Harmful Marine Algal Blooms - P. Lassus, G. Arzul, E. Erard, P. Gentien, C. Marcaillou

The effects of dinoflagellate blooms on the oyster *Crassostrea virginica* in Chesapeake Bay

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Abstract. Dinoflagellate blooms frequent Chesapeake Bay and its tributaries, with non-toxic dinoflagellate blooms characteristic of each season. As part of a larger program to (1) ascertain the importance of bloom production to carbon flux in the system and (2) determine the impact of recurring blooms on oyster production and grazing, feeding experiments were conducted using juvenile *Crassostrea virginica* and two non-toxic dinoflagellates, *Prorocentrum mariæ-lebouriæ* (*P. minimum*) and *Gyrodinium uncatenum*, usually mixed with ambient phytoplankton assemblages. Using a portable flow cytometer (FACScan), changes in particle numbers were followed during short-term incubations of individual oysters and particulate suspensions, corrected for particle dynamics in containers without juvenile oysters. Overall, oysters effectively removed dinoflagellates from suspension, with highest rates when feeding on *Prorocentrum*; pseudofeces production was high. These results suggest that juvenile oyster populations should effectively reduce accumulations of bloom-forming dinoflagellates in the water column prior to or during blooms, transfering surface bloom production to the sediments as feces and pseudofeces, likely increasing benthic oxygen demand over most of the system and shifting dominant oxygen demand from the pelagic to the benthic environment.

Introduction. Chesapeake Bay and its tributaries are typified by frequent, recurring blooms of non-toxic dinoflagellates throughout the year (Loftus *et al.*, 1972; Tyler and Seliger, 1978; Zubkoff *et al.*, 1979; Sellner and Olson, 1985; Gallegos, 1989; Sellner and Brownlee, 1990; Sellner *et al.*, 1991) with the fate of bloom production largely unresolved (Sellner and Brownlee, 1990; Sellner *et al.*, 1993). Benthic suspension feeders, including the previously abundant american oyster, *Crassostrea virginica*,

could conceivably remove large fractions of bloom production (Shumway *et al.*, 1990; Shumway and Cucci, 1987). The present study was undertaken to (1) determine the effects of recurring dinoflagellate blooms on the native oyster populations and (2) estimate the potential importance of oysters in processing bloom production in the bloom-rich estuary.

Methods. One-year-old cohorts of juvenile (2.5-3.8 cm) oysters were transferred to the VIMS oyster hatchery in Gloucester Point, Virginia after initial rearing in ambient waters on the eastern shore of Chesapeake Bay. Prior to the experiments, the oysters were maintained in sand- and charcoal-filtered York River water at ambient temperatures supplemented with daily additions of actively growing *Isochrysis* (clone TISO), *Thalassiosira pseudonana* (clone 3H) and suspensions of *Thalassiosira weisflogii* (clone TFLUV) paste. These phytoplankton as well as the dinoflagellates, *Prorocentrum mariae-lebouriae* (*P. minimum*) and *Gyrodinium uncatenum*, were also reared at the hatchery on a defined nutrient medium with Cool-White fluorescent lighting.

For *Prorocentrum* feeding experiments, 4 L beakers were filled with 0.5-2 L of water, either water from the York River, pre-screened to remove >73 µm particles (zooplankton). For *Gyrodinium*, experiments used sand- and charcoal-filtered hatchery water. Temperatures and salinities for the experiments were 21.5°-25°C, 14‰-18‰ (*Prorocentrum*) and 9°-12°C, 12‰-14‰ (*Gyrodinium*), respectively. These solutions were supplemented with a suite of cultured phytoplankton, including the small flagellate or diatom (TISO or 3H) to the larger diatom TFLUV and the dinoflagellates. Two control beakers were set up in each experiment, containing the food suspension and no oyster. Samples were removed from these beakers at the beginning of each experiment (time zero, t_o, for all beakers) and at the end of the incubation. Individual, juvenile oysters were placed in 6-10 separate beakers with gentle aeration provided by overhead airlines. At the end of the incubation, judged by visible production of feces and/or pseudofeces as well as by quick particle counts from beakers containing actively feeding oysters, feces and pseudofeces from all feeding oysters were pipetted into labelled scintillation vials. Immediately thereafter, subsamples were removed by dipping 20 ml scintillation vials into stirred beakers. These subsamples, as well as the t_o subsamples, feces and pseudofeces, were immediately scanned on a FACScan portable flow cytometer (Becton Dickinson, San Jose, CA), for particle enumeration. Using a 15 mW argon laser (488 nm excitation), chlorophyll and phycoerythrin fluorescence emissions were detected at

>670 and 575-590 nm, respectively. Forward light scatter (FSC) and 90° light scatter (SSC) were simultaneously measured for particle size determination. Size (equivalent spherical diameters) calibration was completed using six types of uniform size microspheres, with a suite of size ranges from 2-20 µm.

Oysters removed from each beaker were sacrificed with soft tissue frozen for subsequent dry weight determinations; other samples were removed and fixed with Lugol's iodine for phytoplankton identifications using inverted light microscope techniques. Particulate carbon, nitrogen chlorophyll *a* and phaeophytin were also determined on Whatman GF/F filtered samples (Strickland and Parsons, 1972). Clearance rates (see Omori and Ikeda, 1984) were estimated for those oysters actively feeding (Coughlan, 1969). Changes in phytoplankton densities observed in control beakers over the incubation were estimated from the differences between initial and final densities for all non-dinoflagellate taxa. Due to widely varying dinoflagellate densities in control beakers, the change in cell numbers in control beakers was assumed to be zero for these motile cells. Initial densities of the dinoflagellates was estimated from the mean of densities noted in initial and final control beakers. To account for changes in a suite of cell concentrations from a single oyster, estimated filtering rates for each oyster were considered as a vector response and were analyzed by multivariate regression or multivariate analysis of variance (MANOVA) using the GLM procedure of SAS (SAS, 1989). Comparisons among filtering rates for each cell size and interactions of each cell size and total density were based on a multivariate repeated measures model.

Results. *Prorocentrum mariæ-lebouriæ* (*P. minimum*): This 11-13 µm dinoflagellate was readily ingested by juvenile oysters. Clearance rates for this alga in unialgal suspensions were similar ($p<0.39$) to rates measured for two other taxa, the 14 µm diatom *T. weisflogii* and the 6 µm *Isochrysis*, when offered at similar concentrations of 10^4 cells·ml^{-1}; rates were 1.95 ± 0.63, 3.73 ± 1.05 and 2.73 ± 0.97 l·oyster^{-1}·h^{-1}, respectively. However, juvenile oyster filtration of the dinoflagellate was not similar to rates measured for other populations within the natural assemblage. When *Prorocentrum* was added to 73 µm screened natural phytoplankton samples collected from the York River, the dinoflagellate was removed from suspension at higher rates than the other similar sized phytoplankton at lower total phytoplankton densities; in contrast, at the highest abundances of total phytoplankton, *Prorocentrum* was filtered at a lower rate relative

Table 1. Mean (and standard error) clearance rates ($l \cdot oyster^{-1} \cdot h^{-1}$) for juvenile *Crassostrea virginica* feeding on natural suites of phytoplankton supplemented with *Prorocentrum* (***Proro***). TOT is all cells >3 µm; cell dimensions are presented below each food item. CRYPT and *Micro* refer to a cryptophyte (**CRYPT**) and *Micromonas* sp. (***Micro***) present in all samples.

TOT ($10^3 \cdot ml^{-1}$)	***Proro*** ($10^3 \cdot ml^{-1}$)	***Proro*** **13 µm**	**17-18 µm CELL**	**CRYPT 12 µm**	**11 µm CELL**	**5 µm CELL**	**3.6 µm CELL**	***Micro*** **1.3 µm**	**TOT**
43.1	1.7	2.74 (1.31)	2.29 (1.33)	2.01 (1.02)	2.40 (1.40)	2.46 (1.34)	0.19 (0.46)	0.14 (0.10)	2.02 (1.18)
42.2	2.5	3.82	2.37	3.02	4.83	2.91	0.73	0.16	2.92
44.9	3.7	3.61 (0.94)	4.00 (0.91)	2.72 (0.85)	4.14 (0.98)	3.61 (0.83)	0.69 (0.38)	0.28 (0.11)	2.91 (0.63)
36.4	5.5	4.16 (0.86)	4.13 (1.26)	3.23 (0.12)	3.81 (1.10)	3.57 (1.05)	0.68 (0.30)	0.40 (1.00)	3.17 (0.85)
43.0	7.9	3.02 (0.55)	2.25 (0.64)	1.24 (0.43)	1.92 (0.54)	1.86 (0.50)	0.39 (0.10)	0.19 (0.03)	1.77 (0.38)
60.0	26.0	0.68 (0.26)	0.90 (0.16)	1.26 (0.31)	0.82 (0.19)	0.88 (0.16)	0.24 (0.10)	0.21 (0.02)	0.64 (0.21)

to the other similar-sized taxa (Table 1). Clearance rates on cells <5 µm were very low, <0.73 l·oyster^{-1}·h^{-1}, suggesting poor filtration of these smallest cells. In addition, clearance rates were inversely related ($p<0.0008$) to total cell numbers (Fig. 1) as highest rates, 4.16±0.86 l·oyster^{-1}·h^{-1} (29.4±4.9 l·g^{-1}·h^{-1}), were recorded at 36.4·10^3 cells·ml^{-1} and lowest rates, 0.68±0.26 l·oyster^{-1}·h^{-1} (5.1±2.0 l·g^{-1}·h^{-1}), were observed at highest total cell numbers 60·10^3·ml^{-1}. Pseudofeces production was high in all containers with elevated *Prorocentrum* densities.

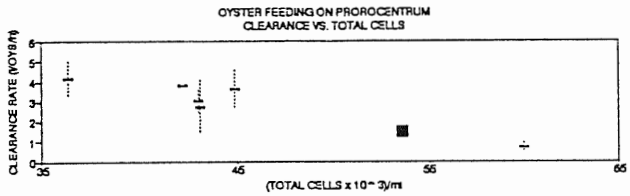

Figure 1. Juvenile oyster clearance rates on *Prorocentrum* as a function of total phytoplankton densities. The square symbol represents the rate for *Thalassiosira weisflogii*, not significantly different ($p<0.54$) from the expected rate for *Prorocentrum* at this density.

Gyrodinium uncatenum: Due to substantial differences in water temperatures between York River and the cultured *Gyrodinium* (19 µm), no grazing experiments were conducted with York River supplemented water samples. Dilutions of the *Gyrodinium* culture were offered to the juvenile oysters, several mixed with aliquots from other cultured phytoplankton (clones TFLUV, TISO, a chain forming diatom, *Chaetoceros calcitrans* and the small dinoflagellate *Heterocapsa triquetra*). Results were quite variable for all experiments with *Gyrodinium* additions and no consistent pattern was evident over the cell concentrations provided. Juvenile oysters ingested the dinoflagellate at 0.25±0.24 l·oyster^{-1}·h^{-1} (1.5±1.8 l·g^{-1}·h^{-1}) when *Gyrodinium* was offered as a sole prey item at 10^3 cells·ml^{-1}; at 3.6·10^3 cells·ml^{-1}, oyster clearance rates declined to 0.07±0.04 l·oyster^{-1}·h^{-1} (0.5±0.3 l·g^{-1}·h^{-1}), a pattern consistent with declining rates with increasing cell density (see Fig. 1). However, clearance rates estimated in mixed diets of *Gyrodinium*, TFLUV and TISO at total cell densities of 4.1· and 35.4·10^3 cells·ml^{-1}, did not follow the same pattern; rates for the dinoflagellate were 0.33±0.07 and 0.27±0.04 l·oyster^{-1}·h^{-1} (3.4±0.7 and 1.7±0.3 l·g^{-1}·h^{-1}), respectively. Oyster clearance rates on the dinoflagellates in a suspension of *Gyrodinium*, *Heterocapsa* and *Chaetoceros* (5 µm) (0.7:3.7:5.6·10^3 cells·ml^{-1}) were negative (no filtering) while the diatom was removed at 0.03±0.02 l·oyster^{-1}·h^{-1} (0.7±0.4 l·g^{-1}·h^{-1}), not significantly different from zero. Pseudofeces and feces were rapidly produced in all experiments.

Discussion: Our results suggest that oysters, if present, can be effective filterers of dinoflagellates (including *Cochlodinium heterolobatum*; unpubl. data) in eutrophic estuaries, exemplified by clearance rates exceeding 4 and 0.7 $l \cdot oyster^{-1} \cdot h^{-1}$ for low and high (bloom) concentrations of *Prorocentrum*, respectively. The dinoflagellates might support oyster growth, although high mortalities were observed at bloom densities of *Prorocentrum* (Luckenbach *et al.*, 1993; Wikfors *et al.*, 1993), but oyster grazing insures rapid transfer of planktonic production to surficial sediments as feces and pseudofeces, vertically segregating nutrient remineralization processes and sediment oxygen demand from euphotic autotrophy in stratified coastal systems.

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