistent differences in final assemblage structure could be detected in relation to grazer absence or presence, number of oysters (treatment), individual bins (containers), or varying incubation periods (ANOSIM permutation tests at $p<0.05$, PRIMER 6).

DISCUSSION

Overall Clearance Rates

Average CRs of *C. virginica* in Bogue Sound were in good agreement with previously reported rates from the Chesapeake Bay, which had CRs of 7 l h^{-1} g^{-1} at 28ºC (Newell and Koch, 2004) and 8.2 l h^{-1} g^{-1} at 25ºC (Gedan *et al*., 2014). Bogue Sound rates also fell within ranges for coastal systems elsewhere and were inversely related to chl *a* concentrations, concurrent with previously reported trends (Table 2). Some of the highest CRs for *Crassostrea* spp. were reported with 18 l h^{-1} g^{-1} at relatively low ambient chl *a* concentrations of 2.6 µg L^{-1} (Sroczynska *et al*., 2012), while Li *et al*. (2012) reported CRs of 0.6 l h^{-1} g^{-1} at relatively high chl *a* levels of ~20 µg L^{-1}. Limited information is available on lower feeding thresholds and how they link to reduced feeding in oysters. Riisgård *et al*. (2003) showed that CRs at 0.5 µg chl *a* L^{-1} (≈ 630 cells ml^{-1} of cultured *Rhodomonas*) eventually caused *Mytilus edulis* to stop feeding, but that adding more algae allowed the bivalves to quickly reestablish clearance activity in the range of 2-3 l h^{-1} $ind.^{-1}$. Prey availability and lower feeding thresholds may have played a role in the treatments that contained 4 oysters or more in this study; CRs decreased quickly with oyster number in both experiments, with chl *a* levels dropping from 2.3 to < 1 and 3.8 to ~2 µg chl *a* L^{-1} within the first hour of our June and September incubations, respectively (Fig. 3). In relation to chl *a* levels detected in Bogue Sound during 2013, the initial chl *a* concentrations in the experiments could have been considered low; however, they reflected phytoplankton dynamics in 2014 rather well, with

weekly levels of ≤ 2.3 and ≤ 3.8 µg chl *a* L^{-1} being detected 32% and 66% of the time, respectively (Fig. 2).

Several studies have examined the effect of temperature on CR, and maximum rates for *C. virginica* were reported around ~28ºC, with diminishing feeding activity occurring as temperature increased beyond this point (Gedan *et al*., 2014, Riisgård, 1988). In our study, CRs were lower in September when temperatures reached up to 32°C, and therefore exceeded the aforementioned 28ºC threshold, and in June, CRs were higher with the maximum temperature reaching 28°C. While lower CRs in September could have been solely due to higher prey availability (less need to clear as much water to obtain the same amount of prey), it could be conjectured that temperature stress contributed to reduced feeding in some oysters treatments with high variability in CRs (Fig. 4).

To the best of our knowledge, only few studies have examined CRs for specific prey categories or taxonomic groups demonstrating the importance of prey type. In Bogue Sound, diatoms were the most abundant microplankton prey (> 20 µm in size) by far, and tended to be cleared at higher rates than ciliates and dinoflagellates (Table 3; Fig. 5). When overall prey abundance was higher in September, CRs for both dinoflagellates and ciliates increased several-fold compared to June and reached values more comparable to those for diatoms despite those prey groups being far less abundant (Table 3). Dupuy *et al*. (2000) also distinguished prey-specific CRs for diatoms, dinoflagellates, ciliates and other flagellates (> 5 µm in size) and derived similar average CRs among these prey types with CRs compatible to rates in our study (Table 3). While Dupuy *et al*. (2000) also considered smaller-sized

flagellates (< 5µm), it was concluded that the larger plankton were more efficiently pursued (Table 3). Over 84.5% of microphytoplankton acted as available carbon sources in this study, with only 1.27 % being represented by picoplankton. Increases in CRs with increasing cell size have been previously reported (Newell, 1988, Newell and Langdon, 1990, Riisgård, 1988). A bivalve feeding study from the Peconic Bay estuary, NY, also yielded similar CRs for varying prey categories including both autotrophs and heterotrophs (CR for dinoflagellates at -0.2 to 3.4 l h^{-1} ind^{-1}, diatoms at 0.8 to 2.3 l h^{-1} ind^{-1} and ciliates at 0.0 – 2.9 l h^{-1} ind^{-1}), albeit the studied bivalves did not include oysters, but instead included *Geukensia demissa, Mercenaria mercenaria,* and *Mya arenaria* (Lonsdale et al., 2009).

In-depth comparison among CRs for the most common diatoms in Bogue Sound allowed for additional insight into prey-oyster dynamics in our study, and indicated that *C. virginica* may clear certain prey types at a higher rate. It is important to note that comparisons here are based on CR estimates and that the fate of prey was not further investigated. In other words, we did not examine whether prey items made it into the digestive track or if material was eventually rejected into the ventral track and subsequently egested as pseudofeces (Ward and Shumway, 2004). In our study, CRs increased dramatically for most of the genera, as abundances dropped below ~1 to 2 cells mL^{-1}, except for *Leptocylindrus,* which kept being cleared at consistently higher rates than the other prey types at similar cell abundances in both experiments (Fig. 6A and B). In September *Skeletonema* was also cleared at slightly higher rates than *Pseudo-nitzschia* at similar abundances. PERMANOVA (testing that took varying cell abundances for each genus into account) indicated that prey type, as well as cell

abundance, were driving factors for CRs in September, and that prey type strongly outweighed the impact of cell abundance (Fig. 6B). In June, cell abundance also impacted genus-specific CRs, but no statistical significance was yielded for genus as a factor (Fig. 6A). Experiments where oysters are exposed to a more even prey assemblage (abundance ranges for each genera overlap) should help elucidate whether differences in CRs are the result of selective feeding. Understanding if selective oyster feeding may drive algal species composition will be important, especially when potentially harmful algae (e.g., *Pseudo-nitzschia*) are members of the prey assemblage.

Oyster Feeding Impact in Bogue Sound

Addressing how oysters can improve water quality (as it pertains to changes in phytoplankton biomass and potentially, composition), is extremely important, as an increasing number of coastal water bodies fail to meet state water quality requirements in North Carolina (Category 5 Water Quality Assessments-303(d) List; NCDENR, 2014). Bogue Sound is one of many bodies of water in the region where symptoms of eutrophication have become an increasing concern due to varying factors which include population growth and tourism (Mallin *et al.*, 2000, Paerl 1988). While high flushing rates and a ~2 day residence time (Bricker *et al.*, 2007) may alleviate some effects of increased nutrient loading within the sound, harmful algae already regularly affect shellfish harvest (see NCDENR Shellfish Sanitation Maps at http://portal.ncdenr.org/web/mf/maps-central-shellfish). Also, major expansion plans are already under development for the Morehead City harbor, which

could result in additional water quality concerns (J. Medlock pers. comm.; U.S. Army Corps of Engineers).

In our experiments, it took 7 to 10 oysters to clear the entire 60 L incubation volumes within an hour. To assess the impact that oyster populations may have on Bogue Sound as a whole, we used information on the Bogue Sound area (274 km^2; Bricker *et al.,* 1999), 2013 data of the percentage area that comprises of subtidal shelled-bottom habitat within Bogue/Core Sound (~3.64%; Coastal Habitat Protection Plan, 2005) and assumed average oyster densities for the entire shelled-bottom area with ~126 oysters m^{-2} (ranges are reported from 0 – 973 oysters m^{-2} in NCDMF, 2015 at http://portal.ncdenr.org/web/mf/shellfish-habitat-mapping). Finally we calculated what percentage of Bogue Sound (volume of 361,680 x 1,000 m^3; Bricker *et al.,* 1999) could have theoretically been cleared, which amounted to 82% (range = 36-108%) and 58% of the bay per day (range = 31-75%) in June and September, respectively. While these estimates are crude approximations and do not consider numerous factors (such as spatiotemporal variability in prey and grazer populations or hydrological dynamics), they can begin to inform oyster population models to guide management decisions and restoration efforts within Bogue Sound and adjacent estuaries. Several studies have extrapolated chl *a* -based CRs to determine the time it would take *in situ* populations to clear particle load within a specific bay (Newell, 1988, Buzzelli *et al.,* 2013, Cerco and Noel, 2007, Ramos *et al.,* 2008) or to estimate the number of bivalves it would take to make improvements in water quality (Cerco and Noel, 2007, Lonsdale *et al.,* 2009).

Oyster CR estimates from this study can help provide a basis to further examine the impact that oyster feeding may have on neighboring sounds, within the Albemarle and Pamlico systems, where shelled-bottom area is estimated to be approximately 1-2%, in most areas (Coastal Habitat Protection Plan, 2005). Water residence times range from slightly more than a week to an entire month (rather than 2 days as seen in Bogue Sound) in many of these areas. These systems tend to have substantial nutrient loading which acts as a major cause for frequent algal blooms and/or oxygen depletion; phenomena that are expected to worsen substantially (Bricker *et al*., 2007). However, with these ecosystem services from oysters, some of these environmental stressors may alleviate water quality issues.

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Table 1. Experimental conditions during field incubations in June and September 2014. Dates, water and air temperatures, general weather conditions, start time and duration of experiments, tides (H = high, L = low). Time points for chl *a* determination were taken more frequently than those for community analysis. Also listed are the number of oysters in each of the treatments, ash free dry weights (average with standard deviations), the contributions of varying taxa to initial protist assemblages and the names of the most abundant genera in each experiment.

Date	June 11, 2014	September 6, 2014
Water Temperature (°C)	26 - 28	29 – 32
Start Time	10:14	10:00
Duration (min)	255	180
Time Points for Chl *a* (min)	0, 60, 90, 135, 165, 195, 255	0, 75, 105, 120, 150, 180
Time Points for Phytoplankton Analysis (min)	0, 135, 195, 255	0, 90, 120, 180
Oyster Treatments (n)	0, 2, 4, 8,16	0, 2, 4, 8
Dry Weights (g oyster $^{-1}$)	0.74 ± 0.33	0.87 ± 0.33
Initial Prey Assemblage*	Diatoms: 97.1% Dinoflagellates: 2.1% Ciliates: 0.7%	Diatoms: 94.6% Dinoflagellates: 4.3% Ciliates: 1.1%
Most Common Genera	*Leptocylindrus, Asterionellopsis, Nitzschia*	*Leptocylindrus, Skeletonema, Melosira, Pseudo-nitzschia*

Table 1 Continued

Tides	H: 6:30 / L: 13:30	H: 5:33 / L: 11:41
Weather	Partly cloudy	Overcast (rained overnight)
Air Temperature (°C)	25 - 29	25 - 31

Table 2. Oyster Clearance Rates (CRs) listed per individual oyster (l h^{-1} $ind.^{-1}$) or normalized to gram oyster dry weight (l h^{-1} g^{-1}). Estimates are all based on changes in chlorophyll biomass. Studies encompass varying experimental approaches (i.e., Lab/Field) and using batch as well as flow-through systems. In several of the studies natural prey was fed (Natural prey type) while others used cultivated prey (Culture). * means maximum value

Location	Dates	Bivalve	Lab/Field	Prey type	CR (l h^{-1} ind^{-1})	CR (l h^{-1} g^{-1})	Temp (°C)	Oyster Size	Study
Bogue Sound, NC	June 2014	*C. virginica*	Field (batch)	Natural	8.2 (4.3 - 13.0)	9.8 (6.5 - 14.6)	27 (26-28)	30-50	*This study*
	Sept 2014				5.6 (3.2-7.8)	6.9 (3.7 - 9.0)	30.5 (29-32)		
Chesapeake Bay, MD	March and Aug 2000	*C. virginica*	Lab (batch)	Natural		1.0, 3.8, 8.2	15, 20, 25	80-100	Newell and Koch, 2004
Chesapeake Bay, MD	Dec 2011	*C. virginica*	Lab (batch)	Culture		7*	28.0	N/A	Gedan et al., 2014
West Neck Bay, NY	June - Sept 2010	*C. virginica*	Field (flow-through system)	Natural		0.6 (0.3 - 2.2)	20.0	3 - 25	Li et al., 2012
Wassaw Sound, GA	July and Aug 1987	*C. virginica*	Lab (batch)	Culture		6.8	28.0	N/A	Riisgard, 1988
Marennes-Oleron Basin, France	Sept 1988, Feb 1989	*C. gigas*	Lab (flow-through system)	Culture	2.0, 4.8, 2.5		5, 20, 32	N/A	Bougrier et al., 1995
Piraquê-açu/Piraquê-mirim estuarine system, Brazil	June 2012	*C. rhizophorae*	Field (flow-through system)	Natural		18.0 (13.6 - 31.5)	25.0	37 - 67	Sroczynska et al., 2012
Ashtamudi Lake, India	N/A	*C. madrasensis*	Lab (batch)	Culture	24.1 ± 0.5		30.0	65 - 105	Rajesh et al., 2001
Peconic Bay Estuary, NY	May and Oct 2003	*G. demissa*	Field (batch)	Natural	2.3 ± 1.5		10-29	80 - 90	Lonsdale et al., 2009
		M. mercenaria			1.4 ± 1.6				
	May and Oct 2004	*G. demissa*			2.9 ± 2.3		10-29	80 - 90	
		M. mercenaria			0.8 ± 1.4				
		M. arenaria			0.6 ± 0.5				

Table 3**.** Taxa-specific CRs (l h $^{-1}$ g $^{-1}$) based on changes in abundances (cells mL $^{-1}$) from Dupuy *et al*., 2000 in comparison to this study. Temperature is listed below the date. nd stands for "not detected".

Study	**Bivalve**	**Date**	**Major Taxa**	**Genera**	**CR (l h^{-1} g^{-1})** ave (range)
This study	*C. virginica*	June 2014	diatoms		21.2 (13.2 - 29.4)
		26-28˚C		*Leptocylindrus*	18.2 (12.1 - 28.4)
				Asterionellopsis	23.7 (21.8 - 27.7)
				Nitzschia	14.9 (4.5 - 22.5)
			dinoflagellates		1.0 (nd - 3.7)
			ciliates		2.9 (2.6 - 3.3)
		Sept 2014	diatoms		13.5 (7.0 - 23.1)
		29-32˚C		*Leptocylindrus*	5.4 (2.5 - 8.4)
				Skeletonema	49.0 (13.6 - 72.4)
				Pseudo-nitzschia	12.0 (0.7 - 19.1)
			dinoflagellates		6.3 (nd - 29.3)
			ciliates		9.0 (nd - 16.8)
Dupuy et al., 2000	*C. gigas*	Aug 1998	diatoms		8.1
		25˚C	dinoflagellates		14.8
			ciliates		12.7
			> 5 µm flagellates		16.7
			< 5 µm flagellates		6.8
			All phytoplankton > 5µm		11.8

Supplementary Table 1. Average cell abundances (cells ml^{-1}) for 43 identified genera and taxonomic groups for June and September 2014.

MAJOR TAXA	**GENUS / GROUP**	**JUNE**	**SEPT**
Ciliates	Ciliates	2.1	5.5
Pennate Diatoms	*Asterionellopsis*	24.8	2.1
	Bacillaria	1.6	0.4
	Cylindrotheca	0.3	2.7
	Entomoneis	0.5	1.5
	Licmorpha	0.3	1.3
	Navicula	2.2	6.9
	Nitzschia	19.4	20.1
	Pleurosigma	1.6	2.7
	Pseudo-Nitzschia	10.6	23.2
	Thalassionema	4.3	17.8
Centric Diatoms	*Bacteriastrium*	0.0	0.3
	Ceratoulina	0.2	0.7
	Chaetoceros	8.6	9.3
	Corethron	1.2	0.9
	Coscinodiscus	3.6	2.8
	Dactyliosolen	0.9	0.2
	Detonula	0.0	1.6
	Ditylum	0.0	0.3
	Eucampia	0.1	2.8
	Guinardia	7.7	1.9

Supplementary Table 1 Continued

	Hemialus	7.1	6.7
	Leptocylindrus	107.6	224.9
	Lithodesmium	0.0	0.6
	Melosira	8.2	25.4
	Odontella	0.5	1.6
	Rhizosolenia	5.3	7.1
	Skeletonema	5.1	50.1
	Thalassiosira	1.2	0.0
	Thalassiosira punctigera	0.0	0.2
	Other Centric Diatoms	13.3	38.9
Other Diatoms	*Other Diatoms*	35.9	12.5
Dinoflagellates	*Ceratium*	0.0	0.1
	Gymnodinium	0.3	0.9
	Gyrodinium	0.0	1.1
	Prorocentrum Gracile	0.8	3.1
	Prorocentrum Micans	0.0	1.6
	Protoperidinium	0.2	0.0
	Other Prorocentrum	4.1	11.3
	Other Dinoflagellates	0.6	3.2
Silicoflagellates	*Dictyocha*	0.1	1.7
Radiolarian	*Radiolarin*	0.0	0.8
Prymnesiophyte	*Phaeocystis*	1.1	1.9

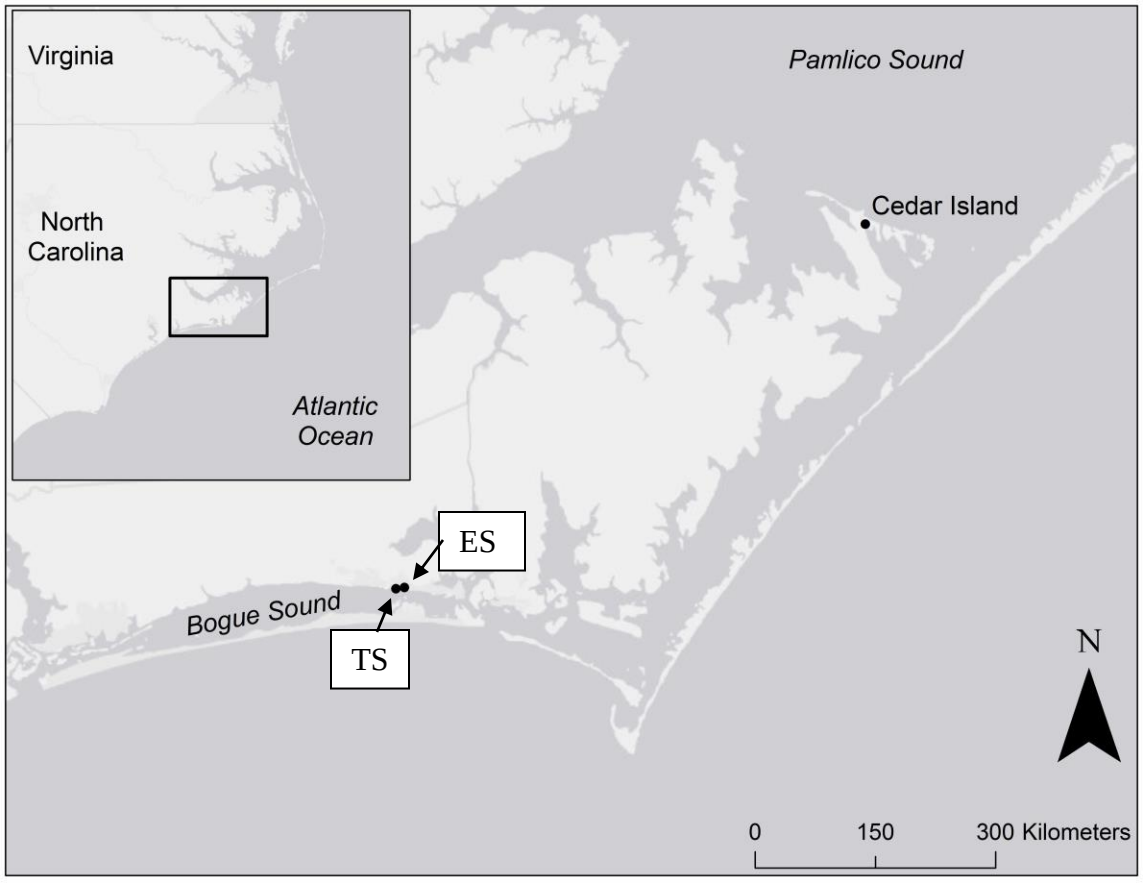

Figure 1: Bogue Sound is located within the southwestern part of the Albemarle-Pamlico Sound estuary. The time-series site (TS) was located in Morehead City (34° 43' 26.651N / 76° 45' 26.964W) just 0.8 km south of the experimental site (ES). Cedar Island is the location of the oyster hatchery where oysters were obtained.

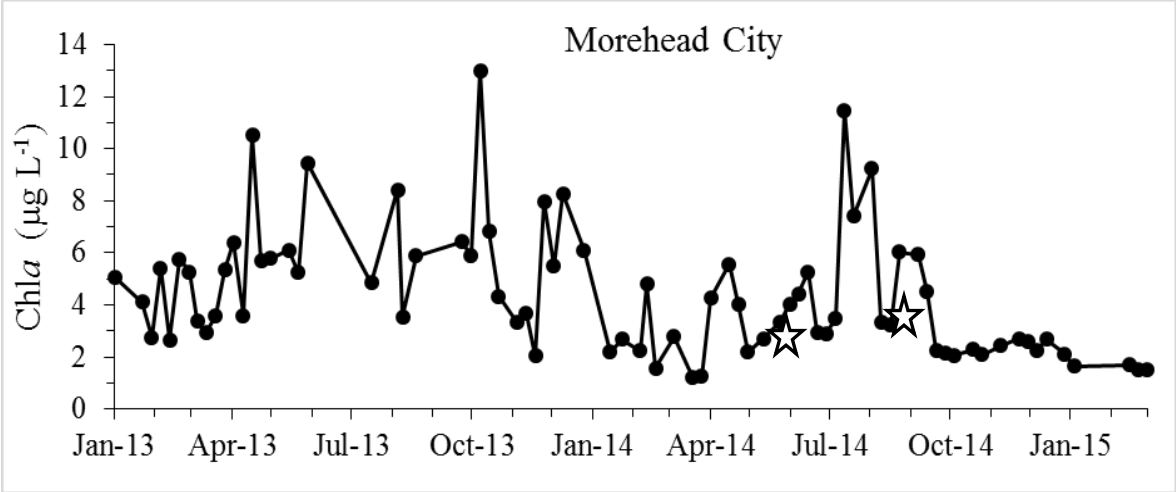

Figure 2: Phytoplankton dynamics based on weekly chlorophyll *a* concentrations (µg chl *a* L^{-1}) determined for the time-series site from January 2013 through March 2015. Stars indicate initial chl *a* levels at the onset of the experiments, with 2.3 and 3.8 µg L^{-1} in June and September, respectively.

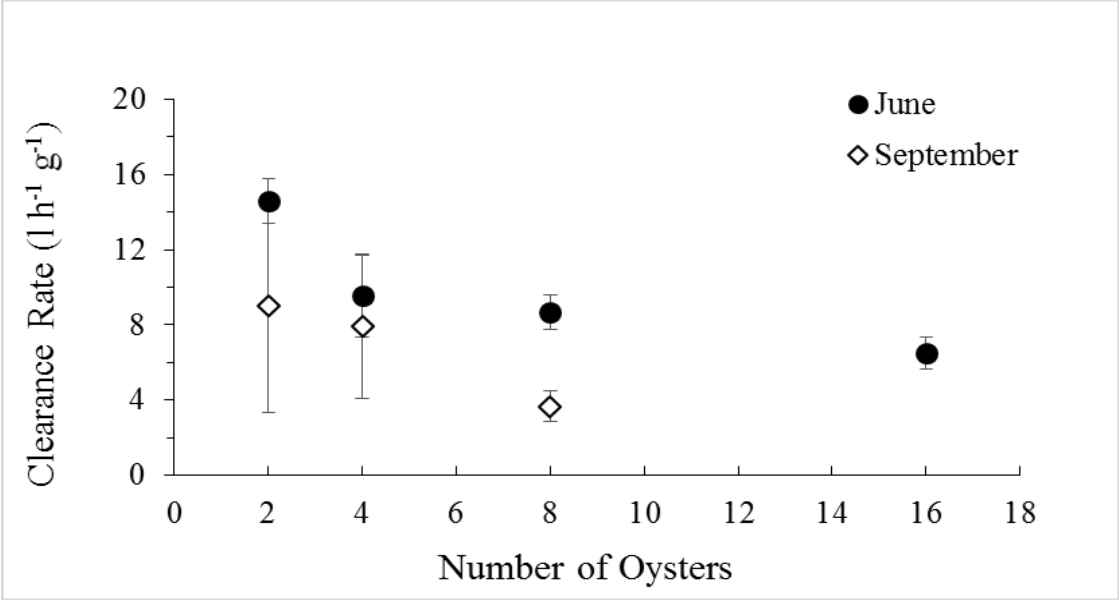

Figure 3: CRs based on changes in chl *a* over first time point and normalized to ash-free oyster dry weights (l h $^{-1}$ g $^{-1}$) within differing treatments (number of oysters = 2, 4, 8, 16 and 2, 4, 8 individuals in June and September, respectively). CRs are shown as means from 3 triplicate containers with their standard deviation.

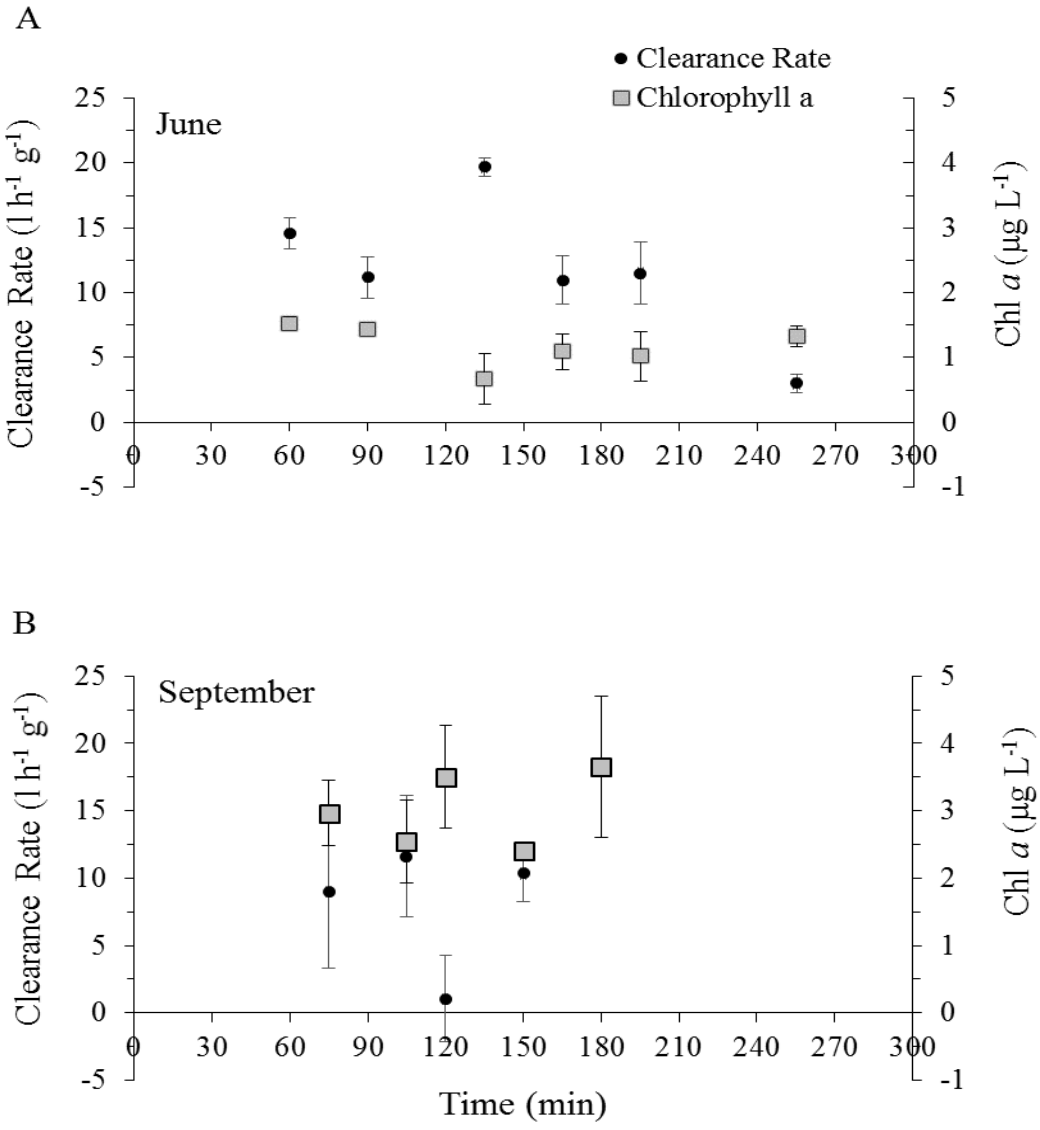

Figure 4: CRs normalized for ash-free dry weight (l h $^{-1}$ g $^{-1}$) in relation to chl *a* concentration (µg l^{-1}, secondary y-axes) shown for the lowest oyster treatment (2) in both experiments. All values are shown as means with their standard deviation. Negative values are not reflected in this graph.

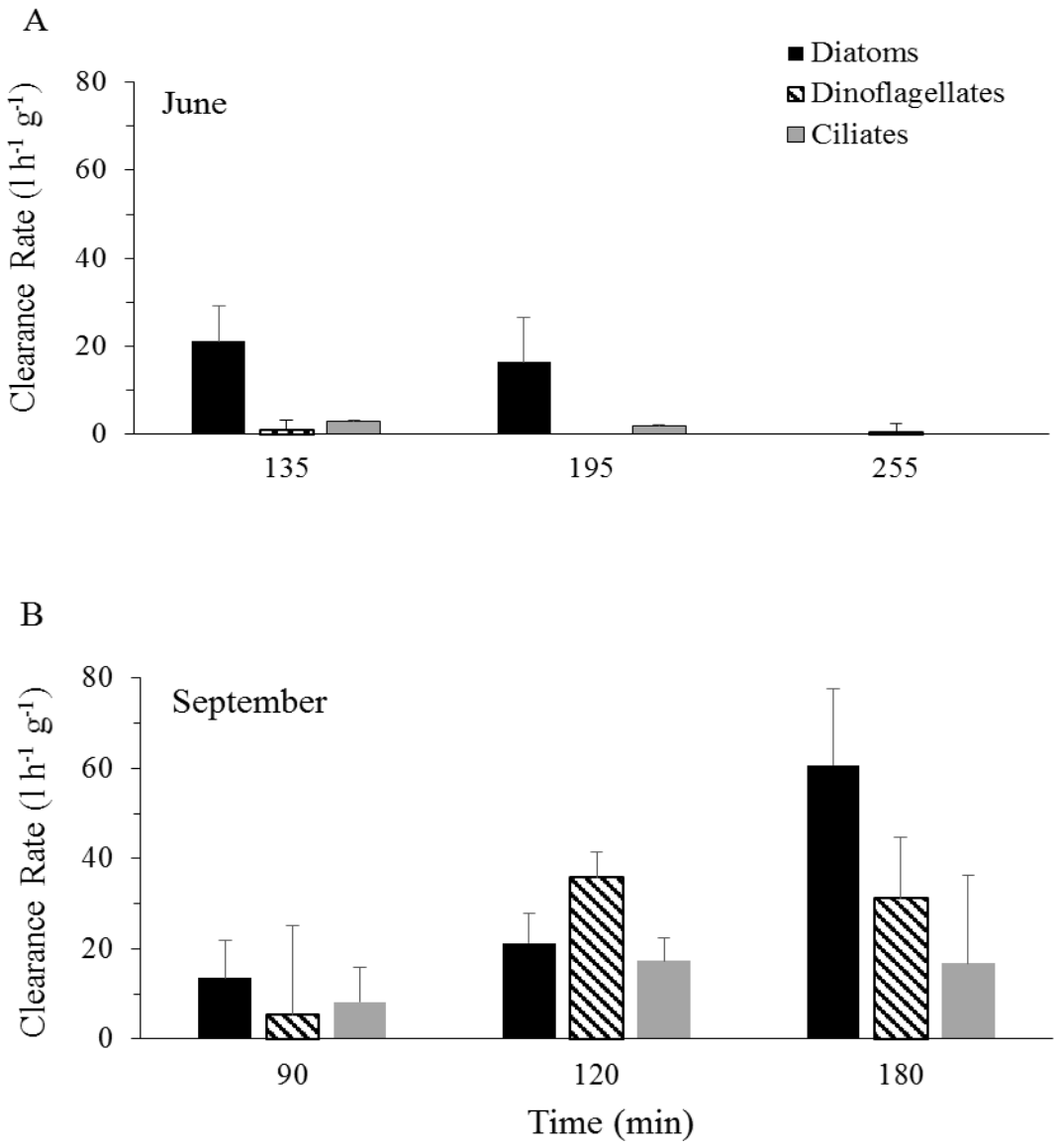

Figure 5: Individual CRs (l h^{-1} g^{-1}) for diatoms, dinoflagellates and ciliates over varying time points for treatments that contained 2 oysters in June (A) and September (B). CRs are shown as means from 3 triplicate containers with their standard deviation.

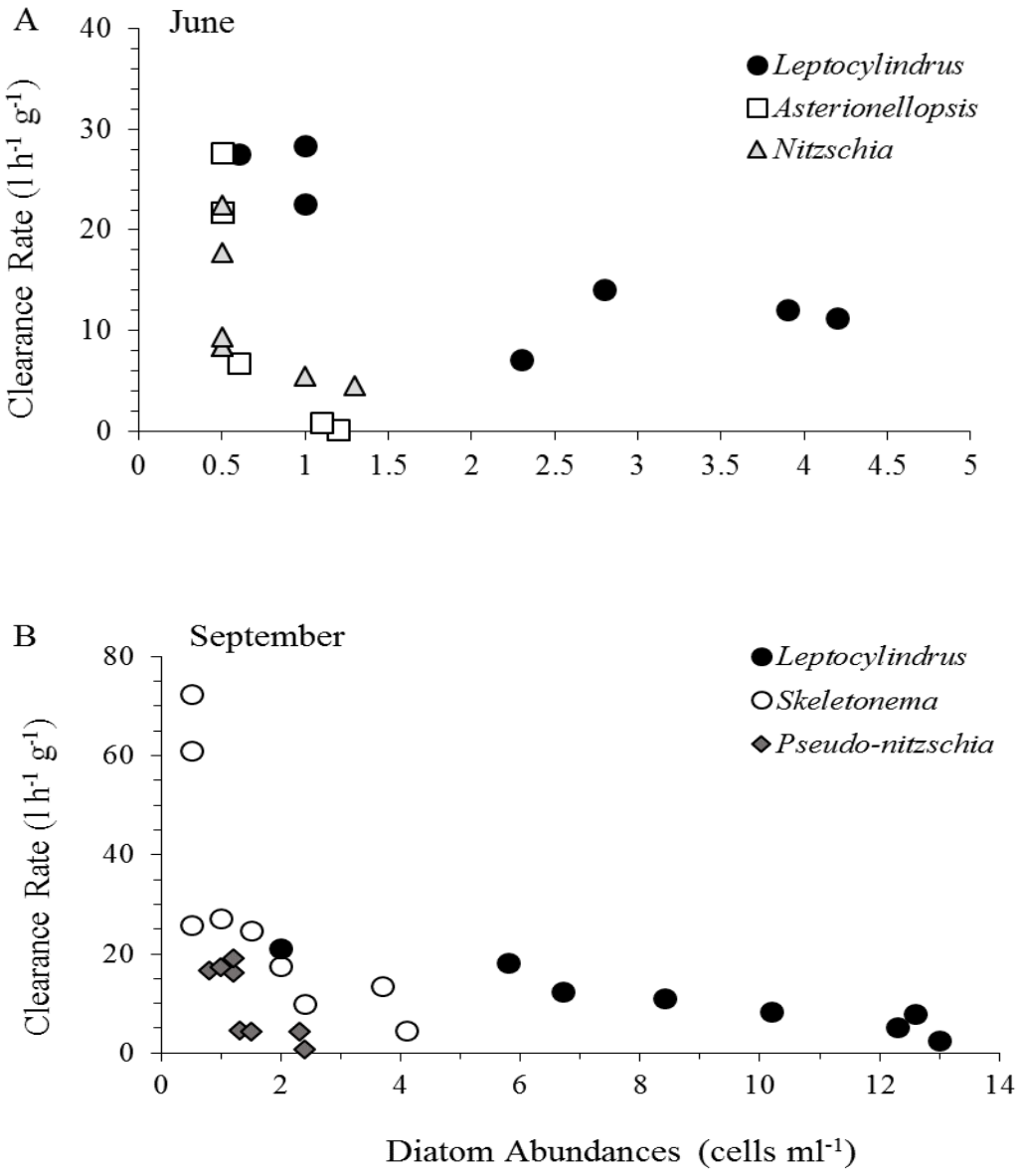

Figure 6: Individual CRs (l h^{-1} g^{-1}) for the most abundant diatom genera from each individual container (all time points combined) in relation to cell abundances (cells mL^{-1}). Note difference in axes range between experiments.